Phyllosphere microbiome-based biocontrol solution against *Botrytis cinerea* and *Plasmopara viticola* in grapevine

Nicolas-David Rappo[1], Sylvain Schnée[2], Clara Chevalley[2], Émilie Michellod[2], Floriane L'Haridon[1,3], Katia Gindro[2], Laure Weisskopf[1,3], Sébastien Bruisson[1,3*]

[1]Department of Biology, University of Fribourg, CH-1700 Fribourg, Switzerland.

[2]Mycology Group, Research Department Plant Protection, Agroscope, Nyon, Switzerland

[3]Food Research and Innovation Center, University of Fribourg, CH-1700 Fribourg, Switzerland

[*]Corresponding author: S. Bruisson; E-mail: sebastien.bruisson@unifr.ch

Chemin du musée 10, CH-1700 Fribourg, Switzerland

Abstract

Plants harbor different microbial communities in their different organs. This difference is due to the various conditions to which the different parts of the plant are subjected. Consequently, the phyllosphere microbiota can differ strongly from root-associated communities. We hypothesize that the grapevine phyllosphere is a valuable source of biocontrol bacteria, whose adaptation to the aerial plant environment may enable them to reach their full protective potential against foliar pathogens. In previous work, we isolated phyllosphere bacteria and showed that many strains were very effective against such pathogens *in vitro*, inhibiting their mycelial and spore development. This work investigates the biocontrol ability of these phyllosphere bacteria in leaf disc assays against two foliar pathogens: *Botrytis cinerea* (gray mold) and *Plasmopara viticola* (downy mildew). Our results show that 40 strains out of 46 affected at least one pathogen by altering their spore physiology and/or reducing disease progression. Among these strains, 27 strains could impact both pathogens and 20 were also capable of stimulating plant defenses. Because bacterial consortia might perform better than single strains, we also compared the protection conferred by individual bacteria and associations of up to three strains. When combined, bacteria showed improved efficacy *in vitro* against *B. cinerea* and *in planta* against *P. viticola*, resulting in a much stronger protection than that obtained by the application of the strains alone. These data suggest that phyllosphere bacteria could be a promising tool to help winegrowers manage grapevine diseases by providing an effective and sustainable protection of their crops.

Keywords: *Vitis vinifera*, *Botrytis cinerea*, *Plasmopara viticola*, plant protection, biocontrol, plant defense.

Funding: co-financed by Innosuisse; co-financed by the Swiss National Science Foundation (grant 310030 207917 to LW).

Introduction

Plant microbiota forms a rich and complex network that is organized into different communities. Each part of the plant provides a specific environment with conditions favorable to the development of different species of bacteria (Compant et al., 2019). Consequently, within the same plant, bacterial communities found in roots are different from those found in leaves and flowers (Zarraonaindia et al., 2015). Furthermore, because the composition of each community is determined by the sum of all conditions they face, the bacteria found at the surface or inside the plant of the same organ are also different (Dastogeer et al., 2020). Many recent studies have shown that the plant microbiota contributes to its host's health by modulating its growth, promoting plant defenses and inhibiting pathogen development (Brader et al., 2017; Rai et al., 2023) and such bacteria are good candidates for developing biocontrol solutions.

However, among the thirty to forty current biological control solutions used in Europe and the USA against crop diseases, most rely exclusively on organisms isolated from the soil or roots, even when the objective is to control pathogens affecting the aerial parts of the plant. Of all the solutions available, relatively few organisms have been isolated from the aerial

56 parts of plants such as the yeast *Aureobasidium pullulans* (Botector® and BlossomProtect™),
57 the yeast *Metschnikowia fructicola* (Noli), and the bacterium *Pantoea agglomerans*
58 (Bloomtime Biological™) (Borges et al., 2021; Pascale et al., 2020; Syed Ab Rahman et al.,
59 2018).

60 In this study, we tested the potential of bacteria isolated from the phyllosphere (the leaves
61 and their surrounding environment) to protect plants against diseases affecting their aerial
62 parts. We selected grapevine as a model plant to test this strategy, for several reasons: i) it is
63 a crop of major economic importance worldwide; ii) it is a perennial plant, and such plants
64 are expected to harbor more adapted phyllosphere microbiota compared to annual plants,
65 since perennial plants have longer-lasting interactions with their associated bacterial
66 communities (Primieri et al., 2022).In such plants, even though leaves are deciduous, the leaf
67 microbiota can be renewed each year from persistent reservoirs present on the plant such as
68 bark (Griggs et al., 2021; Martins et al., 2013; Vitulo et al., 2019); iii) most grapevine cultivars
69 are highly susceptible to leaf-borne pathogens, making this crop one the most fungicide-
70 intensive in the world (W. J. Li et al., 2011). Biocontrol is of particular interest for such
71 cultures that need alternative and novel effective solutions, to limit the environmental
72 toxicity linked to the excessive use of phytosanitary products. In addition, the use of
73 microorganisms that are antagonistic to filamentous plant pathogens is based on multiple
74 modes of action. It is therefore unlikely to lead to the emergence of resistant individuals
75 within pathogen populations and represents a sustainable means of control.

76 Biocontrol bacteria have a wide range of modes of action and can act either directly on the
77 pathogens and/or indirectly, through the plant. Therefore, they can have a direct inhibitory
78 effect through the production of metabolites that can disturb the pathogen's growth and

physiology, impair their virulence, or they can also compete for space and nutrients, e.g. by trapping vital resources such as iron through the secretion of siderophores (H. Chen et al., 2008; Haas & Défago, 2005; Ongena & Jacques, 2008). They can also have an indirect effect on the pathogen by improving plant health through the production of plant growth promoting compounds or the induction of a local or systemic defense response (Legein et al., 2020). In grapevine, one of the well-identified metabolic defense pathways is based on the production of stilbenic compounds, a group of phytoalexins of major importance for this crop due to their high toxicity for fungal pathogens (Pezet et al., 2003, 2004). This family includes a large number of inducible molecules that are produced in several forms from monomers to tetramers (Schnee et al., 2013). Their distribution and concentration are cultivar and organ dependent and the resistant cultivars have been shown to synthesize high amounts of the more active forms such as viniferins (Waffo-Teguo et al., 2008; Viret et al., 2018; Guerrero et al., 2020).

In view of these multiple modes of action, combining different strains with complementary bioactivities into a mixed microbe-based biocontrol solution could provide a multi-layer protection by combining several mechanisms that may lead to improved efficacy and increased plant protection. Several studies already highlighted that consortia of beneficial microbes can help to improve the effectiveness of the protection against pathogens in different cultures such as strawberries and tomatoes (Guetsky et al., 2001; Maciag et al., 2023; Niu et al., 2020). Additionally, the use of consortia can help to make the protection less variable from one plant to another, compared to the use of single strains (Yu et al., 2022; Wang et al., 2023).

101 In a previous study, we demonstrated that the grapevine microbiota isolated from the
102 phyllosphere contained many effective strains that could effectively inhibit the development
103 of leaf pathogens in *in vitro* experiments (Bruisson et al., 2019). In this previous work, several
104 bacteria were observed to strongly inhibit the mycelial growth of the fungus *B. cinerea* and
105 the oomycete *Phytophthora infestans* in *in vitro* conditions. They were also able to disrupt
106 the physiology of these pathogens by affecting their spores. We concluded that the use of
107 such bacteria was a promising strategy to increase the effectiveness of microbe-based
108 biocontrol solutions, but that more advanced testing using whole plants was required.

109 In the present work, we aimed to determine whether grapevine phyllosphere bacteria can
110 confer *in planta* protection and whether the application of phyllosphere bacteria in consortia
111 provide, as hypothesized, superior protection through the addition of individual effects
112 compared to the use of single strains. We tested these hypotheses against two of the most
113 detrimental grapevine diseases: downy mildew caused by the oomycete *Plasmopara viticola*,
114 and gray mold caused by the ascomycete *Botrytis cinerea*. Our results suggest that this
115 protection is achieved by combining direct effects on the pathogens, affecting their spores
116 and their mycelium development, as well as through indirect effects linked to their ability to
117 increase the expression of defense-related genes and the production of specialized
118 metabolites involved in plant defense.

119

120 **Materials and methods**

121 **Biological material and culture media**

122 Bacterial strains isolated from grapevine phyllosphere as described in (Vionnet et al., 2018)
123 and screened for their biocontrol capacity in (Bruisson et al., 2019) were grown on LB
124 (Lysogeny Broth) medium in the dark and at room temperature. LB medium was prepared by
125 mixing 12.5 g/L of LB Broth Miller and 10 g/L of LB Broth Lennox (Fisher Bioreagents) in
126 distilled water. For solid medium, 15 g/L of Agar-Agar Kobe I (Roth) were added.

127 All bacteria are epiphytic or endophytic strains isolated from three different grapevine
128 cultivars. The first two letters of their code name correspond to their cultivar of origin:
129 Chasselas (CH), Pinot Noir (PI) and Solaris (SO). The last letter corresponds to their location of
130 origin: D for endophytic and P for epiphytic.

131 *Botrytis cinerea* strain 2905 was provided by the Agroscope of Changins and was grown in
132 potato dextrose agar (PDA) (Roth). PDA was prepared by dissolving 39 g/L of PDA powder
133 (Sigma-Aldrich) in distilled water.

134 *Plasmopara viticola* sporangia were provided by the Agroscope of Changins. They were
135 harvested from naturally infected leaves collected yearly in local vineyards to reflect the
136 genotypes currently infecting the grapevine at the time of the experiments. These field
137 isolates were maintained by reinoculation on Cabernet Sauvignon.

138 The plant material studied was based on cuttings of *Vitis vinifera* L. cv. Gamay (susceptible to
139 *B. cinerea*) and Gamaret (susceptible to *P. viticola*). After 2 months of growth in greenhouse
140 conditions, plants were transferred into growth chambers for infection assay, with a 16 h/8 h
141 photoperiod, 21/20°C day/night temperature and 40 % relative humidity (RH). A sulfur lamp
142 was provided to prevent the appearance of powdery mildew.

143 **Effect of bacterial strains on pathogens**

144 Experiments to assess the effects of grapevine phyllosphere bacteria on *B. cinerea* and *P.*
145 *viticola* can be divided into three main parts (Fig. 1). The first assays consisted in testing the
146 ability of the bacterial strains to disrupt the pathogens' reproduction by interfering with their
147 spore development. Co-incubation with *B. cinerea* conidia and *P. viticola* zoospores was used
148 to monitor the strains' ability to inhibit their germination and motility, respectively. After co-
149 incubation with the bacteria, *P. viticola* zoospores were used to infect leaf discs to evaluate if
150 the loss of motility impaired their infectivity. In the second assays, the protective abilities of
151 the bacteria were tested by application of the strains on leaf discs two days prior infection in
152 order to let them adapt to their new environment and the possibility of stimulating plant
153 defenses, before measuring their protective effect. After a selection step based on the
154 effectiveness of the strains in both series of assays, their resistance to biofungicides and their
155 compatibility, a last assay on whole plants was conducted. This was a preventive assay to test
156 the local and systemic protection conferred by a selection of bacteria used alone or in
157 combinations of three strains to evaluate their protective abilities.

158 **Effect of bacterial strains on *B. cinerea* spore germination**

159 Spores of *B. cinerea* from 7-day-old mycelium grown on PDA plate were harvested by
160 watering the plate with 5 mL of sterile water and filtration through glass wool to remove
161 hyphae. The spore solution was then adjusted to 5 x 10^4 spores/mL and 12 µL of this solution
162 was mixed in an Eppendorf tube with 36 µL of bacterial suspension adjusted at OD_{600} = 1 in
163 V8 medium that was filtered using a 0.2 µm Millex®-FG filter (Merck). Each single strain from
164 our collection was tested at OD_{600} = 1. Combinations of two to three strains were tested
165 using equal volumes of bacterial solutions with a final concentration of OD_{600} = 1.

166 The mixture was transferred into a 24-well plate, and the plates were incubated at 21°C
167 under light for 8 hours. Three replicates were performed for each modality and the control
168 consisted of spore solutions mixed with filtered V8 without bacteria. After 8 h pictures were
169 taken using an imaging plate reader (Cytation5, Biotek). Spores were counted according to
170 their development stage (not germinated, normal germination, delayed germination). Each
171 assay was replicated twice with n = 3 repetitions per experiment.

172 **Effect of bacterial strains on *P. viticola* zoospore motility**

173 Zoospores of *P. viticola* were released from sporangia harvested by suction from sporulating
174 lesions of artificially inoculated leaves (*V. vinifera* cv. Cabernet Sauvignon) with a filter-
175 containing pipette tip connected to a vacuum device. The sporangia were resuspended in
176 sterile water and their concentration was adjusted to 5 x 10^4 sporangia/mL in a falcon tube.
177 The tube was placed in a shaker at 25 rpm at room temperature and in the dark for zoospore
178 release. After 1 h, the zoospore solution concentration was adjusted to 5 x 10^4
179 zoospores/mL. The effect of bacteria on zoospore motility was assessed by mixing 500 µL of a
180 solution of bacteria diluted at OD_{600} = 2 in sterile water with 500 µL of the zoospore solution
181 in a 1.5 mL tube for a final concentration of OD_{600} = 1 (1:1 mixing). The solution was then
182 incubated at room temperature in a shaker at 25 rpm for 30 minutes. After incubation, the
183 solution was transferred into a counting chamber and observed under a microscope to
184 visually count the number of motile and non-motile zoospores. After counting, the
185 percentages of motile and non-motile zoospores were calculated and compared to the
186 negative control (zoospore mixed with distilled water only) and the positive control
187 (zoospore mixed with a 2 g/L solution of copper hydroxide (Kocide® Opti, Bayer). For each
188 strain, two independent experiments were performed with n = 3 replicates per experiment

189 and each replicate containing at least 50 zoospores. In order to determine the effect of the
190 loss of motility on the zoospore's infectivity an aliquot of the suspension combining
191 zoospores and individual bacteria was then used for leaf disc infection after the 30-minute
192 incubation, following the procedure described below in the leaf disc infection section.

193 **Electron microscope observation of zoospores**

194 Pellets of the different modalities (*P. viticola* zoospores, the bacterial strain PID6 (*Bacillus*
195 cereus), and *P. viticola* zoospores in contact with PID6) from the experiment described above
196 were prepared according to Roland and Vian (1991). Briefly, the pellets were centrifuged for
197 5 minutes at 2000 rpm and resuspended in a solution of 3 % glutaraldehyde–2 %
198 paraformaldehyde in 0.14 M PIPES buffer (pH 7) for prefixation. Samples were then
199 centrifuged for 20 minutes at 2000 rpm, the supernatant was removed, and the samples
200 were washed twice with 0.07 M PIPES buffer. They were embedded in 2 % agarose and
201 postfixed with a solution of 1 % OsO4. The samples were then dehydrated in a graded series
202 of ethanol solutions (30–50–70–95–100 % v/v) and infiltrated in a solution of ethanol mixed
203 with propylene oxide (1:1) and pure propylene oxide, followed by a series of epoxy resin
204 mixed with propylene oxide (1:3, 1:1) for 2 hours each. Finally, they were infiltrated with
205 epoxy resin for 17 hours. After polymerization for 48 hours at 60°C, a staining step was
206 performed by floating on a 50°C solution of 1 % methylene blue, 1 % sodium borate, and 1 %
207 azure II for 10 seconds for semi-thin sections. Thin sections were cut and stained with 2 %
208 uranyl acetate followed by lead citrate, according to Reynolds (1963). The thin sections were
209 observed with a Tecnai G2 Spirit BioTWIN transmission electron microscope (TEM) equipped
210 with a Gatan UltraScan High-resolution CCD camera.

211 **Evaluation of the preventive effect of the application of bacteria on gray mold and downy**
212 **mildew infection on leaf discs**

213 Leaf discs from *V. vinifera* L. cv. Gamay and cv. Gamaret for *B. cinerea* and *P. viticola*
214 respectively were cut from 2-month-old grapevine leaves taken from leaf stages 4 to 7.
215 Leaves were taken randomly from at least ten plants and were rinsed with tap water and
216 surface sterilized by immersion in calcium hypochlorite (40 g/L) for 7 minutes followed by
217 rinsing in sterile water prior to cutting. Leaves were then dried with sterile filter paper, used
218 to cut leaf discs using a 1.7 cm diameter punch and placed in sterile water. For each
219 treatment, three Petri dishes containing ten leaf discs each were used, leaf discs were taken
220 randomly and placed on top of a filter paper moistened with sterile water placed inside a 9
221 cm Petri dish with the abaxial side facing upward and placed in a growth chamber with a 16
222 h/8 h photoperiod, 21/20°C day/night temperature and 40 % RH. After 24 h each plate was
223 inoculated by spraying 500 µL of bacterial solution diluted in sterile water and adjusted to
224 OD_{600} = 1, using a sterile glass atomizer and placed back in the growth chamber for two days,
225 to let them adapt and develop in their new environment. The leaf discs were then infected
226 with *P. viticola* or *B. cinerea*. For *P. viticola*, the infection was performed by spraying 50 µL of
227 solution adjusted at 5 x 10^4 zoospores/mL in each plate. For *B. cinerea*, the infection was
228 performed by depositing a 20 µL droplet of a spore solution adjusted to 1 x 10^5 spores/mL in
229 $PDB^{1/4}$ (quarter-strength potato dextrose broth) on a wound made by a sterile syringe at the
230 center of the leaf disc. The same procedure was used with sterile water only for the negative
231 control. For positive controls, leaf discs were treated with a 2 g/L solution of copper
232 hydroxide (Kocide® Opti, Bayer) against *P. viticola* and a 0.04 % solution of the yeast
233 *Aureobasidium pullulans* (Botector®, Andermatt) against *B. cinerea*. Petri dishes were sealed

234 with Parafilm® and placed back in the growth chamber. For each modality, one plate on the
235 three available containing 10 leaf discs was used to collect material for gene expression
236 analysis by qPCR and stilbene synthesis by UPLC. Five leaf discs were collected, dried and
237 snap frozen in liquid nitrogen 6 h after infection for qPCR analysis to monitor early changes in
238 defense-related genes expression, and the other five 3 days after infection for UPLC analysis,
239 3 days being the time required to observe a representative level of stilbene synthesis.
240 Pictures of each remaining plate were taken one week after infection to assess the
241 development of symptoms. Two independent experiments were performed for each
242 modality.

243 **Whole plant infection with *P. viticola***

244 Half of the leaves from foliar stage 4 to 7 of two-month-old grapevine plants (4 plants per
245 treatment) were sprayed using a sterile glass atomizer with a solution of 4 mL of bacteria
246 adjusted to OD_{600} = 1 on their abaxial face, while the other half was protected with plastic
247 bags to prevent unwanted inoculation. Once the inoculation was complete, plastic bags were
248 removed and uninoculated leaves were sprayed with sterile water. Two days after the
249 bacterial inoculation, all leaves from foliar stage 4 to 7 were sprayed with 200 µL of a solution
250 of *P. viticola* zoospores adjusted to 5 x 10^4 zoospores/mL. After a week, leaves were
251 collected, the level of symptoms was quantified and compared to the control (plants
252 inoculated with water only) in search of local and systemic protection from the bacteria. Two
253 independent experiments were performed for each modality.

254 **Symptom assessment**

255 For symptom measurement on plant tissue, pictures of leaf discs or whole leaves were
256 analyzed in an automated and quantitative manner. A macroinstruction was developed in the

257 freeware ImageJ to measure the necrosis development of *B. cinerea* and another one to
258 measure the formation of sporangiophores of *P. viticola* as described in Guyer et al. (2015).
259 The macro instruction analyzes each leaf tissue in an image. It counts the total number of
260 pixels occupied by the leaf as well as the number of pixels occupied by *B. cinerea* necrosis or
261 *P. viticola* sporangiophores within each leaf tissue. A report is then generated to calculate the
262 percentage of surface area occupied by the pathogen on the surface of each leaf tissue. The
263 level of inhibition was calculated as a percentage of the level of infection observed in the
264 negative control (inoculated with water instead of bacteria). For positive controls, plants
265 were treated with a 0.4 % solution of brown algae (Alginure®, Andermatt) against *P. viticola*
266 to compare the effectiveness of our bacterial treatments against a certified biocontrol
267 solution.

268 **Gene expression analysis**

269 The ability of phyllosphere bacteria to promote defense gene expression on grapevine was
270 analyzed by qPCR in 8-week-old plants. Five leaf discs from the preventive inoculation assays
271 on leaf discs against *B. cinerea* and *P. viticola* described above were sampled 6 h after
272 infection, snap-frozen in liquid nitrogen and stored at −80°C. RNA was extracted with the
273 NucleoSpin RNA Plant from Macherey-Nagel with a modified procedure (Schellenbaum et al.,
274 2008). Reverse transcription was performed using the SensiFAST cDNA Synthesis Kit from
275 Bioline. Quantitative PCR reactions were performed using the SensiFAST SYBR Hi-ROX Kit
276 from Bioline. Each reaction was carried out using a Mic qPCR Cycler (bio molecular systems)
277 and the software micPCR v2.8.13 with 5 µL of cDNA (5 ng/µL), 7.5 µL of SYBR Hi-ROX mix, 0.5
278 µL of each primer and 1.5 µL of sterile water. For amplification, an initial denaturation step at
279 95°C for 15 min was done, followed by 45 amplification cycles (95°C for 15 s, 60°C for 15 s,

280 72°C for 30 s). The experiment was repeated twice, with n = 3 samples in each experiment.
281 For each reaction a technical replicate was performed and values with a standard deviation
282 lower than 0.25 (or 0.5 Cq) were averaged and used for further calculations. All results were
283 analyzed using the double delta Cq method with infected samples treated with water as
284 reference and normalization was performed relative to the expression of the two reference
285 genes Actin and 60SRP (60S ribosomal subunit). After normalization and calculation of the
286 fold change, results were log2 transformed to make fold-changes symmetric.

287 Primers were used to monitor the expression of the following defense-related genes, PR-1
288 (pathogenesis-related protein 1), PR-4 (pathogenesis-related protein 4), STS (stilbene
289 synthase), ROMT (resveratrol O-methyltransferase), PAL (phenylalanine ammonia-lyase) and
290 LOX (lipoxygenase) (Table S1).

291 **Stilbenic compound measurement**

292 Leaf discs were cut in small pieces with a scalpel, weighed and placed into microcentrifuge
293 tubes. Samples were immediately snap-frozen and immersed in 300 µL of methanol. The
294 tubes were then heated at 60°C for 15 minutes with agitation at 800 rpm, followed by rapid
295 cooling in an ice bath for 5 minutes. After cooling, the samples were centrifuged to separate
296 the supernatant. The methanolic extracts were analyzed using Ultra-Performance Liquid
297 Chromatography (UPLC) to quantify the presence of various stilbenic compounds, including
298 trans-piceid, trans-resveratrol, α-viniferin, δ-viniferin, ε-viniferin, isohopeaphenol, and
299 pterostilbene, following the method described by Pezet et al. (2003). The analysis was
300 performed using a Vanquish UPLC system (Thermo Scientific) with a mobile phase consisting
301 of H_2O with 0.1 % formic acid (Phase A) and acetonitrile with 0.1 % formic acid (Phase B). The
302 gradient elution program started with 5 % B and increased to 40 % over 17 minutes, followed

303 by an increase to 50 % B between 17 and 20 minutes. From 20 to 25 minutes, the proportion
304 of B increased to 100 %, which was maintained until 29 minutes. The flow rate was set at 0.3
305 mL/min. Separation was carried out using a Hypersil Gold column (1.9 µm, 100 x 2.1 mm,
306 Thermo Scientific) equipped with a 0.2 µm UPLC filter (Thermo Scientific), maintained at
307 30°C. The injection volume was 2 µL, and detection was performed using a UV detector at
308 280 nm and 310 nm. This method ensures accurate and reproducible quantification of
309 stilbenic compounds in leaf tissues.

310 **Bacterial survival in presence of biocontrol products**

311 For the consortia selection process, the ability of the strains to survive in presence of
312 Armicarb® (potassium bicarbonate, Andermatt) and Thiovit® Jet (wettable sulfur, Syngenta),
313 two biocontrol compounds commonly used in viticulture, was assessed. Bacteria were grown
314 overnight in liquid LB at 28 °C and the solution then adjusted to OD_{600} = 1 and used as
315 inoculum for liquid LB medium containing recommended working concentrations of
316 Armicarb (0.2 %), Thiovit Jet (0.4 %) or a combination of both to test their effects on bacterial
317 growth. After 24 hours, the growth of bacteria in contact with the biocontrol products was
318 compared with the growth of the controls (bacteria grown in medium without biocontrol
319 products). Two independent experiments were conducted for each assay.

320 **Bacterial compatibility assay**

321 Bacteria compatibility was tested in liquid coculture. Each strain was grown overnight in
322 liquid LB at 28 °C and the solution was adjusted to OD_{600} = 1. Then, 50 µL of each strain was
323 used for a coculture in a final volume of 200 µL of LB in a 96-well plate and incubated for 24
324 hours at 28 °C. The mixture was plated on LB agar supplemented with selective antibiotics
325 (kanamycin, cefotaxime, and gentamicin) chosen based on each strain's natural antibiotic

326 resistance. Bacteria were considered compatible when each strain was able to grow
327 successfully in selective media after coculture.

328 **Data analysis**

329 All statistical analyses were performed in GraphPad Prism 10 (GraphPad Software, San Diego,
330 CA, USA). Spore germination assay results (number of spores for each category and for each
331 strain) were analyzed using a Fisher's exact test with Bonferroni's correction for multiple
332 comparisons. For motility assays, the differences in the number of motile zoospores treated
333 with bacteria were assessed by using a One-way ANOVA followed by a Dunnett's test with
334 negative controls (zoospores treated with water) as a reference. For symptom analysis, the
335 effect of the bacteria on symptom development was assessed with an ANOVA on the area of
336 the symptomatic tissue followed by a Dunnett's test with negative controls (leaf discs treated
337 with water) as a reference.

338 For gene expression analysis, an ANOVA analyzing the effects of the presence of bacteria in
339 infected plants followed by a Dunnett's test with negative controls (leaf discs treated with
340 water and infected by pathogen) as a reference, was performed for statistical analysis.
341 Results of all statistical tests are displayed as follows: - = not significant; $* = p < 0.05$; $** = p$
342 < 0.01 and $*** = p < 0.001$.

343

344 **Results**

345 **1. *In vitro* assays/Bacterial effects on pathogen propagules**

346 The ability of phyllosphere bacteria to disrupt the germination of *B. cinerea* spores was
347 assessed by confrontation tests. After being in contact with the bacteria for 8 h, the spores

348 displayed four different morphologies: i) normal germination, with expected formation of a
349 long germ tube, ii) partially inhibited germination, with only the tip of the germ tube visible,
350 iii) complete inhibition of germination, with full inability for the spore to form a germ tube,
351 iv) in a few rare cases, spores could also form a swollen germ tube, revealing an abnormal
352 germination in which the germ tip swelled, stopping its elongation (Table 1, Fig. 2 B-E).

353 Among the 46 bacteria that were tested, 33 strains were able to strongly disturb the normal
354 germination of more than 50 % of *B. cinerea* spores, either by strongly delaying the
355 formation of the germ tube or by fully preventing spore germination (Fig. 2 A). The six most
356 effective strains SOD14, PIP6, PIP4, PID6, PID13 and PID1 were even capable of inhibiting the
357 germination of 100 % of the spores, leaving no spore able to germinate properly. These
358 strains belonged mainly to the *Bacillus* or *Paenibacillus* genus, with the exception of PIP4
359 (*Cupriavidus*) and of the most effective one, PIP6 (*Herbaspirillum*). Five of the six most
360 effective strains were isolated from Pinot Noir.

361 All bacterial strains were tested to assess their ability to disturb *P. viticola* zoospores *in vitro*.
362 Confrontation assays between bacteria and *P. viticola* zoospores showed that the contact
363 with bacteria could strongly affect zoospore motility. A significant reduction was measured
364 with 28 strains out of the 46 tested. Visual assessment revealed that zoospores either
365 completely lost their motility, or remained motile as in the non-treated controls, but we did
366 not observe slower movement or reduced dispersal. However, the proportion of affected
367 zoospores was different depending on the bacterial strain used. Among these 28 motility
368 inhibiting strains, 16 strains caused a very strong reduction, affecting 90 % to 100 % of the
369 zoospores and reaching the level of inhibition achieved with the copper used as a positive
370 control, while 12 strains caused a significant but lower reduction (40 % to 90 %) (Table 1). A

371 large predominance of bacteria from the *Bacillus* genus were found among the active strains,
372 since 19 of the 21 *Bacillus* strains caused a significant effect. However, the two most effective
373 strains affecting both 100 % of the zoospores (PIP10 and SOP5) were a *Pantoea agglomerans*
374 and a *Microbacterium hominis* strain, respectively. The active strains were composed of a
375 balanced proportion of epiphytic (13) and endophytic (15) strains.

376 All the suspensions composed of zoospores and individual bacterial strains were thereafter
377 used to infect leaf discs from the susceptible cultivar Gamaret to monitor pathogen
378 development. This was done to test whether the observed loss of motility would reduce the
379 infectivity of *P. viticola* zoospores. The observations showed that in the case of 16 strains out
380 of 46, the infections performed with the treated zoospores resulted in a significant reduction
381 in symptoms (from ca. 30 % to 80 % of the control) compared with the infection performed
382 with non-treated zoospores (Table 1). From these 16 strains, only 11 were also significantly
383 impairing motility, suggesting that the loss of motility was not always correlated with
384 symptom reduction and that other mechanisms are involved. Indeed, five strains that had
385 not impaired zoospore motility were nevertheless effectively reducing the symptoms caused
386 by the zoospores with which they had been co-incubated. Interestingly, four of these five
387 strains are epiphytes, isolated from Pinot Noir and belonged to different genera than those
388 highlighted previously, namely *Rhodopseudomonas*, *Cupriavidus*, *Variovorax* and
389 *Herbaspirillum.*

390 It seemed that when bacteria (12 strains) isolated from the cultivar Pinot Noir were co-
391 inoculated with zoospores, this almost systematically resulted in significant symptom
392 reduction, whereas strains isolated from the two other cultivars harbored a lesser proportion
393 of protective strains, judging from this leaf disc experiment.

394 The zoospores that had been in contact with the most effective bacterium PID6 (*B. cereus*)
395 were observed using a transmission electron microscope (TEM). The zoospores that were in
396 contact with non-active bacteria and had not lost their motility displayed a standard oval
397 shape comparable to the control zoospores. However, the zoospores that had been in
398 contact with PID6 showed an irregular shape, suggesting that the zoospore cell had lost its
399 integrity and had been strongly affected by the presence of the bacteria (Fig. 3).

400 **2. Bacteria from the grapevine phyllosphere inhibit symptom development caused by *B.***
401 ***cinerea* and *P. viticola* infection in preventive leaf discs assays**

402 In this next experimental part, we tested if the inoculation of bacteria in plant tissue prior to
403 infection could reduce the development of symptoms caused by *B. cinerea* and *P. viticola*
404 applied directly on leaf tissue. The protection conferred by bacteria was tested by inoculating
405 leaf discs before infecting them with either pathogen.

406 To assess their protective activity, strains were applied to leaf discs before infection with *B.*
407 *cinerea* and *P. viticola* to let them adapt to their new environment. In leaf discs infected with
408 *B. cinerea*, preventive bacterial treatment with 15 strains reduced necrosis significantly by 38
409 to 81 % on average (Table 2, Fig. 4). In our conditions, seven strains, with a majority of
410 epiphytic bacteria were able to achieve a better reduction in symptoms (more than 61 %)
411 than the Botector® (*Aureobasidium pullulans*) used as positive control: CHP29
412 (*Microbacterium proteolyticum*), SOP47 (*Variovorax paradoxus*), CHP2 (*Cupriavidus*
413 *metallidurans*), PIP6 (*Herbaspirillum huttiense*), PIP36 (*Bacillus cereus*), SOP8 (*Cupriavidus*
414 *metallidurans*) and PID2 (*Bacillus simplex*) (Fig. 4).

415 In leaf discs infected with *P. viticola,* 14 strains were able to significantly reduce the disease
416 symptoms, to a level ranging from 24 to 59 % on average (Table 2, Fig. 5), while all other

417 strains did not result in any significant symptom reduction. These 14 strains included 7
418 strains already identified as active in the first infection assay carried out with exposed
419 zoospores described above (Table 1), while seven other strains had not shown activity in the
420 previous experiment, but revealed their anti-oomycete abilities when pre-inoculated on the
421 leaf discs (Table 2) : CHP15 (*Micrococcus yunnanensis*), SOD6 (*Bacillus cereus)*, SOD2 (*Bacillus*
422 *halotolerans*), CHP2 (*Cupriavidus metallidurans*), CHP20 (*Bacillus cereus*), CHP29
423 (*Microbacterium proteolyticum*) and SOD31 (*Bacillus zhangzhouensis*).

424

425 **3. Phyllosphere bacteria stimulate the expression of several plant defense genes and**
426 **promote the synthesis of several stilbenic compounds after infection**

427 Beyond the direct effects against pathogens observed above, we next tested whether the
428 most protective strains would also stimulate plant defenses. Infected leaf discs preinoculated
429 with bacteria were collected to evaluate their effect on plant defenses. We monitored
430 defense genes expression and stilbenic compound production. A set of defense genes linked
431 to the systemic acquired resistance (PAL), the induced systemic resistance (LOX), the stilbene
432 biosynthesis pathway (STS and ROMT), as well as two pathogenesis related protein encoding
433 genes (PR-1 and PR-4) were used for the qPCR analysis.

434 For *B. cinerea*, we tested the strains that triggered an inhibition higher than that observed in
435 the positive control. These strains exhibited diverse effects on the expression of defense-
436 related genes, with the majority either downregulating or not significantly affecting their
437 expression (Fig. 6). Except for the strain PIP36 (*Bacillus cereus*), which was able to promote
438 the expression of almost all genes, SOP8 (*Cupriavidus metallidurans*), which only promoted
439 the expression of PR-4, and ROMT, CHP29 and CHP2 which promoted the expression of

440 ROMT and PR-1 respectively but had moderate to no effect on the other genes, all the other
441 strains had no significant effect on the expression of the tested set of defense-related genes,
442 suggesting that they affected the pathogen through another mechanism.

443 Among the 14 strains that were effective against *P. viticola*, 11 significantly upregulated the
444 expression of at least three defense genes and only three strains had no positive impact on
445 the defense gene expression (Fig. 6). These latter strains (CHP20, CHP29 and SOD31), showed
446 the lowest level of inhibition of symptoms on leaf discs and mainly downregulated gene
447 expression or had no significant effect on the monitored genes. Among the strains that
448 significantly upregulated the expression of several defense-genes, we observed a tendency
449 for upregulation of the genes from the SAR and the stilbene synthesis pathways (PR-1, PAL,
450 STS and ROMT). In contrast, the expression of the genes LOX and PR4 were induced by fewer
451 strains. Furthermore, it seems that when these two genes were upregulated, the fold change
452 was globally lower compared to the genes from the SAR or the stilbene pathway.
453 Interestingly, the strains PIP7, PIP17, SOD13, SOD2 and CHP2 were able to stimulate all or
454 almost all genes tested without downregulating any single gene, except to PIP17 which
455 dowregulated only STS. Finally, regarding the two best performing strains in terms of
456 symptom reduction, PIP27 and CHP15, they had an opposite effect on gene expression: PIP27
457 upregulated ROMT, PR-1 and PR-4 and slightly downregulated LOX, PAL and STS, while CHP15
458 caused the opposite effect.

459 Regarding the production of stilbenic compounds, differences were only observed in plants
460 infected with *P. viticola* (Fig. 7). In infected plants inoculated with protective bacteria, the
461 production of piceid (an inactive storage form), resveratrol (a precursor of more active forms)
462 and δ viniferin (an oxidized form with antimicrobial activity) was stimulated by most of the

463 bacteria. On the contrary, the production of the active compounds, isohopeaphenol, α-
464 viniferin and ε-viniferin was negatively impacted by the inoculation with most strains.
465 However, few strains were able to stimulate the production of the different active forms.
466 Accordingly, CHP15 (*Micrococcus yunnanensis*), SOD13 (*Paenibacillus xylanexedens*), SOD2
467 (*Bacillus halotolerans*), SOD6 and PID6 (two *Bacillus cereus*) were able to promote the
468 synthesis of at least three of the active forms in infected leaves. Except for CHP15, which
469 triggered an inhibition of symptoms of 46 %, these strains were responsible for an inhibition
470 of 37, 36, 39 and 32 %, respectively, corresponding to a moderate protection level among our
471 active strains.

472 **4. Selection of bacteria for the consortia**

473 A selection of strains that had shown significant effects against *B. cinerea* and *P. viticola* was
474 performed for a use in combination, based both on effectiveness and on the resistance to
475 phytosanitary products. The goal was to build consortia of three strains which would be
476 effective against *B. cinerea, P. viticola* and display plant defense stimulation abilities.

477 **4.1 Effectiveness threshold against *B. cinerea* and *P. viticola***

478 Regarding the effects on propagules *in vitro*, we selected strains able to at the same time
479 fully inhibit the germination of at least 50 % of *B. cinerea* spores, inhibit *P. viticola* zoospore
480 motility and provide a significant level of symptom inhibition with the subsequent infection.
481 Eight strains met these criteria, five *Bacillus,* two *Microbacterium* spp. and one
482 *Sphingomonas* sp. (Table 3).

483 For effects against *B. cinerea* symptoms on leaf discs, we only considered strains with an
484 effectiveness higher than the 61 % reached by the positive control Botector® (*Aureobasidium*

485 *pullulans*). For *P. viticola*, since the reduction of symptoms was lower in the preventive leaf
486 disc assays, the threshold of effectiveness was set to 30 %. These criteria were met by seven
487 strains for *B. cinerea* and by 10 strains for *P. viticola* (Table 3).

488 **4.2 Resistance to plant protection products**

489 Even though in this study, the effects of the bacteria were tested under laboratory
490 conditions, it is important to consider that ultimately, the bacteria would be used in the field
491 under real agricultural conditions. They could be in contact with chemical plant protection
492 solutions, and it is therefore important that the selected strains are not susceptible to such
493 products. We therefore decided to select strains based on their ability to survive in presence
494 of two biofungicides used in organic viticulture, potassium bicarbonate and wettable sulfur,
495 alone or in combination (Table S2).

496 Seven strains were capable of both affecting pathogen propagules and/or providing a
497 significant plant protection as well as of surviving in presence of the tested phytosanitary
498 products: PID6 (*Bacillus cereus*), PID13 (*Bacillus endophyticus*), SOD2 (*Bacillus halotolerans*),
499 CHP2 (*Cupriavidus metallidurans*), CHP14 (*Bacillus subtilis*), PIP6 (*Herbaspirillum huttiense*)
500 and SOP8 (*Cupriavidus metallidurans*) (Table 3). No incompatibility was observed between
501 these strains when grown together in liquid coculture, as tested by their subsequent
502 cultivation on selective media plates. It appeared that they were all able to survive
503 adequately when grown together in the same mixture.

504 **5. Protective effects of bacteria consortia against pathogens**

505 Combinations of the seven selected bacteria were tested against *B. cinerea* spore
506 germination *in vitro* and *P. viticola* infection on whole plants to compare their protective
507 effect with that of single strains.

508 **5.1. The inhibition of *B. cinerea* spore germination was higher with consortia than with**
509 **bacteria alone**

510 Consortia containing two to three of the seven selected strains were tested. The solution
511 containing the combination of bacteria had the same final cell concentration as the one used
512 in the previous experiment with single strains, so any increase in effectiveness could not be
513 simply due to the increased bacterial density in the mixture. This combination resulted in an
514 overall increase of the strains' germination inhibition abilities. All consortia were able to
515 repress the germination process, with the least effective one (CHP14, PIP6 and SOD2)
516 affecting 98 % of the spores and 20 out of the 23 tested consortia affecting 100 % of the
517 spores (Fig. 8). Consortia differed mainly in the proportion of spores that were fully vs.
518 partially inhibited in their germination. The abilities of consortia to fully inhibit the
519 germination of *B. cinerea* conidia were compared to the best-performing strain within each
520 combination using the Highest Single Agent (HSA) method (Table S3). Among the 23
521 combinations tested, 7 displayed a reduced effectiveness (≤ 10 percentage points lower than
522 HSA), 9 showed a similar level of inhibition (within ± 10 percentage points compared to HSA),
523 and 7 performed better (≥ 10 percentage points higher than HSA). The highest improvements
524 were observed in consortia of two strains containing bacteria with low to medium effects
525 regarding full inhibition of spore germination: PID6/SOP8 and CHP2/PID6 with an increase of
526 67 and 82 % compared to their HSA respectively. Accordingly, three-strains consortia
527 including these four strains like, performed similarly to, or better than their HSA. In contrast,

528 all three strains consortia including SOD2 performed not better, and even significantly worse
529 than their HSA.

530 **5.2 Strains combinations were more efficient and consistent than single strains for whole**
531 **plant protection against *P. viticola***

532 The seven selected strains were assembled to build four consortia each containing at least
533 one strain capable of impairing *B. cinerea* and *P. viticola* propagules, inhibiting symptom
534 development of both pathogens, and stimulating plant defenses. Consortia were sprayed on
535 half the leaves of grapevine plants (leaf stages 4 to 7), while the other half was sprayed with
536 water prior to whole plant infection with *P. viticola* zoospores. After a week, symptoms were
537 measured on bacterial-inoculated and uninoculated leaves in search of local and systemic
538 protective effects of the bacteria. Results showed that the strains CHP14, CHP2 and PID13
539 alone provided the lowest reduction of symptoms along with the highest variability (38, 34
540 and 31 % respectively), the reduction triggered by CHP2 and PID13 being not even
541 significant. However, all combinations were able to significantly reduce the level of
542 symptoms except for SOP8/CHP14/PID6 (Fig. 9 and Table 4). For all plants and for all
543 treatments except for PIP6, the severity of symptoms was statistically similar on bacteria-
544 inoculated leaves and in leaves that were sprayed with only water, suggesting that the
545 presence of bacteria triggered a systemic response. However, the level of symptom reduction
546 was different between the different treatments. The three best combinations
547 (CHP2/PID6/PIP6; CHP14/PID13/PIP6 and CHP2/CHP14/PID13) led to a symptom reduction
548 ranging from 88 to 94 % on average, reaching an almost complete protection, however still
549 lower than the positive control (Table 4). The combination (CHP2/CHP14/SOD2) provided a
550 reduction of 58 % which, although significant, was not very consistent (standard deviation of

551 39.2) and remained lower than the protection generated by the three best single strains
552 PID6, SOP8 and PIP6 alone (82, 68 and 63 % respectively). The last combination
553 (SOP8/CHP14/PID6) did not cause a significant reduction of symptoms either locally and
554 systemically despite the fact that the single strains PID6 and SOP8 are the most effective
555 strains.

556

557 **Discussion**

558 The overall results of this study indicate that a significant number of phyllosphere bacteria
559 have complementary multi-level properties that significantly reduced the symptoms of two
560 diseases affecting grapevine. When assembled into consortia, the strains provided stronger
561 and more consistent protection of the plant.

562 **Disruption of spore germination in *B. cinerea* and of zoospore motility in *P. viticola***

563 The first layer of protection consists in affecting the spores, the pathogens' unit of dispersion
564 and infection, which will prevent the infectious process. In our study, among the 28 strains
565 able to impair the motility of *P. viticola* zoospores, almost 20 strains belonged to the genus
566 *Bacillus*. This genus is well known for containing many biocontrol species and several studies
567 have highlighted the effect of *Bacillus* strains on zoospores. *Bacillus* sp. 109GG020 and *B.*
568 *licheniformis* were shown to affect *Phytophthora capsici* zoospore motility (Tareq et al., 2015;
569 Y. Li et al., 2020). Comparable effects have been described using *Bacillus amyloliquefaciens*
570 against *Phytophthora sojae* (D. Liu et al., 2019) and *Bacillus cereus* against the pathogen
571 *Pythium torulosum* (Shang et al., 1999). However, in our study, except only two *Bacillus*
572 strains (PID6 and PID2), the most active strains which, after co-incubation with the

573 zoospores, induced a reduction in the subsequent development of symptoms, belonged to
574 the *Pantoea*, *Microbacterium* and *Rhodopseudomonas* genera which have so far not been
575 reported to harbor strains affecting zoospores of oomycetes (Mondol et al., 2017). In our
576 work, no abnormal swimming was observed after contact with the bacteria, and we did not
577 observe any decrease in the number of retrieved zoospores compared with the non-treated
578 controls. However, the TEM observations of zoospores exposed to the most effective strain
579 (*B. cereus* PID6) showed that the zoospores were strongly affected since they displayed a
580 complete loss of their typical oval shape, suggesting a severe loss of integrity. Despite the
581 strong effects of several bacteria on zoospore motility, the surviving zoospores were still able
582 to infect grapevine leaf discs, although with a significant reduction in symptoms compared to
583 the control. Motility is an essential function of the zoospore for plant infection in oomycetes
584 (Chepsergon et al., 2020). We therefore expected that zoospores unable to swim would be
585 less likely to infect plants. However, several zoospores were still able to cause infection
586 despite this loss of function. It seems that they were still able to reach an infection site. Thus,
587 zoospores treated with strains like SOD2 and CHP14, despite an almost full loss of motility,
588 were still able to successfully infect the plants. In contrast, some zoospores treated with
589 strains such as PIP27 and PIP4 that did not inhibit spore motility showed a limited capacity to
590 infect plants. These results suggest that loss of motility alone is not enough to fully hinder
591 the infection of *P. viticola* zoospores and that the contact with bacteria not only affects the
592 motility but also other key functions in the zoospores, as highlighted by the TEM observation
593 (Fig. 3).

594 The phyllosphere bacteria not only affected the spore motility of *P. viticola* but also the spore
595 germination of *B. cinerea*. The most effective strains regarding this effect belonged to the

596 genera *Herbaspirillum*, *Cupriavidus* and *Bacillus.* The biocontrol abilities for these two first
597 genera have not been well studied so far, but the effect of *Bacillus* strains on fungal
598 pathogens including *B. cinerea* is well known. *Bacillus subtilis* has been reported to affect *B.*
599 *cinerea* hyphal morphology and to inhibit spore germination through the release of
600 antifungal volatiles (H. Chen et al., 2008). The use of a broth from *B. cereus* strains also
601 caused comparable effects (Li et al., 2012), as did the application of *B. mycoides* (from the *B.*
602 *cereus* group) as a biocontrol agent against *B. cinerea* in strawberry (Guetsky et al., 2001a).
603 Interestingly, one strain of *B. thuringiensis* (SOD14) was found among the four best
604 performing *Bacillus* strains in the present study. This species is also a member of the *B.*
605 *cereus* group and a source of powerful biocontrol agents against insects, mainly due to the
606 production of Cry and Cyt toxins by several of its members (Bravo et al., 2011), but also
607 because of their production of chitinase (Honda et al., 2017). Since *B. cinerea* hyphae and
608 spores are also composed of chitin (Chardonnet et al., 1999), chitinases could be responsible
609 for this germination inhibition. Indeed, *B. thuringiensis* strains were recently suggested to be
610 very effective biocontrol agent against fungal pathogens (Martínez-Zavala et al., 2024).
611 Beyond Cry and Cyt toxins, *Bacilli* produce other compounds with antifungal or anti-
612 oomycete activity like cyclic lipopeptides harboring membrane disruption abilities, such as
613 iturin, bacillomycin and fengycin. These compounds could also be involved in the
614 germination disruption capacity of *B. thuringiensis* (SOD14) as well as its potency to inhibit
615 successfully the motility of *P. viticola*(Balleux et al., 2025; Markelova & Chumak, 2025;
616 Romero et al., 2007).

617 **Preventive inoculation of phyllosphere bacteria on leaf discs prior to infection led to**
618 **significant protection against *P. viticola* and *B. cinerea***

619 When applied on leaves two days prior infection, fewer bacterial strains caused a significant
620 reduction of symptoms against *P. viticola* compared with when bacteria were mixed with
621 zoospores prior to leaf inoculation. This reduction in the number of effective strains is
622 probably due to the infection method; since zoospores were deposited directly on the leaf
623 tissue and without preexposure to bacteria, their motility was still intact. Moreover, in this
624 setup, zoospores may have started to infect the leaves before being impaired by bacteria. If
625 we compare the list of effective strains from the first assay that were at the same time able
626 to affect motility and/or to reduce symptom development, with the strains effective in the
627 bacterial preinoculation experiment, seven strains, SOD13, PID6, SOP5, PIP7, PIP17, PIP27
628 and CHP14 (*Paenibacillus xylanexedens*, *Bacillus cereus*, two strains of *Microbacterium*
629 *hominis, Staphylococcus arlettae, Rhodospeudomonas boonkerdii* and *Bacillus subtilis*
630 respectively) proved effective in both experimental setups. Once again, the genera from the
631 previous experiment *i.e.*: *Cupriavidus, Paenibacillus Rhodopseudomonas, Micrococcus* and a
632 large representation of *Bacillus* species were found to be active, but we also found three
633 different strains of *Microbacterium* among active strains. So far, this genus has not been
634 often mentioned in the literature as effective against *P. viticola,* but some strains have been
635 shown to stimulate plant defense and inhibit the growth of oomycetes and several fungal
636 pathogens (Barnett et al., 2006; Freitas et al., 2019; Ray et al., 2021; Suárez-Estrella et al.,
637 2023).

638 The same observations were made with *B. cinerea*: fewer strains caused a reduction in
639 symptoms compared to the test performed on spores, suggesting that for both oomycetes
640 and fungi, activities on spores, although helpful, are not sufficient for achieving effective
641 biocontrol. All genera containing strains active against *P. viticola,* except *Micrococcus*, also

642 exhibited activity against *B. cinerea*, with the addition of one *Herbaspirillum* (PIP6), one
643 *Variovorax* (SOP47) and one *Plantibacter* (PIP26). Strains belonging to these two last genera
644 are known for their plant growth promoting abilities (Giannelli et al., 2024; Han et al., 2013;
645 Mayer et al., 2019; Sun et al., 2018), but their biocontrol abilities are poorly studied.
646 *Variovorax* strains have shown moderate biocontrol activities in tomatoes against *B. cinerea*
647 and in wheat against *Fusarium* (Besset-Manzoni et al., 2019; Köhl et al., 2020), but to our
648 knowledge, no study has yet reported any biocontrol effects of *Plantibacter* strains.

649 **Several phyllosphere bacteria contribute to enhance grapevine defenses**

650 Bacterial strains successfully inhibiting the development of symptoms when they were
651 inoculated in leaf discs prior to infection were tested for their ability to stimulate the
652 expression of several genes belonging to different defense pathways. Most of the strains
653 effective against *P. viticola* were able to upregulate multiple defense genes, but several
654 bacteria such as CHP20, CHP29 and SOD31 showed little to no significant effect regarding the
655 selected defense gene upregulation and seemed to even slightly downregulate plant
656 defenses. Most of the strains shared the downregulation of the PR-4 gene, a gene encoding a
657 protein with chitinase and chitin-binding activities (Enoki & Suzuki, 2016; M. Y. Li et al., 2020; G.
658 T. Liu et al., 2021). The expression of this protein has been shown to be upregulated in
659 grapevine cultivars that are resistant to *P. viticola* or in susceptible cultivar upon transgenic
660 overexpression, but not in wild-type susceptible cultivars (Enoki & Suzuki, 2016; M. Y. Li et
661 al., 2020; G. T. Liu et al., 2021). For most bacteria stimulating plant defenses against *P.*
662 *viticola*, the genes that were most frequently upregulated, PAL, STS, ROMT and PR1, were all
663 markers of the SA pathway as expected against a biotrophic pathogen (J. Y. Chen et al., 2006;
664 Chong et al., 2008). Interestingly, it seems that the most effective strains against *P. viticola*,

665 PIP27 and CHP15, were not the best at stimulating plant defenses, so we may assume that
666 they have a direct and strong effect on the pathogen.

667 When testing for induction of defense genes upon infection with *B. cinerea*, we observed
668 that except for PIP36 and SOP8 that triggered the upregulation of LOX, PR-4 and ROMT
669 (which is consistent with the results obtained with other strains by Trusov et al., 2009 and
670 Van Loon et al., 2006), the other phyllosphere isolates negatively affected the expression of
671 most of the defense-related genes we monitored. If we compare this observation with the
672 results obtained for *P. viticola*, we observe that *Cupriavidus metallidurans* CHP2 stimulated
673 the expression of defense-related genes in presence of *P. viticola*, but mostly inhibited the
674 expression of defense-related genes in presence of *B. cinerea*. Yet, the reduction in plant
675 defense did not appear to be detrimental to the plant, since despite the lack of plant defense
676 stimulation, the bacteria were able to successfully protect the plant against the pathogen,
677 suggesting that their effect was more likely acting on the pathogen rather than stimulating
678 plant defenses. (Zhu et al., 2019; Rahman et al., 2024)

679 The inoculation of bacteria also affected the production of stilbenes in leaves. These
680 compounds are phytoalexins with different levels of antifungal properties depending on their
681 structure (Viret et al., 2018). In plants infected with *P. viticola*, multiple strains were able to
682 stimulate the production of several of these compounds. Most of the strains promoted the
683 production of resveratrol, synthesized by the stilbene synthase (STS), an active form of
684 stilbene and precursor of several more active compounds such as viniferins and
685 pterostilbenes (Coutos-Thévenot et al., 2001). CHP15, SOD13, CHP2, SOD6 and PID6 (*M.*
686 *yunnanensis*, *B. xylanexedens*, *C. metallidurans* and two *B. cereus* strains) were able to
687 strongly increase the production of at least two different forms of viniferins, one of the most

688 active stilbenic compounds against *P. viticola* (Alonso-Villaverde et al., 2011; Pezet et al.,
689 2003; Van Leeuwen et al., 2013). The correlation between the increased resveratrol and
690 viniferin production and the higher expression of STS genes induced by several active strains
691 suggests that the protection mediated by these strains is indeed mediated, at least partly, by
692 the induction of this pathway. Similar effects of beneficial microbes on the grapevine stilbene
693 biosynthesis pathway have been described in Rühmann et al. (2013) with the yeast
694 *Aureobasidium pullulans*. In contrast to viniferins, we could not detect pterostilbenes, which
695 are compounds with higher antifungal effects than viniferins, in any sample, despite the
696 increase in the expression of the ROMT gene encoding the enzyme responsible for their
697 synthesis from resveratrol.

698 On the contrary, no significant increase of any stilbenic compound was found upon *B. cinerea*
699 infection, but these compounds have not yet been shown to be effective against *B. cinerea*
700 dormant conidia (Pezet & Pont, 1990; Pont & Pezet, 1990).

701 **Combinations of phyllosphere bacteria are more effective than single strains in protecting**
702 **grapevine plants against *P. viticola***

703 The combination of strains selected based on their complementary effects on pathogen
704 spores, symptom development and plant defenses provided high protection with low
705 variability on whole plants. This protection was observed at comparable levels both locally
706 and systemically (Table 4). Interestingly, as observed in experiments targeting *B. cinerea*
707 spore germination (Fig. 8), the combination of strains containing three different genera was
708 the most effective one against *P. viticola* compared to the other combinations containing at
709 least two *Bacillus* strains. The combination CHP2/PID6/PIP6 (*Cupriavidus metallidurans*,
710 *Bacillus cereus* and *Herbaspirillum huttiense*) was the most effective one and was able to

711 reach an almost full inhibition of symptoms (94 %) with very little variability (SD 5.5). The
712 best level of protection (98 %) was obtained by the positive control Alginure®, a product
713 certified for field use against *P. viticola* in Switzerland containing a combination of brown
714 algae, potassium phosphonate and amino acids, acting as a direct fungicide and as a plant
715 defense enhancer. Our best combination of bacteria, despite the absence of any formulation,
716 was therefore able to match the protection conferred by a mixture of biocontrol and
717 chemical agents. However, two combinations performed less well than expected. The
718 CHP2/CHP14/SOD2 consortium conferred a weaker reduction in symptoms than the three
719 best single-strains. This result is consistent with our observations on *B. cinerea* spores, where
720 the presence of the strain SOD2 (*Bacillus halotolerans*) seemed to limit the performance of
721 consortia in which it was included. Finally, the weakest consortium, SOP8/CHP14/PID6,
722 showed no visible reduction in symptoms despite containing two of the best single strains
723 (SOP8 and PID6). This consortium also exhibited the highest variability of all combinations.

724 In the whole plant assay against *P. viticola*, the effective consortia not only significantly
725 reduced the symptoms in treated leaves, but also conferred a systemic protection against the
726 pathogen. All single strains, except for PIP6, and combinations provided a comparable level
727 of inhibition in untreated leaves, suggesting that protection was mediated by the plant's
728 defense response and could therefore be transferred to distant parts of the plant. Compared
729 with single strains, the effects of consortia showed a higher stability as reflected by the lower
730 standard deviation even at the systemic scale, with the exception of the combination
731 CHP2/CHP14/SOD2 (*Cupriavidus metallidurans, Bacillus subtilis, Bacillus halotolerans*) whose
732 variability was comparable to strains used alone both at a local and systemic level, probably
733 because of the negative impact of SOD2, as discussed above. While mechanisms involved in

734 this systemic response triggered by consortia were not analyzed in the whole plant
735 experiment, the data obtained from single strains suggest that they rely on the induction of
736 the SAR pathway and on increased production of stilbenic compounds.

737 The use of combinations of bacteria with other bacterial strains, microorganisms from
738 different kingdoms or even chemical compounds has been reported as a successful strategy
739 against several plant pathogens in the past. This solution has several advantages, as it allows
740 the addition of several effects such as direct pathogen inhibition, accumulation of more
741 diverse antipathogenic compounds, plant defense stimulation and higher competition for
742 space and resources (Niu et al., 2020). Strains of *Bacillus mycoides* and *Pichia guillermondii*
743 were more effective at inhibiting *B. cinerea* spore germination and reducing necrotic lesion
744 size in strawberry leaves when used in combination compared to their use alone, showing
745 that organisms from different kingdoms can synergize and also provide more consistent
746 protection (Guetsky et al., 2001). In this study, the level of protection conferred by the
747 consortia often matched or exceeded the protection conferred by the best performing strain
748 alone, and decreased the variability despite the dilution (OD_{600} = 0.3 for each strain in a
749 consortium instead of OD_{600} = 1 for single strains), suggesting that strains assembled in a
750 consortium have synergies and display better performance in terms of protection against
751 diseases. From literature data, some genera seem to harbor more effective strains than
752 others in terms of plant protection, as e.g. for bacteria, the genera *Bacillus* and *Pseudomonas*
753 (Niu et al., 2020). The genus *Bacillus* has been reported as one of the four bacterial taxa
754 whose presence was correlated with lower disease incidence severity in vineyard soils
755 (Fournier et al., 2025). In our study, several different *Bacillus* species were proven effective
756 but no *Pseudomonas* were present. Instead, other genera such as *Cupriavidus*,

757 *Herbaspirillum*, *Microbacterium*, and members of other less commonly studied genera
758 showed high protective potential. These results are consistent with the idea that optimal
759 protection with bacteria-based biocontrol solutions may benefit from the use of
760 combinations including diverse genera of beneficial microorganisms with complementary
761 effects, and that strains showing only moderate effects when used alone could be of high
762 importance to stabilize or increase the effectiveness of a consortium (Maciag et al., 2023;
763 Nunes et al., 2024).

764

765 **Conclusion**

766 Bacteria isolated from the grapevine phyllosphere represent a wide range of strains that,
767 alone or in combination, significantly reduced symptoms caused by the two pathogens *P.*
768 *viticola* and *B. cinerea* and were also able to interfere with the pathogens' spore motility and
769 germination. These strains were also able to stimulate their host defenses through the
770 upregulation of defense-related genes and through the increased production of stilbenes.

771 The most effective bacteria consortia: CHP2/PID6/PIP6 (*C. metallidurans/B. cereus/H.*
772 *huttiense*) and CHP14/PID13/PIP6 (*B. subtilis/B. endophyticus/H. huttiense*) caused a
773 symptom reduction of 88 to 94 % *in planta* under growth chambers conditions, similar to
774 that achieved with commercially available solutions used as a benchmark. This work suggests
775 that the use of bacterial combinations is a valid strategy to increase biocontrol effectiveness.
776 Such strategy combined with an adapted should be tested under field conditions to evaluate
777 its suitability as a sustainable alternative to current crop protection practices.

778

Acknowledgements

The authors are grateful to Alisson Gillon and Gaëlle Ruffieux for their help in the experimental work with grapevine infection and bacterial inoculum preparation.

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Table 1: Effect of grapevine-associated phyllosphere bacteria on propagules of *B. cinerea* and *P. viticola*. *B. cinerea* conidial germination inhibition is expressed as the percentage of spores with impaired germination. Activity against *P. viticola* zoospores is reported as the percentage of non-motile zoospores and as the inhibition of symptom development on grapevine leaf discs using the same zoospore suspension exposed to bacteria as in the motility assay. Statistical significance: * = p < 0.05; ** = p < 0.01; *** = p < 0.001; - not significant.

				B. cinerea		***P. viticola***					
				germination		**motility**			**symptoms**		
habitat	**host cultivar**	**strain**	**organism / treatment**	**inhibition (%)**	**sig.**	**inhibition (%)**	**SD**	**sig.**	**inhibition (%)**	**SD**	**sig.**
		ctrl -	water	4.8	-	0	0	-	0	0	-
		ctrl +	copper hydroxide (*P. viticola*)	-	-	100	0	***	92	3.8	***
endophytic	Chasselas	CHD1	*Bacillus cereus*	95	***	98.5	2.6	***	30.1	58.7	-
		CHD2	*Bacillus cereus*	90.7	***	95.2	4.4	***	33.5	36.9	-
		CHD4	*Bacillus subtilis*	86.8	***	97.1	5.0	***	25.4	36.1	-
		CHD6	*Micrococcus aloeverae*	11.1	-	39.7	11.1	**	23.1	46.7	-
	Pinot Noir	PID1	*Paenibacillus amylolyticus*	100	***	64.1	22.9	***	8.2	48.8	-
		PID2	*Bacillus simplex*	84.6	***	77.9	13.6	***	74.5	28.4	***
		PID5	*Bacillus altitudinis*	93.3	***	98.7	1.3	***	18.5	61.3	-
		PID6	*Bacillus cereus*	100	***	99.0	1.7	***	83.3	10.8	***
		PID10	*Micrococcus aloeverae*	36.8	***	17.9	15.3	-	-9.7	60.1	-
		PID13	*Bacillus endophyticus*	100	***	51.9	16.4	**	52.3	41.1	***
	Solaris	SOD2	*Bacillus halotolerans*	69.8	***	99.0	1.7	***	22.7	39.0	-
		SOD5	*Bacillus licheniformis*	83.5	***	79.9	28.3	**	10.3	48.6	-
		SOD6	*Bacillus cereus*	79.4	***	92.2	4.7	***	39.4	47.6	-
		SOD7	*Bacillus psychrosaccharolyticus*	60.5	***	18.2	22.2	-	4.1	42.2	-
		SOD13	*Paenibacillus xylanexedens*	62.4	***	35.6	13.9	-	47.3	31.6	***
		SOD14	*Bacillus thuringiensis*	100	***	97.5	2.1	***	4.4	40.5	-
		SOD20	*Bacillus stratosphericus*	87.4	***	75.5	3.3	*	19.3	50.2	-
		SOD29	*Staphylococcus pasteuri*	47.2	***	31.8	7.3	-	14.3	55.3	-
		SOD31	*Bacillus zhangzhouensis*	68.2	***	95.7	4.6	***	26.0	46.7	-
epiphytic	Chasselas	CHP2	*Cupriavidus metallidurans*	87.5	***	56.0	38.6	-	-47.7	97.3	-

	CHP6	*Rhodopseudomonas boonkerdii*	9.2	-	56.3	13.8	***	13.4	55.6	-
	CHP14	*Bacillus subtilis*	98.2	***	98.8	2.1	***	38.5	39.2	**
	CHP15	*Micrococcus yunnanensis*	33.3	**	19.0	11.8	-	54.3	34.9	-
	CHP16	*Micrococcus yunnanensis*	17.5	-	51.1	26.3	-	25.4	44.6	-
	CHP19	*Methylobacterium pseudosasicola*	19.0	-	0	0	-	-16.3	104.9	-
	CHP20	*Bacillus cereus*	98.3	***	95.3	7.3	***	18.1	51.1	-
	CHP26	*Bacillus drentensis*	5.0	-	16.1	26.8	-	11.7	61.2	-
	CHP29	*Microbacterium proteolyticum*	62.8	***	76.3	30.7	**	5.0	55.0	-
	CHP33	*Frigoribacterium sp.*	3.9	-	23.7	29.9	-	-16.7	56.6	-
	CHP35	*Bacillus aryabhattai*	74.5	***	60.2	17.5	*	35.0	58.6	-
Pinot Noir	PIP2	*Sphingomonas zeae*	95.0	***	84.0	27.7	**	50.6	36.5	**
	PIP3	*Variovorax paradoxus*	93.5	***	7.7	28.0	-	50.6	62.6	***
	PIP4	*Cupriavidus metallidurans*	100	***	28.0	18.3	-	60.3	32.3	***
	PIP6	*Herbaspirillum huttiense*	100	***	3.8	22.6	-	31.8	36.5	*
	PIP7	*Microbacterium hominis*	70.6	***	99.4	1.1	***	66.2	29.9	***
	PIP10	*Pantoea agglomerans*	8.9	-	100	0	***	64	21.9	***
	PIP17	*Staphylococcus arlettae*	10.1	-	81.4	12.3	**	39.1	42.8	**
	PIP26	*Plantibacter flavus*	53.9	***	0	0	-	-34.2	94.3	-
	PIP27	*Rhodopseudomonas boonkerdii*	14.9	-	36.3	31.2	-	63.7	24.5	***
	PIP36	*Bacillus cereus*	80.2	***	98.2	3.2	***	46.7	42.2	***
Solaris	SOP5	*Microbacterium hominis*	80.6	***	100	0	***	61.8	29.3	***
	SOP8	*Cupriavidus metallidurans*	96.3	***	10.6	20.0	-	6.4	75.2	-
	SOP16	*Bacillus butanolivorans*	97.4	***	43.1	11.1	**	14.9	49.8	-
	SOP47	*Variovorax paradoxus*	84.1	***	0	0	-	11.2	39.6	-
	SOP48	*Staphylococcus xylosus*	65.9	***	17.2	16.0	-	22.5	64.5	-
	SOP51	*Bacillus pumilus*	45.4	***	98.7	1.2	***	49.4	34.4	***

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Table 2: Effect of grapevine-associated phyllosphere bacteria on *B. cinerea* and *P. viticola* symptom inhibition one week after infection in grapevine leaf discs inoculated with bacteria two days before infection. Activity against *B. cinerea* is reported as the reduction of the area covered by the necrosis compared to the infected water control. Activity against *P. viticola* is reported as the reduction of the area covered by the mycelium compared to the infected water control. Statistical significance: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; - not significant.

				B. cinerea			***P. viticola***		
habitat	**host cultivar**	**strain**	**organism / treatment**	**inhibition (%)**	**SD**	**sig.**	**inhibition (%)**	**SD**	**sig.**
endophytic		ctrl -	water	0	0	-	0	0	-
		ctrl +	Botector® (*B. cinerea*) / copper hydroxide (*P. viticola*)	61.1	14.8	***	89.9	4.9	***
	Chasselas	CHD1	*Bacillus cereus*	39.4	22.2	*	10.5	42.2	-
		CHD2	*Bacillus cereus*	-4.8	52.6	-	12.9	29.9	-
		CHD4	*Bacillus subtilis*	27.1	40.5	-	4.7	46.1	-
		CHD6	*Micrococcus aloeverae*	26.8	58.8	-	-18.5	43.2	-
	Pinot Noir	PID1	*Paenibacillus amylolyticus*	-12.7	56.4	-	15.7	51.5	-
		PID2	*Bacillus simplex*	62.4	11.6	*	5.7	83.0	-
		PID5	*Bacillus altitudinis*	14.5	33.2	-	-12.2	35.5	-
		PID6	*Bacillus cereus*	1.4	36.1	-	32.4	42.7	*
		PID10	*Micrococcus aloeverae*	-3.8	69.3	-	-2.6	41.8	-
		PID13	*Bacillus endophyticus*	5.4	50.3	-	15.7	68.0	-
	Solaris	SOD2	*Bacillus halotolerans*	26.1	53.0	-	36.6	45.5	**
		SOD5	*Bacillus licheniformis*	21.9	46.8	-	-17.8	98.7	-
		SOD6	*Bacillus cereus*	4.8	80.2	-	39.0	41.7	**
		SOD7	*Bacillus psychrosaccharolyticus*	38.6	33.6	*	4.5	34.6	-
		SOD13	*Paenibacillus xylanexedens*	54.2	25.5	**	37.9	52.5	*
		SOD14	*Bacillus thuringiensis*	-19.9	41.4	-	17.5	39.5	-
		SOD20	*Bacillus stratosphericus*	1.8	69.4	-	16.0	43.7	-
		SOD29	*Staphylococcus pasteuri*	10.0	76.8	-	16.2	37.8	-
		SOD31	*Bacillus zhangzhouensis*	11.7	70.0	-	23.8	31.5	*

	Chasselas	CHP2	*Cupriavidus metallidurans*	70.8	19.1	***	35.5	47.2	*
		CHP6	*Rhodopseudomonas boonkerdii*	4.8	56.0	-	0.6	30.6	-
		CHP14	*Bacillus subtilis*	28.4	56.8	-	39.7	32.8	**
		CHP15	*Micrococcus yunnanensis*	-4.3	90.9	-	46.1	49.1	*
		CHP16	*Micrococcus yunnanensis*	-15.2	68.8	-	-3.3	63.4	-
		CHP19	*Methylobacterium pseudosasicola*	2.9	78.0	-	-1.3	43.6	-
		CHP20	*Bacillus cereus*	49.8	43.7	**	24.8	42.4	*
		CHP26	*Bacillus drentensis*	50	40.6	**	11.0	53.4	-
		CHP29	*Microbacterium proteolyticum*	81.4	10.5	***	24.0	28.3	*
		CHP33	*Frigoribacterium sp.*	-11.0	79.2	-	-7.5	70.1	-
		CHP35	*Bacillus aryabhattai*	-10.6	71.2	-	-7.9	50.8	-
epiphytic	Pinot Noir	PIP2	*Sphingomonas zeae*	21.5	75.4	-	17.7	93.1	-
		PIP3	*Variovorax paradoxus*	2.1	83.8	-	12.8	75.9	-
		PIP4	*Cupriavidus metallidurans*	2.1	51.2	-	18.8	50.9	-
		PIP6	*Herbaspirillum huttiense*	69.3	19	***	-24.2	58.6	-
		PIP7	*Microbacterium hominis*	-49.0	95.8	-	41.6	34.1	**
		PIP10	*Pantoea agglomerans*	13.6	62.5	-	6.2	74.2	-
		PIP17	*Staphylococcus arlettae*	8.8	78.7	-	39.1	47.3	*
		PIP26	*Plantibacter flavus*	59.2	25.2	*	7.7	26.7	-
		PIP27	*Rhodopseudomonas boonkerdii*	20.2	67.1	-	58.6	41.8	**
		PIP36	*Bacillus cereus*	64.8	16.1	**	-16.0	42.5	-
	Solaris	SOP5	*Microbacterium hominis*	21.0	57.4	-	28.9	24.3	**
		SOP8	*Cupriavidus metallidurans*	63.8	19.4	*	13.1	55.1	-
		SOP16	*Bacillus butanolivorans*	55.8	25.4	**	-4.7	36.9	-
		SOP47	*Variovorax paradoxus*	75.4	9.7	***	8.0	112.6	-
		SOP48	*Staphylococcus xylosus*	52.1	25.3	*	0.1	115.0	-
		SOP51	*Bacillus pumilus*	20.2	64.7	-	9.0	41.1	-

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Table 3: Summary of the selection criteria used to select bacteria consortia. Strains were evaluated for i) *in vitro* inhibition of *B. cinerea* conidial germination and *P. viticola* zoospore motility, ii) inhibition of symptoms caused by *B. cinerea* and *P. viticola* on grapevine leaf discs, and iii) resistance to biofungicide products. A cross indicates that the strain met the following selection thresholds. Propagules: at least 50% inhibition of *B. cinerea* conidial germination and inhibition of *P. viticola* zoospore motility with significant symptom reduction in the subsequent infection assay; leaf tissue: reduction of symptoms of at least 61 % for *B. cinerea* and reduction of symptoms of at least 30 % for *P. viticola*; biofungicide resistance: survival in the presence of potassium bicarbonate (0.2 %) and wettable sulfur (0.4 %), tested individually and in combination. The seven strains fulfilling the selection criteria are marked in bold.

strain	organism / treatment	*P. viticola* propagule inhibition	*B. cinerea* propagule inhibition	*B. cinerea* inhibition on leaf discs	*P. viticola* inhibition on leaf discs	resistance to biofungicides
PID2	*Bacillus simplex*	x	x	x		
PID6	***Bacillus cereus***	x	x		x	x
PID13	***Bacillus endophyticus***	x	x			x
SOD2	***Bacillus halotolerans***		x		x	x
SOD6	*Bacillus cereus*		x		x	
SOD13	*Paenibacillus xylanexedens*		x		x	
CHP2	***Cupriavidus metallidurans***		x	x	x	x
CHP14	***Bacillus subtilis***	x	x		x	x
CHP15	*Micrococcus yunnanensis*				x	
CHP29	*Microbacterium proteolyticum*		x	x		
PIP2	*Sphingomonas zeae*	x	x			
PIP6	***Herbaspirillum huttiense***		x	x		x
PIP7	*Microbacterium hominis*	x	x		x	
PIP10	*Pantoea agglomerans*	x				
PIP17	*Staphylococcus arlettae*	x			x	
PIP27	*Rhodopseudomonas boonkerdii*				x	
PIP36	*Bacillus cereus*	x	x	x		
SOP5	*Microbacterium hominis*	x	x			
SOP8	***Cupriavidus metallidurans***		x	x		x
SOP47	*Variovorax paradoxus*		x	x		
SOP51	*Bacillus pumilus*	x				

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Table 4: Local and systemic *P. viticola* symptom inhibition one week after infection in whole plants pre-inoculated with phyllosphere bacteria relative to uninoculated control. Statistical significance: * = p < 0.05; ** = p < 0.01; *** = p < 0.001; - not significant.

		local			**systemic**		
Strain	**organism / treatment**	**inhibition (%)**	**SD**	**significance**	**inhibition (%)**	**SD**	**significance**
Alginure®	brown algae	98,2	0,9	***	98,2	0,9	***
PID6	*Bacillus cereus*	82,4	21,7	***	68,5	38,5	***
SOP8	*Cupriavidus metallidurans*	68,3	29,5	***	74	19,8	***
PIP6	*Herbaspirillum huttiense*	63,4	35,9	***	29,6	55,5	-
SOD2	*Bacillus halotolerans*	52,6	31,9	***	58,3	18,1	***
CHP14	*Bacillus subtilis*	38	64,4	*	31,1	73,5	*
CHP2	*Cupriavidus metallidurans*	33,8	46,6	-	25,8	44,6	-
PID13	*Bacillus endophyticus*	31,2	70,8	-	32,2	53,4	-
CHP2 / PID6 / PIP6	*Cupriavidus metallidurans / Bacillus cereus / Herbaspirillum huttiense*	94,2	5,5	***	94	5,2	***
CHP14 / PID13 / PIP6	*Bacillus subtilis / Bacillus endophyticus / Herbaspirillum huttiense*	88,1	8,6	***	90	16,2	***
CHP2 / CHP14 / PID13	*Cupriavidus metallidurans / Bacillus subtilis / Bacillus endophyticus*	84,9	13,1	***	87,7	15,7	***
CHP2 / CHP14 / SOD2	*Cupriavidus metallidurans / Bacillus subtilis / Bacillus halotolerans*	57,6	39,2	**	67	26,8	**
SOP8 /CHP14 / PID6	*Cupriavidus metallidurans / Bacillus subtilis / Bacillus cereus*	3,4	52,6	-	0,0	48,5	-

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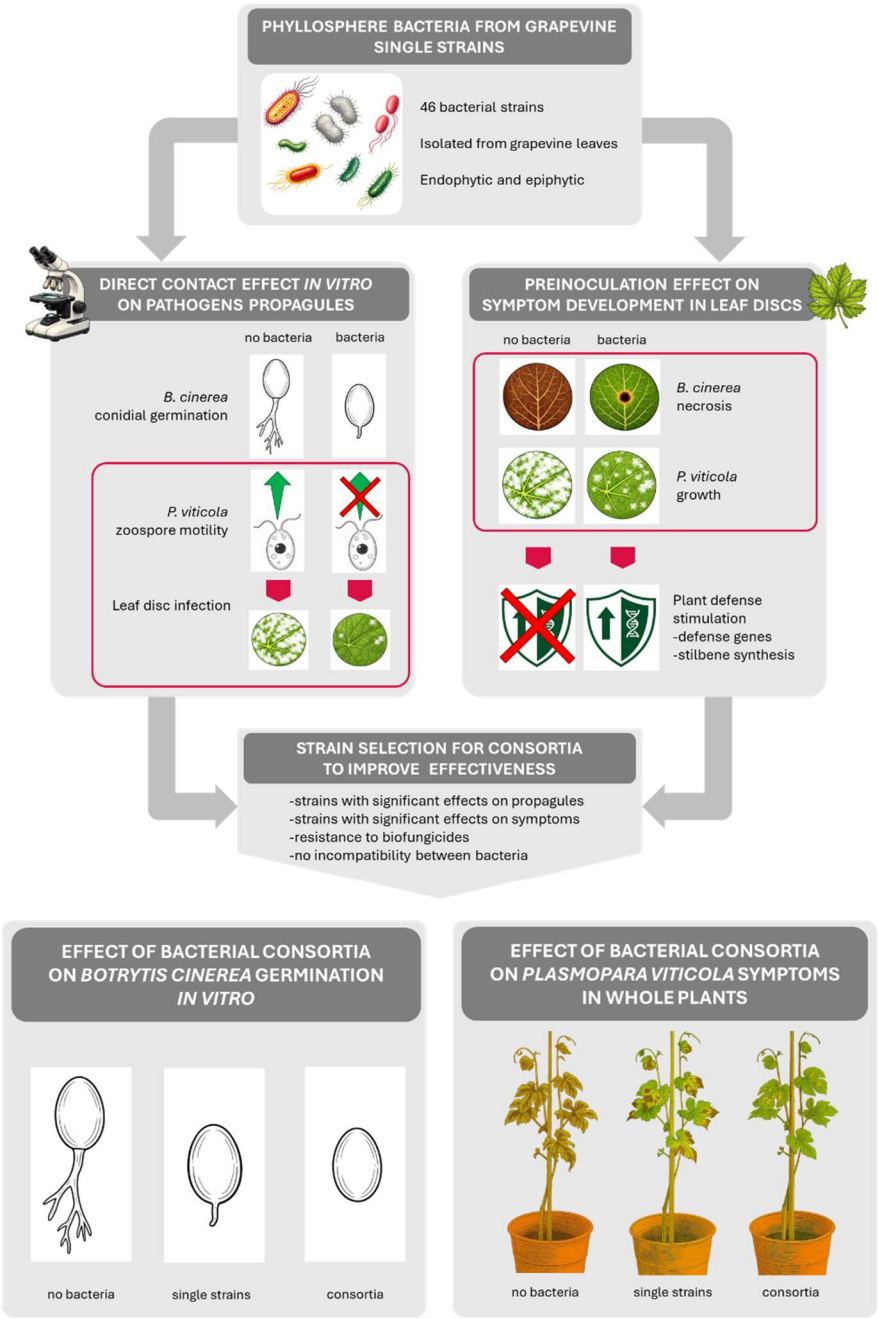
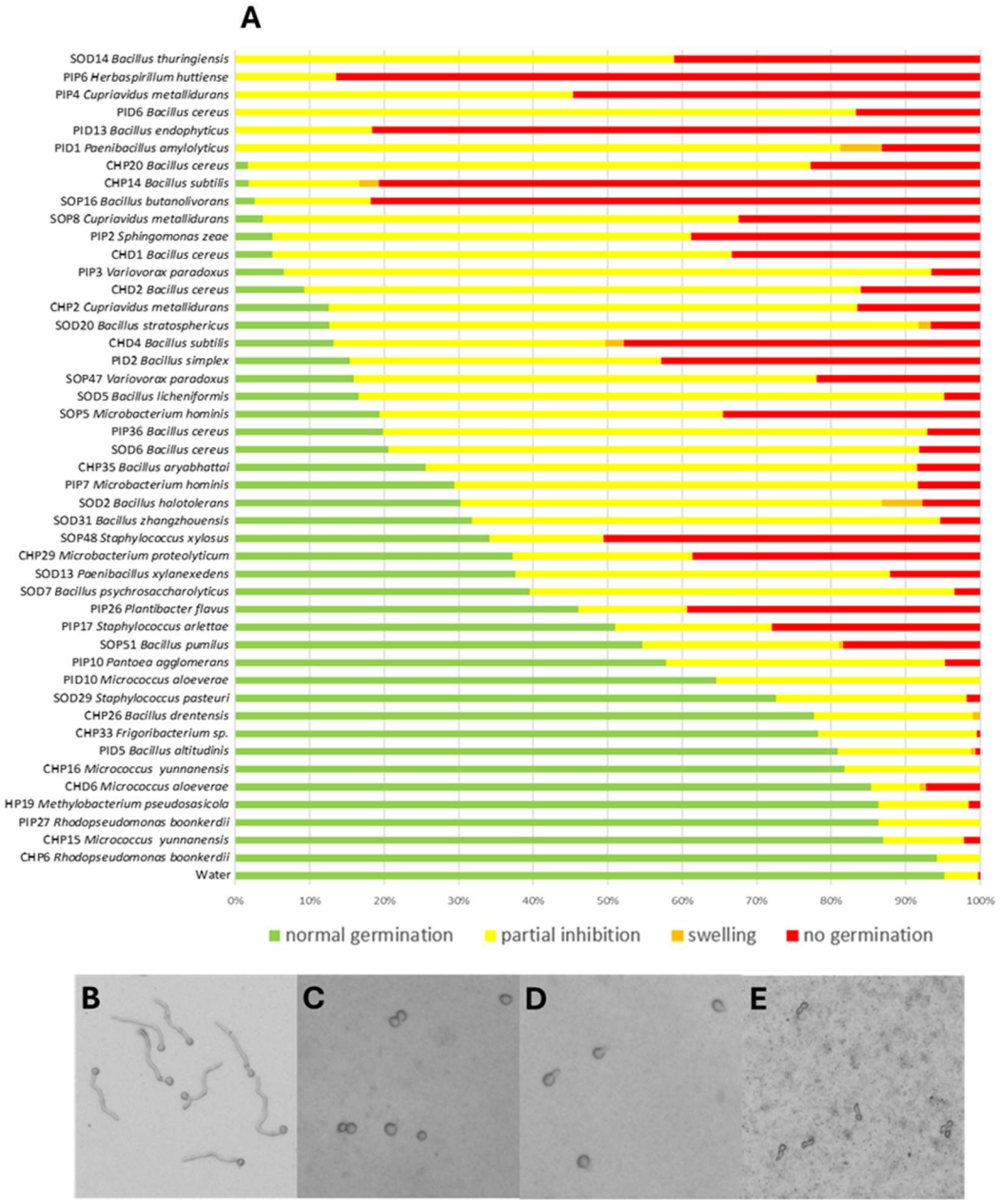
Figure 1: Summary of the experimental workflow: *in vitro* experiments against pathogen propagules, leaf disc assays, selection of bacteria for consortia and consortia effect on propagules and whole-plant infections. Red boxes indicate experiments that are part of the same experimental setup; a red cross over an icon indicates absence of the respective effect. Illustration developed with assistance from Sora (OpenAI), with final graphics produced by the authors.



1045 **Figure 2:** Effect of phyllosphere bacteria on *B. cinerea* spore germination. A: Each colored bar
1046 represents the proportion of spores showing the corresponding morphology. Green, normal
1047 germination; yellow, partial inhibition (only the tip of the germ tube is visible); orange,
1048 swelling; and red, no germination. The different types of germination morphologies are
1049 shown in (B to E); B: normal germination; C: absence of germination; D: partial germination
1050 and E: swelling.



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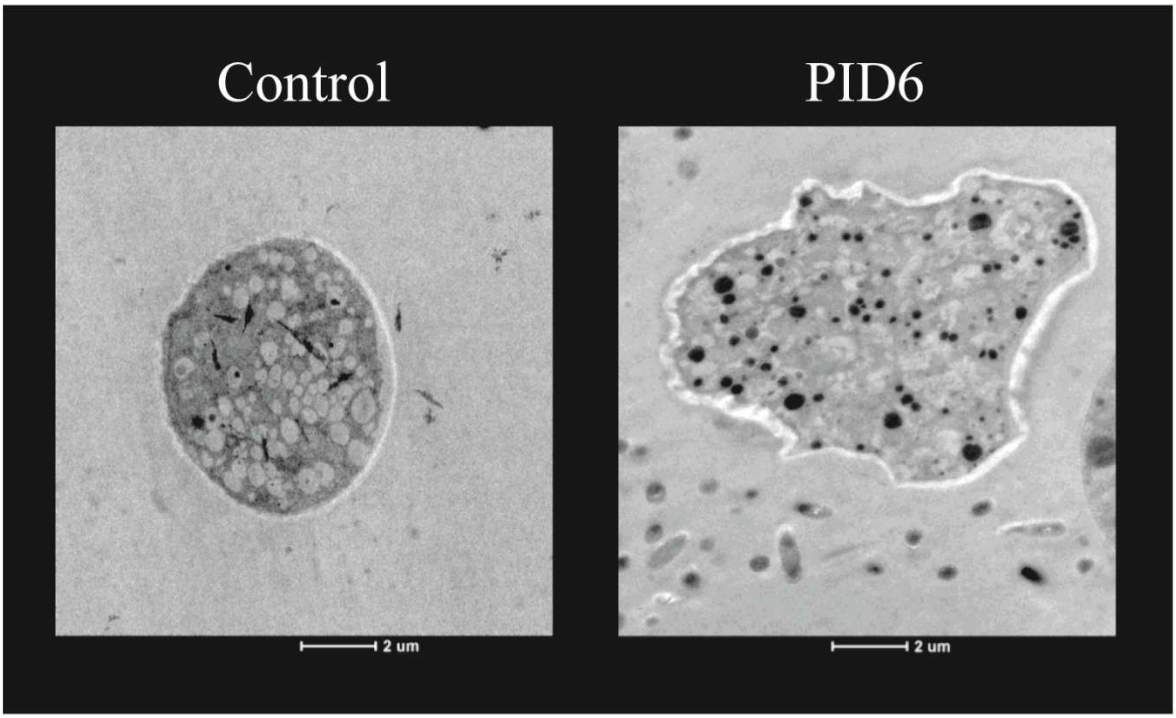
1052 **Figure 3**: Transmission electron microscopy image of *P. viticola* zoospores placed for 30 min

1053 in contact with water (control), or a solution of the *Bacillus cereus* strain PID6 OD_{600} = 1.



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1062 **Figure 4:** Representative pictures of grapevine leaf discs inoculated with phyllosphere
1063 bacteria prior to infection with *B. cinerea*. Control: leaf disc not inoculated with bacteria but
1064 infected with *B. cinerea* spores; CHP29, PID2 and SOD7: leaf discs inoculated with
1065 *Microbacterium proteolyticum* (CHP29), *Bacillus simplex* (PID2) and *Bacillus*
1066 *psychrosaccharolyticus* (SOD7), respectively, prior to infection. Pictures were taken one week
1067 after infection.

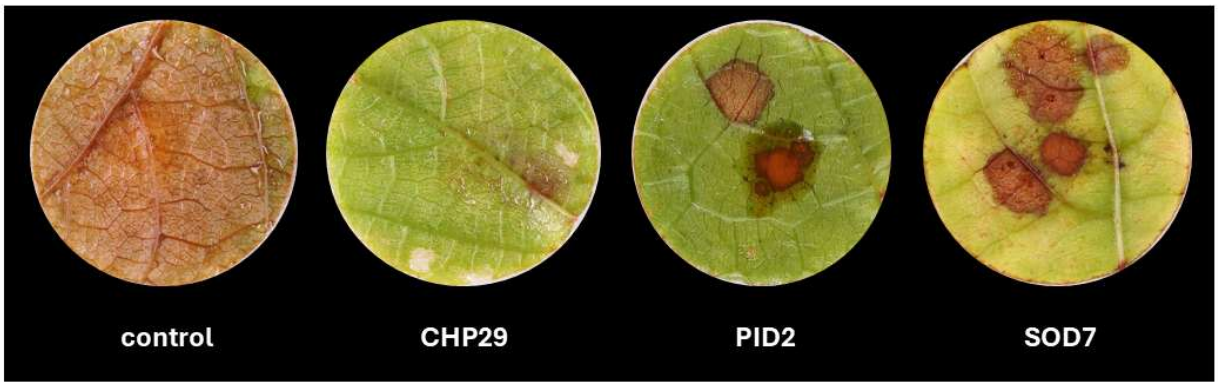

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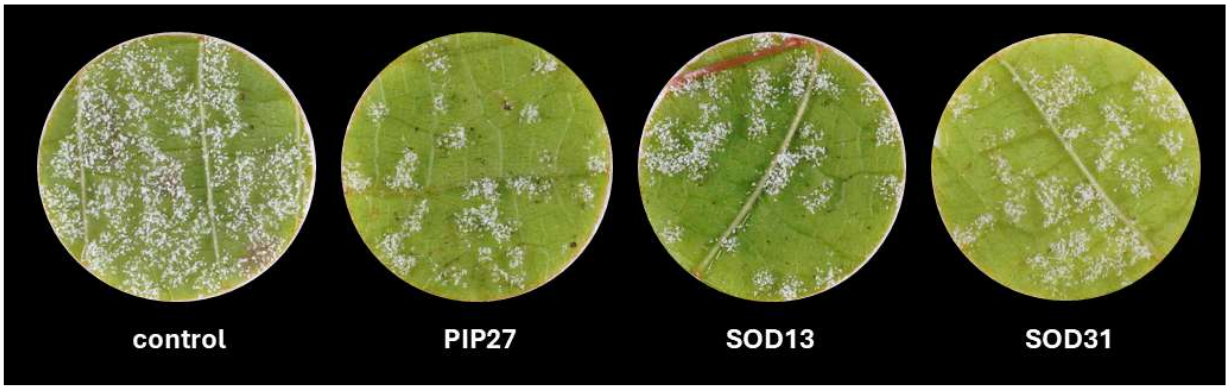
Figure 5: Representative pictures of grapevine leaf discs inoculated with phyllosphere bacteria prior to infection with *P. viticola*. Control: leaf disc not inoculated with bacteria but infected with *P. viticola* zoospores; PIP27, SOD13 and SOD31: leaf discs inoculated with *Rhodopseudomonas boonkerdii* (PIP27), *Paenibacillus xylanexedens* (SOD13) and *Bacillus zhangzhouensis* (SOD31), respectively, prior to infection. Pictures were taken one week after infection.



1093 **Figure 6:** Level of expression of defense-related genes in grapevine leaf discs inoculated with
1094 phyllosphere bacteria that significantly protected the plant against *P. viticola* or *B. cinerea* in
1095 leaf disc assays. The levels of expression were normalized with the two reference genes Actin
1096 and 60SRP, and expressed relative to infected control leaf discs that were not inoculated with
1097 bacteria. Strains are ordered by their inhibition percentage, which corresponds to the
1098 reduction in symptom development caused by each strain in the leaf disc experiments.

pathogen	strain	organism	LOX	PAL	STS	ROMT	PR-1	PR-4	inhibition (%)
B. cinerea	CHP29	*Microbacterium proteolyticum*							81.4
	SOP47	*Variovorax paradoxus*							75.4
	CHP2	*Cupriavidus metallidurans*							70.8
	PIP6	*Herbaspirillum huttiense*							69.3
	PIP36	*Bacillus cereus*							64.8
	SOP8	*Cupriavidus metallidurans*							63.8
	PID2	*Bacillus simplex*							62.4
P. viticola	PIP27	*Rhodopseudomonas boonkerdii*							58.6
	CHP15	*Micrococcus yunnanensis*							46.1
	PIP7	*Microbacterium hominis*							41.6
	CHP14	*Bacillus subtilis*							39.7
	PIP17	*Staphylococcus arlettae*							39.1
	SOD6	*Bacillus cereus*							39.0
	SOD13	*Paenibacillus xylanexedens*							37.9
	SOD2	*Bacillus halotolerans*							36.6
	CHP2	*Cupriavidus metallidurans*							35.5
	PID6	*Bacillus cereus*							32.4
	SOP5	*Microbacterium hominis*							28.9
	CHP20	*Bacillus cereus*							24.8
	CHP29	*Microbacterium proteolyticum*							24.0
	SOD31	*Bacillus zhangzhouensis*							23.8

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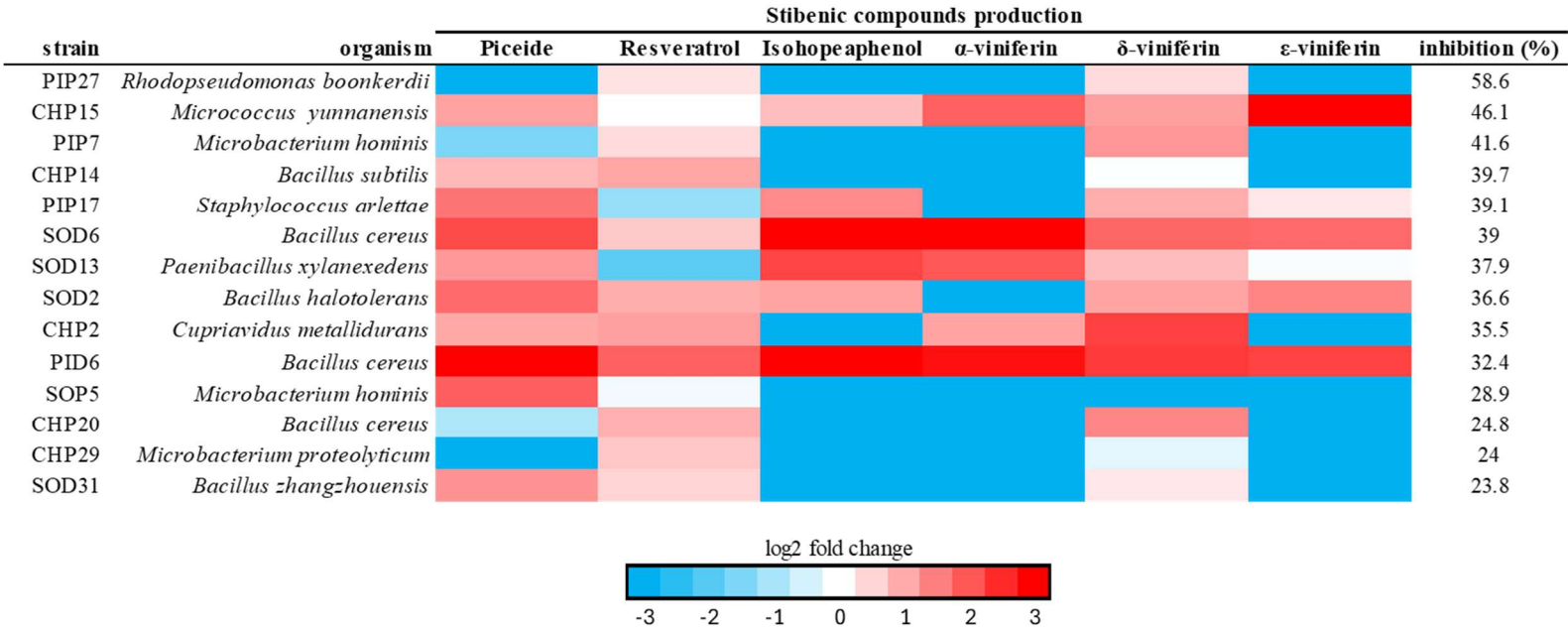
1102 **Figure 7:** Stilbene production levels in grapevine leaf discs inoculated with phyllosphere

1103 bacteria 3 days after infection with *P. viticola* zoospores. Levels of production are based on

1104 dry weight and are expressed relative to infected leaves that were not inoculated with

1105 bacteria. Strains are ordered by their inhibition percentage, which corresponds to the



1106 reduction in symptom development achieved with each strain in leaf disc assays.

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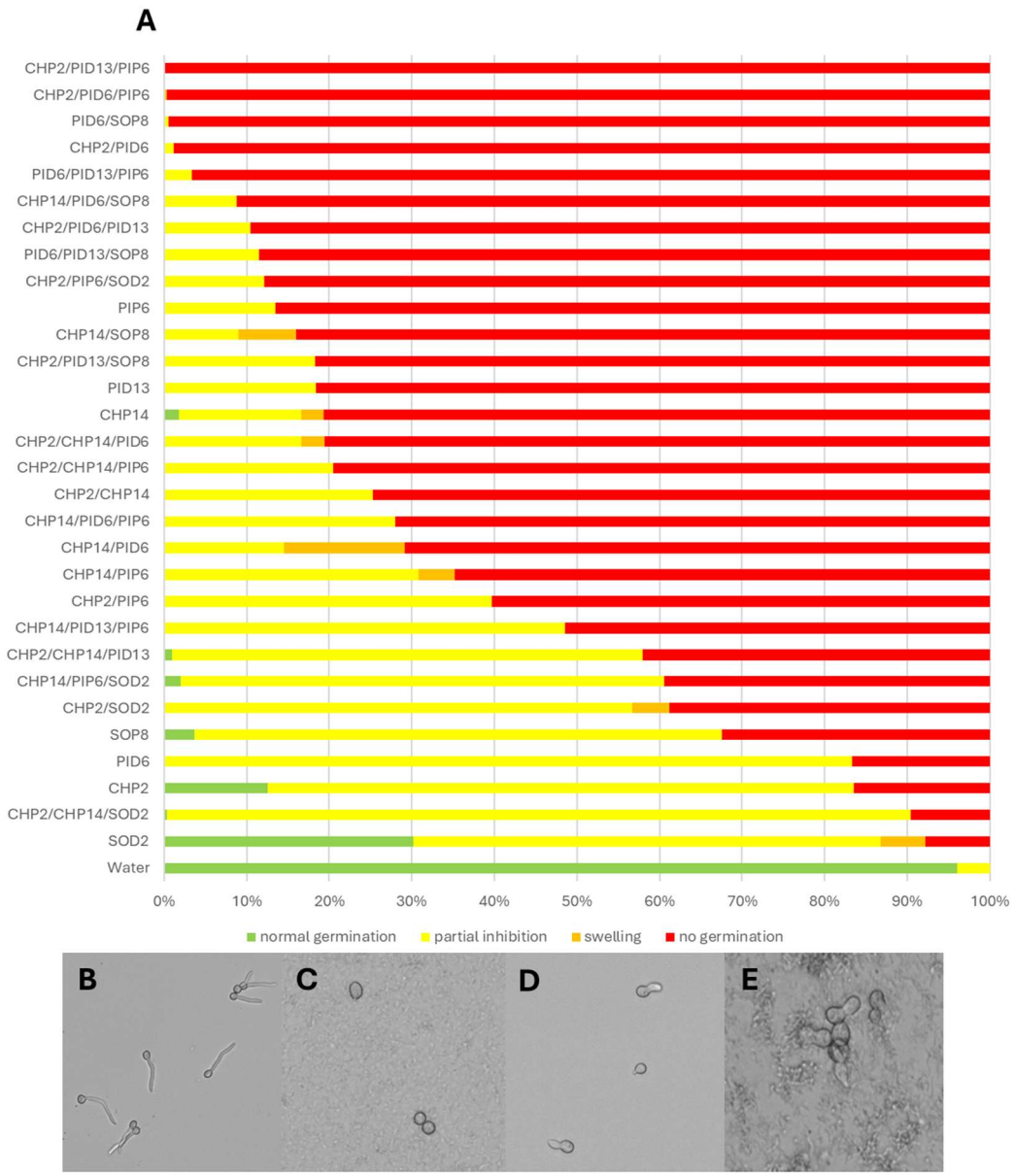
1114 **Figure 8**: Effect of consortia of phyllosphere bacteria on *B. cinerea* spore germination. A: Each
1115 colored bar represents the proportion of spores showing the corresponding morphology.
1116 Green: normal germination; yellow; partial inhibition (only the tip of the germ tube is
1117 visible); orange: swelling and red: no germination. Each treatment with bacteria significantly
1118 affected the physiology of the spores, with $p < 0.05$ according to a Fisher's exact test with
1119 Bonferroni's correction. The different types of germination morphologies are shown in (B to
1120 E); B: normal germination; C: absence of germination; D: partial germination and E: swelling.



1121

Figure 9: Representative pictures of grapevine leaves from whole grapevine plants inoculated with phyllosphere bacteria prior to infection with *P. viticola*. Leaves were inoculated with different mixtures of the following six strains: *Cupriavidus metallidurans* (CHP2), *Bacillus cereus* (PID6), *Herbaspirillum huttiense* (PIP6), *Bacillus subtilis* (CHP14), *Bacillus endophyticus* (PID13) and *Bacillus halotolerans* (SOD2). Control: non-inoculated leaf infected with *P. viticola* zoospores. Pictures were taken one week after infection.

CONTROL **CHP2/PID6/PIP6** **CHP14/PID13/PIP6** **CHP2/CHP14/PID13** **CHP2/CHP14/SOD2**