iVES OENO One vine & wine

DOI: https://doi.org/10.20870/oeno-one.2023.57.3.7463

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

The potential role of *Aureobasidium pullulans* in the development of foliar symptoms of Esca disease in grapevine

Zoltán Karácsony[1*], Vincenzo Mondello[2], Florence Fontaine[2], Kálmán Zoltán Váczy[1]

[1] Eszterházy Károly Catholic University, Food and Wine Research Institute, Leányka utca 6/G, Eger, H3300, Hungary

[2] Université de Reims Champagne-Ardenne, Unité de recherche Résistance Induite et Bioprotection des Plantes RIBP EA 4707 – USC INRAE 1488, BP 1039, 51687 Reims Cedex 2, France

*correspondence:
karacsony.zoltan@uni-eszterhazy.hu

Associate editor:
Alonso Pérez-Donoso

Received:
10 March 2023

Accepted:
3 August 2023

Published:
28 August 2023

This article is published under the **Creative Commons licence** (CC BY 4.0).

Use of all or part of the content of this article must mention the authors, the year of publication, the title, the name of the journal, the volume, the pages and the DOI in compliance with the information given above.

ABSTRACT

Esca belongs to the group of grapevine trunk diseases - fungal diseases present worldwide in all wine-growing regions. Some aspects of the disease, like the development of external symptoms, have still not been completely discovered and are believed to be affected by several factors, including interactions within the vine microbiome. The examination of the occurrence of the yeast-like fungus *Aureobasidium pullulans* in the healthy wood of Esca-diseased grapevines via both isolation and qPCR measurements showed a positive correlation between its abundance and the severity of foliar symptoms, suggesting the contribution of this fungus to Esca pathogenesis via an indirect action. *In vitro* confrontation tests revealed antagonistic interaction between *A. pullulans* and the Esca pathogen *Phaeomoniella chlamydospora*. Mutual growth inhibition and the induction of asexual sporogenesis were observed for both fungi without cytotoxic effects. *In planta* confrontation tests revealed that *A. pullulans* in combination with *P. chlamydospora* can lead to severe foliar damage in a strain-dependent manner. This phenomenon could be explained by the altered metabolism of the Esca pathogen in the presence of *A. pullulans*, or by the cumulative/synergistic effects of the secreted polysaccharides and/or proteins of the two fungi. The present study shows the importance of microbial interactions in the development of plant diseases, highlighting that even a non-pathogenic microorganism can act as a disease-enhancer.

KEYWORDS: grapevine trunk disease, pathogenesis, *Phaeomoniella chlamydospora*, microbial interaction

INTRODUCTION

Esca disease belongs to the group of grapevine trunk diseases (GTDs) which are devastating in vineyards worldwide. The syndromes of GTDs (Esca complex, Eutypa- and Botryosphaeria- diebacks, Phomopsis disease and Black foot disease; for a review see Gramaje *et al.* (2018)) are caused by filamentous fungi of different genera (*Phaeoacremonium*, *Phaeomoniella*, *Diplodia*, *Neofusicoccum*, *Lasiodiplodia*, *Eutypa*, *Ilyonectria,* etc.), often leading to the death of the affected plants (Surico *et al.*, 2006; Mugnai *et al.*, 1999; Mondello *et al.*, 2018). GTD pathogens infect vines, especially through wounds, and they colonise woody tissues, developing internal wood discolorations and necrosis (Bertsch *et al.*, 2013). Even though the pathogens grow inside the trunk, there are typical GTD external symptoms that develop on the green organs of the vine; i.e., on the shoots, leaves and, in some cases, the berries. Shoots and leaves can dry rapidly, often affecting the whole plant (apoplexy). On leaves, chlorotic and necrotic areas appear, while berries can develop small black spots (Black measles) or can become completely dry, depending on the GTD (Bertsch *et al.*, 2013). To date, following the ban of sodium-arsenite and some benzimidazoles, there is no simple and reliable GTD control method in neither the vineyard nor the nursery (Gramaje *et al.*, 2018; Mondello *et al.*, 2018).

The main agents of the Esca disease complex are the ascomycetous fungi *Phaeoacremonium minimum* (Phm) and *Phaeomoniella chlamydospora* (Pch), sometimes associated with *Fomitiporia mediterranea* (Fomed), and other basidiomycetous fungi of the genus *Fomitiporia* (Moretti *et al.*, 2021), which cause white rot in the Esca itself (Graniti *et al.*, 2000a; Fischer, 2002; Cloete *et al.*, 2014). The typical Esca internal symptoms are vascular discoloration with the production of dark tarry drops and wood necrosis. In leaves, both chlorotic and necrotic areas have typically been described as tiger-striped leaves (Mugnai *et al.*, 1999), and later as Grapevine Leaf Strip Disease (GLSD - Surico, 2009). The absence of the pathogens in symptomatic leaves, their presence as endophytes in healthy vines (Bruez *et al.*, 2014; 2015; Del Frari *et al.*, 2019; Mondello *et al.*, 2022) and the erratic symptoms expression on Esca-affected plants clearly indicate that there is no direct and simple relationship between the presence of pathogens and the development of the Esca external symptoms. Therefore, Esca disease expression (and those of other GTDs, in general) is believed to be strongly affected by other factors, such as abiotic stresses, weather conditions and microbial interactions (Claverie *et al.*, 2020; Fischer and Peighami-Ashnaei, 2019; Songy *et al.*, 2019). Thus, the main hypothesis for explaining the complexity of foliar symptoms expression in Esca-affected vines is based on the toxicity of some pathogens' secreted metabolites. These phytotoxins, whose production can be modulated by internal (fungus) and /or external (environmental) factors, can spread throughout plants as a result of the sap flux reaching the leaves, thus determining metabolic disorders and, finally, the symptoms (Mugnai *et al.*, 1999; Claverie *et al.*, 2020; Trotel-Aziz *et al.*, 2019).

The study of this molecular background has led to the identification of several toxic effector molecules of differing chemical nature. The results of studies have suggested that secreted polypeptides of Pch and Phm have a direct toxic effect on plant grapevine cells (Luini *et al.*, 2010) or act as degrading enzymes, damaging the colonised vascular tissues (Bruno and Sparapano, 2006b). It has also been suggested that secondary metabolites secreted by Esca pathogens take part in symptom development. According to the results of analyses of naturally infected grapevines and phytotoxicity tests, two Esca-associated phytotoxic secondary metabolites have been found to be scytalone and isosclerone (Bruno and Sparapano, 2006a; Bruno *et al.*, 2007). Polysaccharides have also been found to contribute to Esca foliar symptom development, especially pullulan (Bruno and Sparapano, 2006a; Bruno *et al.*, 2007), which is produced by Pch and Phm. Its phytotoxicity has also been hypothesised in the cases of other fungal plant diseases, like chestnut blight caused by *Cryphonectria parasitica* (Forabosco *et al.*, 2006). This suggests that pullulan may act as a non-host-specific phytotoxin. Even if secreted proteins, secondary metabolites (e.g., scytalone, isosclerone, naphthalenone, and mellein) and extracellular polysaccharides (e.g., pullulan) have proved to be toxic to grapevines (Bruno *et al.*, 2007; Burruano *et al.*, 2016; Graniti *et al.*, 2000b; Andolfi *et al.*, 2011; Abou-Mansour *et al.*, 2015; Trotel-Aziz *et al.*, 2019), their mode of action and their role in the development of the symptoms in natural conditions are not completely clear. The fact that GLSD foliar symptoms have never been observed in grapevine artificially infected with Phm, Pch and Fomed, either alone or in combination, raises questions about the real impact of their effector molecules in Esca disease, confirming that other factors could promote or block foliar symptoms expression.

Recent studies indicate a possible role of the vine microbiome in GTD symptoms expression (Bettenfeld *et al.*, 2020; 2021; Niem *et al.*, 2020). According to this hypothesis, healthy vines are the result of a balance of the different elements of the vine microbiome. Therefore, a diseased vine could be the result of an unbalanced microbiome, in which interactions between microorganisms have negative effects for plant health, especially in the case of pathogen attack.

Aureobasidium pullulans (Apu) is a cosmopolitan dimorphic fungus and a relevant member of the epiphytic (Barata *et al.*, 2012) and endophytic (Knapp *et al.*, 2021) microbiome of the aboveground parts of grapevines. Apu is mostly known for its biocontrol capacities and its ability to produce industrially important enzymes and the polysaccharide pullulan (Prasongsuk *et al.*, 2018). The effects of this fungus on the grapevine host are also beneficial according to previous studies. Apu has proved to be a potential biocontrol agent against *Botrytis cinerea*, the causal agent of grapevine bunch-rot (Galli *et al.*, 2021), and *Greeneria uvicola*, the pathogen of bitter rot in grapes (Rathnayake *et al.*, 2018). Apu is an effective inhibitor of *Diplodia seriata* (Botryosphaeria dieback), directly inhibiting and possibly elicitating defense responses in the host, as has been suggested by the elevated

expression of *PR6* pathogenesis-related gene in Apu-treated plants (Pinto *et al.*, 2018). In contrast to the abovementioned beneficial effects of Apu on plant hosts, its phytopathogenicity has also been reported in certain cases (Guo *et al.*, 2020; Lee *et al.*, 2019; Prashanthi and Kulkarni, 2005; Wu *et al.*, 2017; Xie *et al.*, 2022). Moreover, the fact that Apu is a potent producer of the aforementioned phytotoxin pullulan raises further doubts about whether it is generally a beneficial microorganism.

Our initial investigations on Esca-affected grapevines in the field produced surprising results: the abundance of Apu was found to positively correlate with the severity of the foliar symptoms (Figure 1B). Since Apu is a well-characterised inhabitant of the grapevine microbiome that does not have any known damaging effects on the host, we hypothesised that the disease-promoting effect of this fungus is based on its interaction with the known Esca pathogens Pch and Phm. Interactions between the fungal species were studied *in vitro* confrontation assays, revealing a modulatory effect of Apu and Pch on each other's development. Their direct and indirect effects on the development of foliar symptoms were examined by fungal artificial inoculation. Strain-dependent differences in the combined pathogenicity of Apu and Pch strains are suggested to be related to the measured differences in the ability of strains to produce known virulence factors of Esca disease like exopolysaccharides (EPS) or extracellular proteins.

MATERIALS AND METHODS

1. Isolation and identification of fungal strains

The wood endophytic fungal population of Esca-affected vines was isolated in 2017 from *Vitis vinifera* cv. Cabernet-Sauvignon plants in the vineyard of Eszterházy Károly Catholic University. To isolate the wood-colonising fungi small pieces of the vine trunk were cut out. Since there is a large variance in the severity of foliar symptoms between the canes of a plant, the wood trunks were sampled just below the canes monitored for foliar symptom severity. The obtained wood chips were placed on Potato Dextrose Agar (PDA) plates supplemented with 10 µg/ml oxytetracycline to prevent bacterial growth. The plates were incubated at 25 °C for 4 weeks. The emerging mycelia were first placed individually on PDA plates, then pure cultures were obtained by subculturing a small portion of actively growing mycelia or by streaking conidia when available. The strains were stored in 50 %v/v glycerol at -80 °C. A total of 43 trunks with the foliar symptoms of Esca disease were sampled.

Total DNA was extracted from the fungal biomass using DNeasy Plant Mini Kit (Qiagen) for molecular identification. Internal transcribed spacer regions were amplified using ITS1-f(5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers. Reaction products were checked by agarose gel electrophoresis. The obtained amplicons were sequenced (BaseClear B.V. -Netherlands) and the sequences were compared in online databases using BLAST (www.ncbi.nlm.nih.gov). Fungal strains were identified based on sequence similarity and morphological characteristics.

2. Measurement of Apu DNA in grapevines by Quantitative Real Time PCR

In September 2018, to quantify the presence of Apu in the wood of Esca-disease-expressing vines, 10 cv Cabernet-Sauvignon grapevines showing foliar symptoms of disease and 10 asymptomatic plants were sampled in the same vineyard of 2017; this was done by drilling the trunk at the base of canes being examined for symptom severity. The samples were lyophilised and grounded in a tissue-lyser (50 Hz, 2×5 min) with the aid of a stainless-steel ball. DNA was extracted with DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions and measured by Nanodrop 2000 (Thermo Fisher Scientific) spectrophotometer. The amount of Apu DNA was determined by using species-specific primers as described by Martini *et al.* (2009).

3. Investigation of the severity of foliar symptoms on Esca-affected grapevines

The symptomatic leaves of Esca-diseased vines (3 per each symptomatic cane) were removed ans photographed using a table scanner. The symptomatic areas were analysed by image analysis software (Photoshop CS5) to calculate symptom severity. For each cane, the symptom severity was calculated as the mean of 3 leaves.

4. Study of *in vitro* fungal interactions

4.1. Direct assay: dual in vitro culture assays

To verify any possible interactions between Apu (8 isolates) and Pch (6 isolates) or Phm (3 isolates), the fungi isolated from Esca-diseased vines were inoculated in dual culture assay. Mycelial plugs (diameter of 3 mm) from the margins of 1-week-old colonies were placed on a PDA plate at a distance of 5 cm from each other. The controls consisted in plates individually inoculated with the used strains (3 replicates per combination). The petri dishes were incubated at 25 °C in the dark for two weeks.

4.2. Indirect assay: cultural filtrate assays

To verify the nature of the observed antagonism, the strains of the pathogen-Apu combinations showing interaction were used in the culture filtrate assay. Apu strain Y60 and Pch strain P3 were inoculated separately in 3×100 ml liquid YG medium (2 % m/v glucose, 1 % m/v yeast extract) at 25 °C and 180 rpm for 7 days. Afterwards, the fungal mycelia were removed by filtration, and the cultural filtrate (CF) was sterilised using a membrane with a 0.2 µm pore size. CFs were mixed with PDA medium to obtain plates of CF at concentrations of 50, 25, 12.5, and 0 % v/v. PDA plates with 50 % v/v YG medium were also prepared as a control. Mycelial plugs (diameter of 3 mm) were inoculated on the plates for the measurement of the effect of culture filtrates on growth rate and sporulation, while 100 conidia were streaked on the plates for the assessment of germination rates. Fungal cultures were incubated at 25 °C in the dark for 8 days. Mycelial growth and conidia germination were monitored

in order to calculate the respective growth and germination rate. The number of formed conidia for Pch and of single-cell forms (conidia and yeast) for Apu was obtained using a Burker-chamber. Three isolates of Apu and three isolates of Pch were used for the indirect assays. The experimental design is summarised in Supplementary Table S1. All tests were done in triplicates.

5. Artificial inoculation of grapevine detached green shoots

To also test the interaction between Apu and Pch *in planta*, artificial inoculations were performed on detached green shoots. Three strains of Apu (Y37, Y43 and Y60) and three of Pch (P3, P37, and P46) were used in this test. In detail, green canes of cv. Cabernet-Sauvignon were collected at the beginning of July and cut into small sections containing a node, an internodal section and a leaf. The cuttings were surface sterilised using 70 %v/v ethanol and then wounded with a sterile scalpel in the upper third part. Apu and Pch mycelial plugs (diameter of 3 mm) from the margin of a 1-week-old colony were placed within the wound. Each strain was inoculated alone and in combination with a strain of the other fungus. In the latter case, strains were inoculated at 1 cm distance from each other. Each of these inoculation conditions was replicated 6 times. The controls were inoculated with sterile PDA plugs. The cuttings were placed in water and incubated without artificial lighting and with full exposure to sunlight for 15 days in July. The relative area of the leaves in all conditions was measured after photographing as described in Section 3 of Materials and methods. The experiment was repeated three times.

6. Analytical methods

The production capacity of the EPS and extracellular proteins (potential virulence factors) of Apu and Pch strains was determined to explain the strain-dependent differences in pathogenicity. To further investigate the possible interaction of Apu and Esca pathogens in disease expression, we also studied the possible role of Apu and Pch exopolysaccharides (EPS) in the development of Esca foliar symptoms.

6.1. Purification of exopolysaccharides from fungal culture filtrates

To obtain EPS, culture filtrates of Apu (Y37, Y43 and Y60) and Pch (P3, P37 and P46) strains were added to two volumes of 96 % v/v ethanol and incubated at 4 °C overnight. EPS were pelleted by centrifugation (4000 rpm, 30 min) and washed twice with 70 %v/v ethanol. EPS were dried and weighed to prepare 5 mg/ml stock solutions.

6.2. Proteins and polysaccharides quantification

The concentrations of proteins were assayed by a dye-binding method (Bradford, 1976) using BSA (bovines serum albumin) as a standard. Polysaccharide content was evaluated by the phenol-sulfuric acid method (Dubois *et al.*, 1956) using purified EPS of the Y60 Apu strain (described above) as a standard. Absorbance was measured by Nanodrop 2000 (Thermo Fisher Scientific). All measurements were done in triplicate.

6.3. Measurement of reducing sugars

The reducing sugar content of purified fungal EPS was determined using the neocuproine method (Dygert *et al.*, 1965). Absorbance was measured by Nanodrop 2000 (Thermo Fisher Scientific) using glucose as a standard. All measurements were done in triplicate.

6.4. Foliar disc assays

The effect of the interaction of Apu and Pch EPS, alone or in combination, on foliar symptom development was tested using foliar disc assays. Foliar discs (diameter: 1 cm) were obtained from leaves of cv Chardonnay and Cabernet-Sauvignon using a cork-borer and put in aqueous solution containing single (Pch or Apu) or coupled (Pch+Apu) EPSs at 50, 250 and 500 µg/mL each. The controls comprised water without any EPS and, as a positive control, water with mellein (same concentrations), which is a secondary metabolite with ascertained phytotoxic effect on grapevine leaves. In each of these conditions three discs were used. The foliar discs were observed at 24, 48 and 96h for necrosis development. To obtain quantitative phytotoxicity data, image analysis was conducted on photographs of the foliar discs, using Fiji software (Schindelin *et al.*, 2012). The images were converted to black and white, processing only red and yellow pixels (reflecting on necrosis and discoloration) and omitting the green ones. Afterwards, pixel density was determined.

7. Statistical analysis

Significances of differences were determined by ANOVA analysis with Tukey post-test, while correlations were assessed by applying Spearman's test. In both tests, results with $p < 0.05$ were considered to be significant.

RESULTS

1. The presence of *A. pullulans* in the endophytic microbiome is linked to increased severity of foliar symptoms on Esca-affected grapevines

A total of 43 Esca-symptomatic grapevines was sampled to evaluate their endophytic fungal population. Apu and Pch were isolated at frequencies of 16 % and 14 % respectively. Interestingly, Apu seemed to be associated with vines with higher (46-81 %) foliar symptom severity (Figure 1A), and in accordance with this observation, grapevines with isolated Apu showed significantly higher severity values (Figure 1B).

The measurement of Apu DNA by qRT-PCR showed a significantly increased value in the diseased plants (Figure 2A). This increase was due to the occurrence of high values in the diseased plants rather than a general increase in Esca-affected ones. Moreover, the severity of the foliar symptoms showed a correlation with Apu DNA content according to Spearman's test ($p < 0.05$) in diseased plants (Figure 2B).

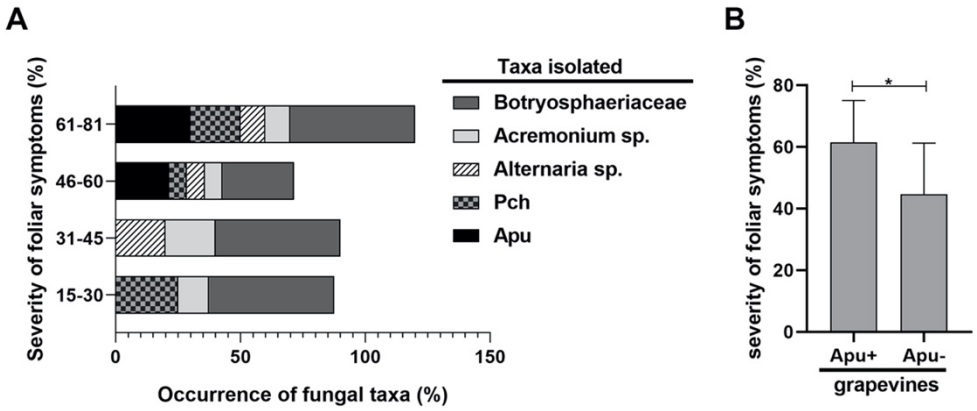


FIGURE 1. Correlation between Apu abundance and severity of Esca foliar symptoms based on fungal isolation.

A) Isolation frequency of most abundant fungal taxa in Cabernet-Sauvignon grapevines with different severity of Esca foliar symptoms in field conditions. B) Severity of Esca foliar symptoms on Cabernet-Sauvignon grapevines in field conditions in which Apu was present (Apu+) or absent (Apu-). Asterisks indicate the significance of difference (* $p < 0.05$). Statistical analysis was done by ANOVA test.

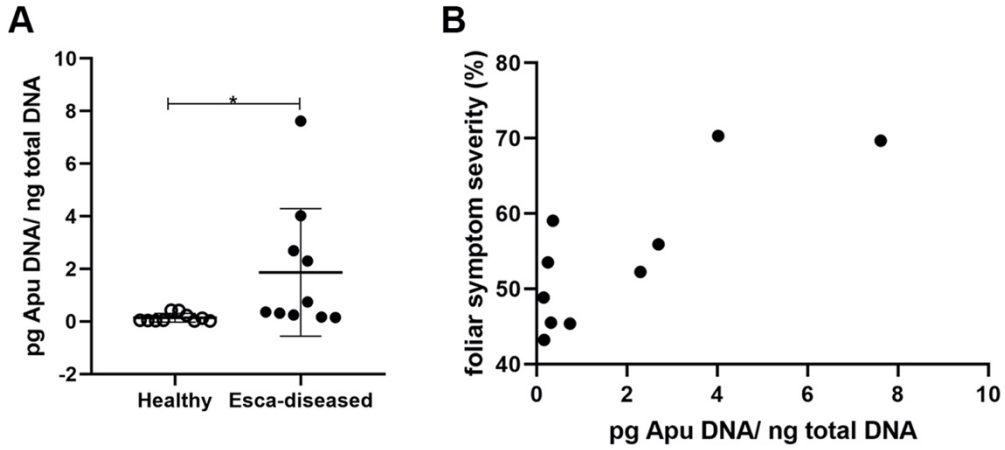


FIGURE 2. Abundance of Apu in healthy and Esca-diseased grapevines in correlation with the severity of foliar symptoms based on DNA quantification.

A) Amount of Apu DNA in healthy and Esca-affected grapevines, and B) severity of foliar symptoms plotted against Apu DNA content in Esca-affected plants. Asterisk indicates the significance of difference (*$p < 0.05$). Statistical analysis was done by ANOVA test.

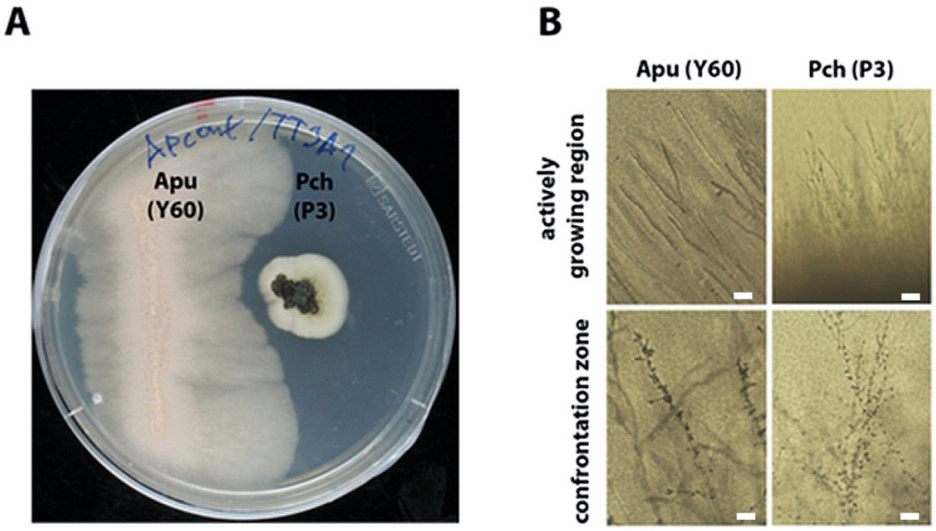


FIGURE 3. Effects of *in vitro* interaction between Y60 Apu and P3 Pch strains at the macro- and microscopic level.

A) Confrontation test with Y60 and P3 on PDA medium at 25 °C and 15 dpi. B) Microscopic examination (100 × magnification) of mycelia of Y60 and P3 in the confrontation zone and in actively growing regions. Scalebars represent 20 µm.

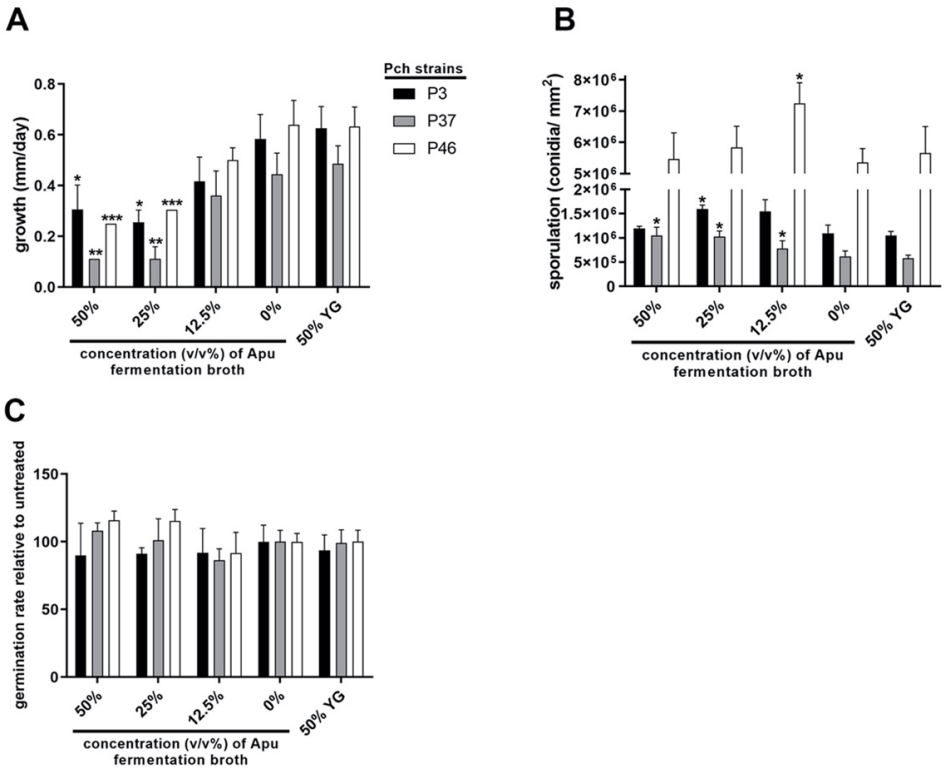


FIGURE 4. Effects of Apu secreted molecules on Pch development.

Growth rate (A), sporulation (B) and germination rate (C) of P3, P37 and P46 Pch strains growing on PDA medium supplemented with different concentrations of the Apu culture filtrate Y60 strain or with 50 % v/v YG medium. Asterisks mark significant differences (ANOVA test) relative to the untreated (0%) controls. *p < 0.05; **p < 0.005; ***p < 0.0005.

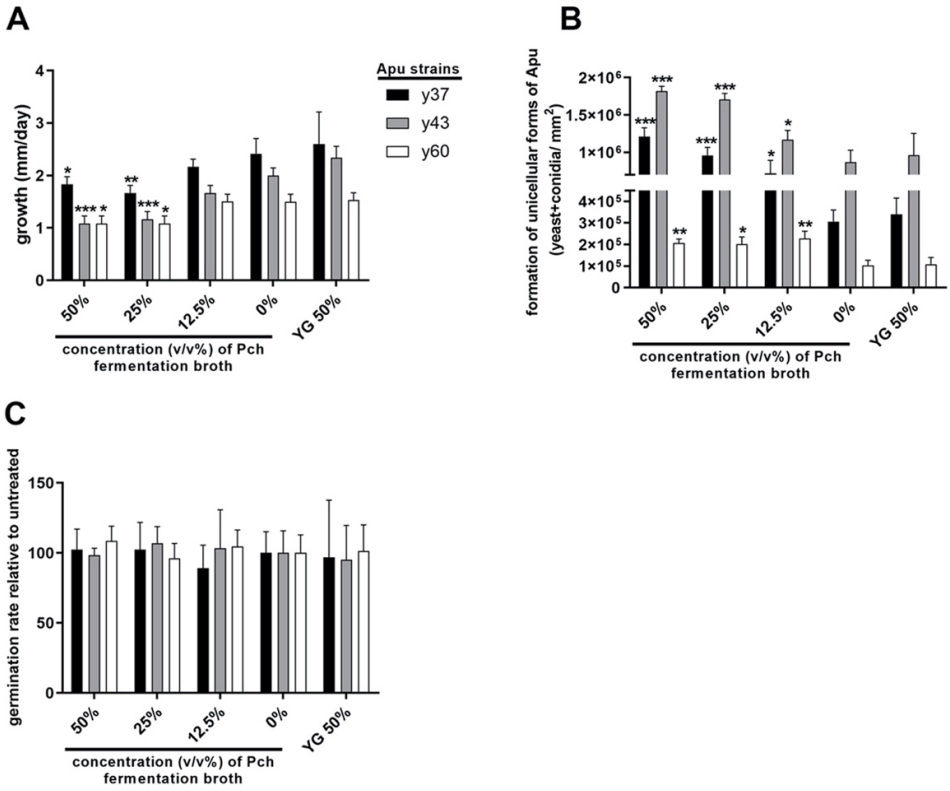


FIGURE 5. Effects of Pch secreted molecules on Apu development.

Growth rate (A), formation of yeast and conidia (B) and germination rate (C) of Y37, Y43 and Y60 Apu strains growing on PDA medium supplemented with different concentrations of the Pch strain P3 culture filtrate or with 50 %v/v YG medium. Asterisks mark significant differences (ANOVA test) relative to the untreated (0 %) controls. *p < 0.05; **p < 0.005; ***p < 0.0005.

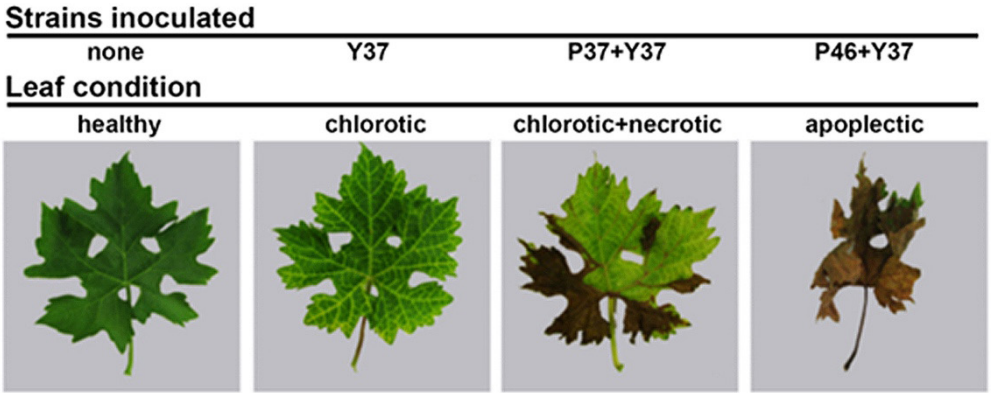


FIGURE 6. Leaf damages observed on shoots inoculated with Pch and/or Apu strains.

Representative photographs of leaves of Cabernet-Sauvignon shoot sections inoculated with Pch and/or Apu mycelia, incubated in water and examined at 15 dpi.

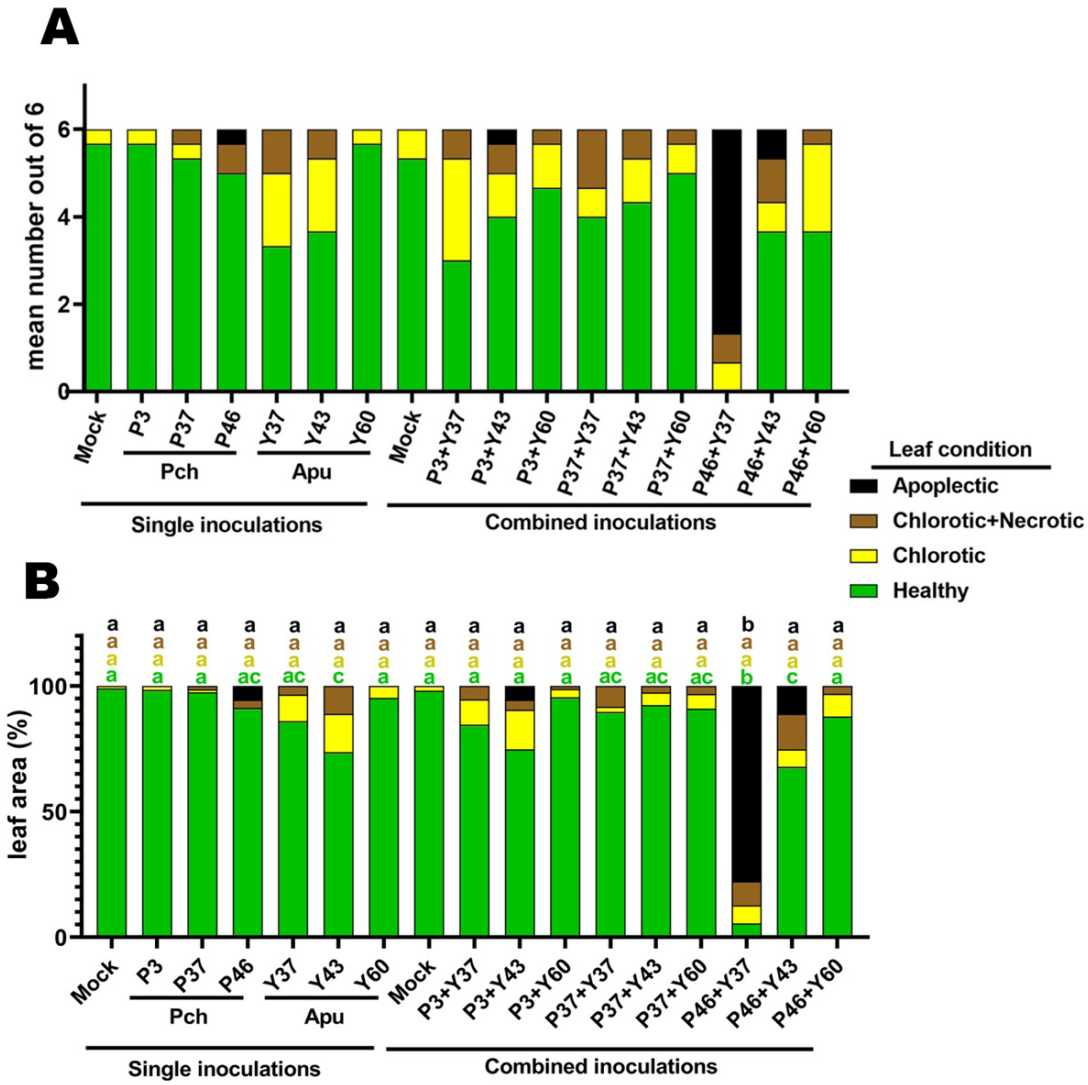


FIGURE 7. Effects of Apu and Pch on leaf damages depending on the test conditions.

Cabernet-Sauvignon shoot sections inoculated with Pch and/or Apu mycelia, incubated in water and examined at 15 dpi. A) Average number and kind of damaged leaves in different conditions out of 6 tested plants in three artificial infection experiments. B) Average leaf area with different kinds of symptoms. Letters mark significantly differing ($p < 0.05$, ANOVA with Tukey post-test) datasets for the same foliar health status. The colour of letters reflects the analysed health status.

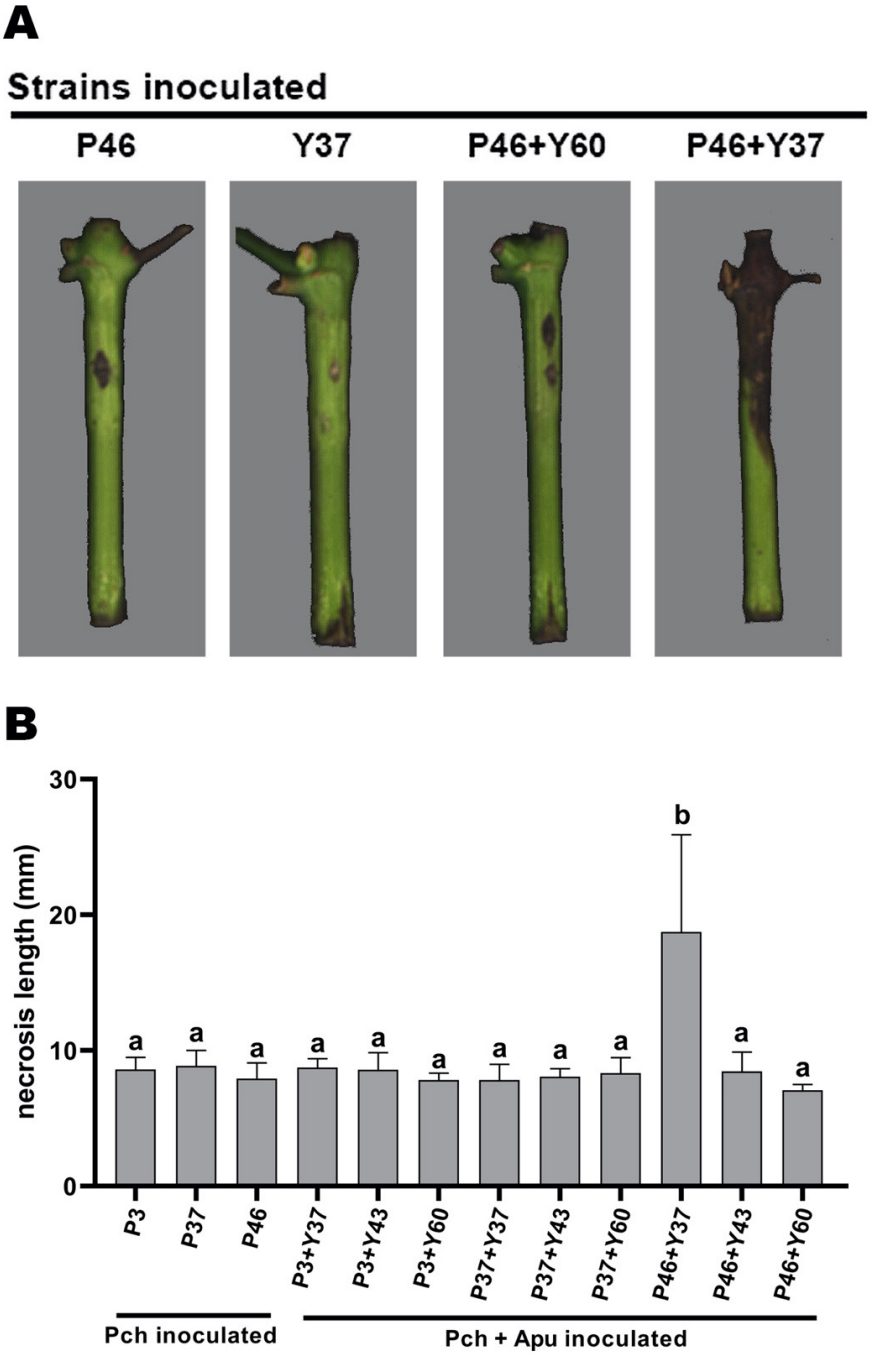


FIGURE 8. Effects of Apu and Pch strains on the development of necrosis on green canes.

Cabernet-Sauvignon shoot sections were inoculated with Pch and/or Apu mycelia, incubated in water and photographed at 15 dpi. A) Representative photographs of different effects of inoculated fungi on canes. B) Mean values and standard deviations of necrosis length on canes inoculated with Pch strains only, or in combination with Apu strains. Letters mark significantly differing ($p < 0.05$, ANOVA with Tukey post-test) datasets.

2. *In vitro* interaction of Apu with Pch and Phm, causal agents of Esca disease

Antagonistic activity of Phm against Apu was observed in the *in vitro* confrontation tests. While the growth of all Phm isolates was stimulated by the Apu colonies, the latter showed death of cells (condensation of cytoplasm and outflow at the mycelial tips) in the confrontation zones. Because of this inhibition of Apu growth by Phm (not related to the observed Apu disease-promoting effect), the interactions between these fungi were excluded from further analysis. In the Apu-Pch confrontation tests, both species showed a decreasing growth rate close to the confrontation zone (Figure 3A). However, no signs of cell death (e.g., cytoplasm condensation and cytoplasm outflow) were detected at the microscopic level. By contrast, all the tested Apu and Pch strains showed increased sporulation close to the confrontation areas (Figure 3B).

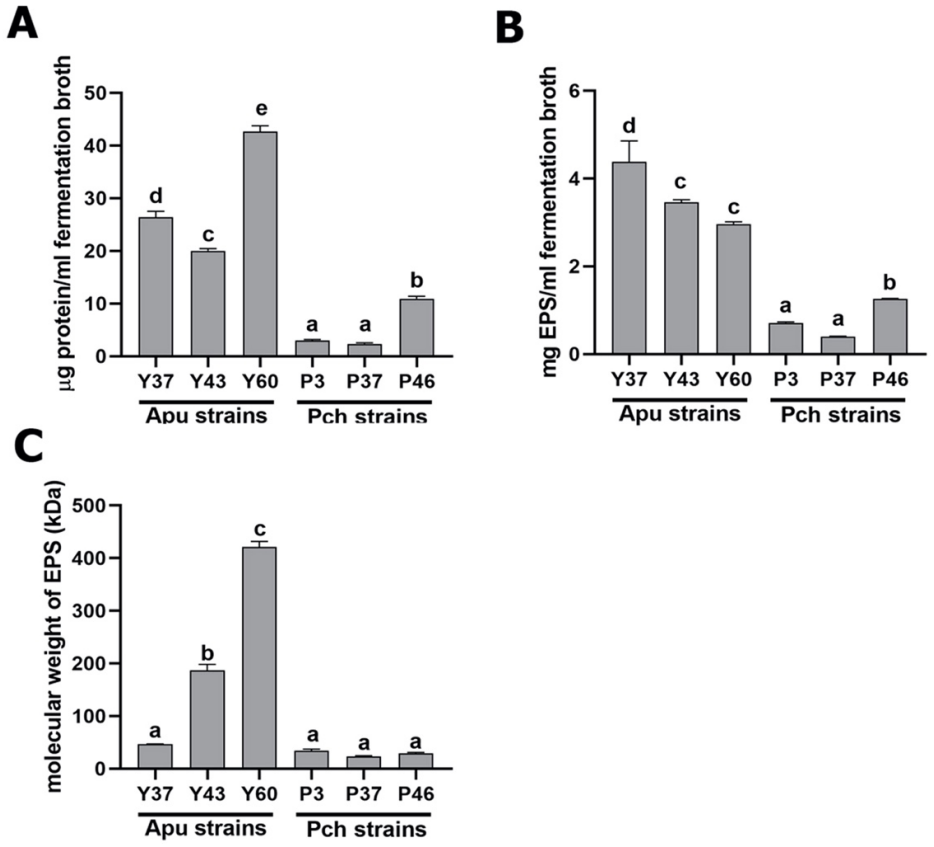


FIGURE 9. Comparison of protein and EPS secretion from Apu and Pch strains.

Production of secreted proteins (A), EPS (B) and the average molecular weight of secreted EPS (C) in case of Apu (Y37, Y43, Y60) and Pch strains (P3, P37, P46) in liquid cultures (YG medium, 25 °C, 7 days). Significantly differing ($p < 0.05$) values are represented by different letters. Statistical analysis was done by ANOVA with Tukey post-test.

For further investigation of the interaction between Apu and Pch, three isolates of each species were selected (Supplementary Table S2). The Pch strains formed white colonies when cultured on PDA plates at 25 °C in the dark, and showed different rates of greenish melanisation when grown at room temperature (21 °C) in ambient light, P37 having the lowest pigmentation. Differences were also observed in the sporulation of Pch strains with the highest value for P46, followed by P3 and P37. All the strains produced unicellular ellipsoidal conidia. Not only did the observed morphological characteristics agree with the description of Pch (Gams and Crous, 2000), but the ITS sequences were highly similar to the CBS 229.95 ex-type strain. All the Apu strains formed white-pinkish colonies with even margins on PDA medium at 25 °C. After two weeks of incubation at room temperature in ambient light conditions, strong black melanisation was observed in Y60, and a lesser pigmentation in Y43, while Y37 showed brownish coloration after about four weeks. Microscopic examinations revealed hyaline mycelia and the presence of yeast forms, conidia and chlamydospores in agreement with the general characteristic of the species (Ciferri *et al.*, 1957). The ITS sequences of all Apu isolates showed a high degree of similarity to the CBS 584.75 ex-type strain. Morphological characteristics of the selected Apu and Pch strains showed considerable differences, suggesting that they represent both physiological and genetical variants of the same species.

3. Study of the crossed antibiosis between Apu and Pch using culture filtrates

The Y60 Apu culture filtrate reduced the growth rate and increased the sporulation of the tested Pch strains without inhibiting their germination (Figures 4A, B, C). At values of between 12.5 and 50 % v/v concentrations of Apu culture filtrate, the number of Pch conidia significantly increased (Figure 4B).

The same effects were observed for Apu strains growing on medium supplemented with the culture filtrate of Pch strain P3. The Apu germination rate was not affected (Figure 5C) while the growth decreased (Figure 5A) and the production of conidia and yeast cells increased (Figure 5B) in the presence of P3 Pch culture filtrate.

4. Effects of co-inoculation of Apu and Pch on green canes of grapevine

After 15 dpi, the leaves on the artificially-infected green canes showed leaf damages (Figure 6) that differed in terms of severity and type depending on the inoculated strain (Figure 7). No or very slight symptoms were observed on mock-inoculated canes, which remained mostly asymptomatic (Figure 7A).

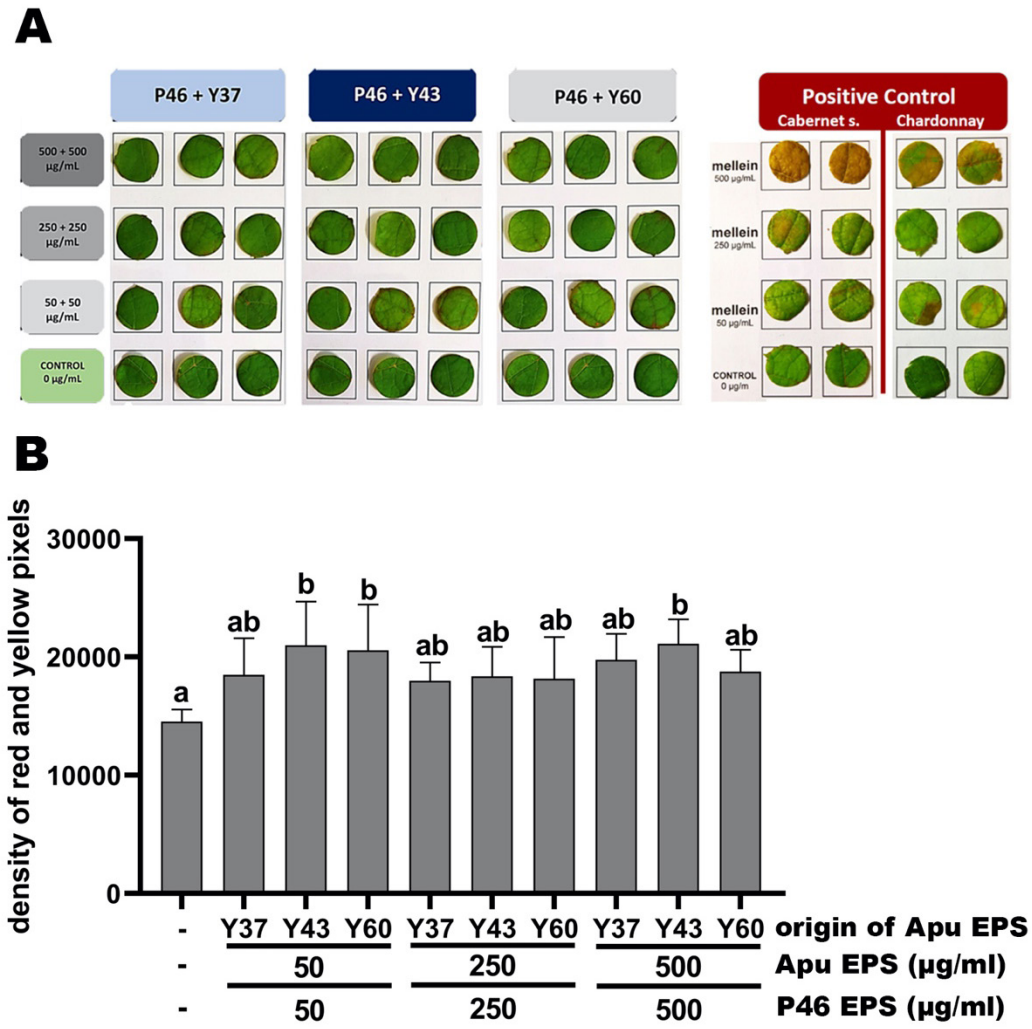


FIGURE 10. Toxicity of combined EPS from Pch and Apu on grapevine leaf discs.

A) Left panel, effects of EPS from P46 Pch strain combined with the EPS from Y37, Y43 and Y60 Apu strains (in concentrations of 0, 50, 250 and 500 µg/ml) on Cabernet-Sauvignon leaf discs after 48 h incubation. Right panel, effects of mellein (in concentrations of 0, 50, 250 and 500 µg/ml) on Cabernet-Sauvignon or Chardonnay leaf discs after 48 h incubation. B) Relative red and yellow pixel densities (reflecting to foliar damage) of control and EPS-treated foliar disks from panel A. Significantly differing ($p < 0.05$) values were labelled by different letters. Statistical analysis was done by ANOVA with Tukey post-test.

Two out of the three inoculated Apu strains (Y37, Y43) caused mild damage, significantly reducing the healthy leaf area in case of Y43 (Figure 7B). Both the occurrence and severity of foliar damage increased in the Apu+Pch inoculated canes in a strain-dependent manner. The highest severity was observed in the Y37 Apu and P46 Pch co-inoculation (Figure 7B), which resulted in the death of most of the leaves. A significant decline was also observed in the shoots inoculated with Y43 Apu and P46 Pch strains, with uniform chlorotic, necrotic and apoplectic areas appearing (Figure 7B).

In single inoculated canes, all Pch strains and none of the Apu strains caused necrosis at the inoculation point. Interestingly, canes with apoplectic leaves in combined Y37 Apu and P46 Pch inoculations showed massive necrosis (Figure 8A), resulting in significantly higher necrosis length in this inoculation than in the other groups (Figure 8B).

The results of the artificial infections agree with the hypothesis that Apu could promote the development of foliar symptoms of Esca disease, highlighting the strain-dependent nature of this interaction. Moreover, it seems that Apu may also contribute to the development of local vascular necrosis, as was seen in the case of Y37 Apu and P46 Pch combined inoculations.

5. Production of potential virulence factors by Apu and Pch strains

The secretion of proteins by the Apu strains showed significant differences among strains. Y37, Y43 and Y60 produced 26.4, 20, and 42.7 µg/ml respectively (Figure 9A). Of the Pch strains, P46 was the greatest protein producer, with 11 µg/ml, while P3 and P37 secreted 3 and 2.3 µg/ml of proteins respectively (Figure 9A).

The EPS produced by Apu strains were greater than Pch ones (Figure 9B). Y37, Y43 and Y60 Apu strains secreted 4.4, 3.5 and 3.0 mg/ml EPS, while P3, P37 and P46 Pch strains secreted only 0.7, 0.4 and 1.3 mg/ml.

The average molecular weight of EPS produced by the examined fungal strains was calculated as the rate of measured EPS mass, and the reducing sugar molarity was expressed as a glucose equivalent. Differences between the tested strains were observed in this aspect (Figure 9C). Of the Apu strains, Y60 produced EPS with the highest molecular weight (183 kDa) followed by Y43 (84.3 kDa) and Y37 (33.4 kDa). In the Pch strains, the highest EPS molecular weight was measured in P46 (15.2 kDa), followed by P3 (11.2 kDa) and P37 (4.4 kDa).

6. Effect of Apu and Pch EPS on grapevine foliar discs

No necrotic areas were observed in any of the foliar discs treated with single EPS after 96 hours. By contrast, the combination of P46 EPS and Y37, Y43 and Y60 EPS induced edge necrosis on Cabernet-Sauvignon foliar discs after 48h (Figure 10A). No effect was observed on Chardonnay, while the positive control based on mellein content induced necrotic areas for both cultivars at all tested concentrations. The image analysis of the photographed foliar disks revealed higher red and yellow pixel densities on the EPS-treated samples than on the untreated control (Figure 10B), indicating the negative effect of fungal EPS on the leaves. This difference was significant when 50 µg/ml P46 EPS was combined with 50 or 500 µg/ml EPS of Y43 Apu strain, or 50 µg/ml EPS of Y60 strain (Figure 10B).

DISCUSSION

In the present study, our results indicate that there is a positive relationship between the presence of Apu in the vine microbiome at the base of the analysed canes and the severity of Esca disease foliar symptoms. This is a surprising result, since Apu is a well-characterised fungus to date with very limited information on its phytopathogenicity. Apu has been reported to be a pathogen on some hosts and to cause spot blight on pear (Xie *et al.*, 2022), anthracnose on *Paeonia suffruticosa* (Lee *et al.*, 2019), and stem and fruit spot on pitaya (Wu *et al.*, 2017). Apu is also a rare but possible causal agent of post-harvest rot of fruits, like sweet cherries (Kim, 2014), pears (Dobra *et al.*, 2014), or even grapes (Lorenzini and Zapparoli, 2015). In addition to cultivated plants, Apu has also showed pathogenicity against some weeds (Prashanthi and Kulkarni, 2005; Guo *et al.*, 2020). It is worth noting that all the abovementioned diseases are the result of the infection of green and annual plant parts, unlike in the case of GTDs. Despite its low relevance as a phytopathogen, Apu is considered to have potential as a biocontrol agent against plant pathogenic fungi (Prasongsuk *et al.*, 2018). Data on the occurrence of Apu in Esca-affected grapevines are very controversial. Some studies did not find relevant incidences of Apu in symptomatic grapevines (Bruez *et al.*, 2014), others reported similar incidences in both symptomatic and asymptomatic plants (Hofstetter *et al.*, 2012) or considered its presence as typical on healthy wood in symptomatic plants (Rusjan *et al.*, 2017). To date, the only known relationship between Apu and Esca pathogens is the production of pullulan. This non-toxic and widely used polysaccharide (in pharmaceutical and food industries) is believed to participate in the pathogenesis of Esca disease (Bruno *et al.*, 2007; Graniti *et al.*, 2000b). Some researchers suggest that pullulan blocks the water and nutrient flows in grapevine by obstructing the vascular tissues, but this theory needs to be validated.

Regarding the possible role of Apu in Esca disease expression, two possible modes of action were examined: i) the direct interaction of Apu with the Esca pathogens, increasing their virulence in grapevine; and ii) the production of effector molecules (e.g. pullulan) by Apu that may contribute, in synergy with Esca pathogens effectors, to increasing the severity of the symptoms.

In the *in vitro* confrontation tests, Pch and Apu mutually inhibited each other's growth, increased sporulation and did not secrete molecules that were toxic to one another. In addition to the direct confrontation tests, these results were further confirmed in the culture filtrate tests. For these latter experiments, three strains of Apu and Pch fungal species were chosen from our collection. The characterisation of these strains in terms of traits like growth rate, sporulation and pigmentation suggests that they represent different pheno- and genotypes of their species. This allowed us to identify some strain-dependent differences, as well as possible general attributes of these fungal species. It seems that these two fungi can mutually modulate each other's development causing a shift to the stationary phase, possibly resulting in an altered metabolism. This may be achieved by the so called "quorum sensing" mechanism, which allows both bacteria (Miller and Bassler, 2001) and fungi (Albuquerque and Casadevall, 2012) to change their development and metabolism according to their cell density. The biosynthesis of fungal phytotoxins (as secondary metabolites) generally occurs during the stationary growth phase (Calvo *et al.*, 2002); therefore, the modulatory effect of Apu on Pch development may enhance the pathogenicity of the latter. In contrast to Pch, Phm greatly inhibited Apu growth. Due to the molecules it secretes, Phm is also able to kill Apu cells, while the latter have no effect on Phm.

Our artificial co-inoculations allowed us to study possible interactions between Pch and Apu that affect the severity of Esca disease. As expected, in the artificial inoculations, Pch strains alone developed necrosis at the inoculation point, but neither foliar symptoms nor damages. Apu strains did not cause necrosis at the inoculation point or any foliar symptoms, except for the Y37 and Y43 strains, which caused mild foliar symptoms (partial necrosis and chlorosis) in about 50 % of inoculated plants. This result suggests that, depending on the strain, Apu can cause damage to grapevine leaves. The discolorations on Apu-treated leaves were yellow, suggesting a decrease in chlorophyll content, rather than the stress response of the plants that results in an accumulation

of red anthocyanine pigments (Kaur *et al.*, 2022). This phenomenon is also in accordance with the decreased chlorophyll content in Esca-affected leaves reported previously (Petit *et al.*, 2006). The Apu/Pch co-inoculation revealed other pathogenesis-related effects. The Apu strains Y43 and Y60, in combination with all the tested Pch strains, showed the same symptoms as single Apu inoculations (chlorosis and partial necrosis for Y43; no symptoms for Y60) with the exception of Y43/P46 co-inoculations, which caused low but significant foliar damage. In Y37/P46 co-inoculations, about 80 % of shoots were apoplectic, with a significant necrosis of both leaves and canes. These results suggest that the hypothesised effect of the Apu/Pch interaction on Esca foliar and vascular symptom expression could be strain-dependent. The possible strain-specificity of the Apu/Pch interaction was evaluated by studying the phytotoxicity of EPS. Phytotoxicity tests with leaf discs showed that the Apu and Pch EPS fractions are not toxic at the concentrations tested. Only the combination of the Apu EPS+P46 EPS determined the development of necrosis on the leaf discs. This result suggests that some molecules in EPS derived from Apu and Pch strains may interact synergistically to damage grapevine leaves. The phytotoxicity of the combined EPS of the two species further reinforces the importance of pullulan in Esca disease development and may explain the contribution of Apu to this process (Bruno *et al*., 2007; Graniti *et al.*, 2000b).

The production of extracellular polysaccharides and proteins by Apu and Pch strains in liquid cultures was also investigated. The characteristic feature of the Y37 strain among the tested Apu strains was that it produced the highest amount of EPS and had the lowest average molecular weight. The latter trait may facilitate the transport of Y37-derived EPS from the wood to the leaves, allowing it to cause foliar symptoms. The negative correlation between the molecular weight of a microbial EPS and its ability to affect a plant host has already been reported in a previous study (Zou *et al.*, 2018). Of the Pch strains, P46 produced the highest amount of extracellular proteins, about 4 times more than both the P3 and P37 strains. Since we know that the secreted proteins of Pch are involved in Esca symptom expression (Luini *et al.*, 2010), the latter result may explain the high pathogenicity of P46 among the examined Pch strains. These results suggest that the secreted polysaccharides of Apu and the secreted proteins of Pch are responsible for the severe foliar damage observed in the Y37-P46 co-inoculations. To confirm the role of these molecules in the development of foliar symptoms, further experiments should be carried out by measuring their phytotoxicity before and after specific enzymatic decomposition. This should be followed by the identification of individual phytotoxic proteins and polysaccharides and their subsequent characterisation.

Our surprising finding that Apu may promote Esca disease development is not unprecedented. Regarding their complex nature, GTDs are believed to be partly the result of microbial interactions in the vine microbiome. The interactions of known GTD pathogens have been previously discussed in several studies. Possibe interactions between the Esca pathogens Pch, Phm, and Fmed (Bruno and Sparapano, 2006b; Sparapano *et al*., 2001) were examined in great detail, while the disease-promoting effects of non-Esca GTD pathogens on Esca pathogenesis have also been suggested (Martín *et al.*, 2022). Recent have suggested that microorganisms that frequently occur in grapevine without remarkable pathogenicity also contribute to GTD pathogenesis. DNA-based analyses of the grapevine microbiome have found a significantly high abundance of the fungus *Acremonium alternatum* (Bekris *et al.*, 2021), as well as the bacterial taxa Xanthomonadales, Rhizobiales and Enterobacteriales (Bruez *et al.*, 2015), in symptomatic plants. While disease-promoting interactions have been suggested by several of the above studies, only a limited number of papers have focused on the validation and characterisation of such interactions: Haidar *et al*. (2021) confirmed that the synergistic interaction between a *Paenibacillus sp.* isolate and Fmed was associated with the degradation of grapevine wood in *in vitro* experiments; in addition, a synergistic interaction between the virulent GTD pathogen *Neofusicoccum parvum* and the bacterial taxa *Bacillus pumilus* and *Xanthomonas* sp. was found to be linked to the development of vascular necrosis in artificially inoculated grapevine cuttings (Haidar *et al.*, 2016).

CONCLUSIONS

According to the results of this study, even fungal species, which are generally considered to be beneficial members of the plant microbiome, may promote the pathogenesis of Esca disease. The fungal species Apu showed a strain-dependent disease-promoting effect, probably based on the secretion of polysaccharides. The strain-dependence of pathogenesis in the case of Pch strains suggests that the proteins secreted by this fungus may also play a role in symptom development. In addition to the possible synergistic action of Pch proteins and Apu polysaccharides, direct interaction between these two fungi may also contribute to the development of Esca disease.

ACKNOWLEDGMENTS

This research was supported by the National Research Development and Innovation Office (NKFIH) through the OTKA Grant K-143453. It was also supported by project TÉT-16-1-2016-0131, PHC Balaton programme 3857XJ, by the academic Chaire supervised by Pr Fontaine Florence and by Grand Reims and the University of Reims Champagne-Ardenne. The authors would like to thank Eliane Abou-Mansour for the production of mellein.

REFERENCES

Abou-Mansour, E., Débieux, J., Ramírez-Suero, M., Bénard-Gellon, M., Magnin-Robert, M., Spagnolo, A., Chong, J., Farine, S., Bertsch, C., L'Haridon, F., Serrano, M., Fontaine, F., Rego, C., & Larignon, P. (2015). Phytotoxic metabolites from *Neofusicoccum parvum*, a

pathogen of Botryosphaeria dieback of grapevine. *Phytochemistry*, 115, 207–215. https://doi.org/10.1016/j.phytochem.2015.01.012

Albuquerque, P., & Casadevall, A. (2012). Quorum sensing in fungi–a review. *Medical Mycology*, 50(4), 337-345. https://doi.org/10.3109/13693786.2011.652201

Andolfi, A., Mugnai, L., Luque, J., Surico, G., Cimmino, A., & Evidente, A. (2011). Phytotoxins produced by fungi associated with grapevine trunk diseases. *Toxins*, 3(12), 1569-1605. https://doi.org/10.3390/toxins3121569

Barata, A., Malfeito-Ferreira, M., & Loureiro, V. (2012). The microbial ecology of wine grape berries. *International Journal of Food Microbiology*, 153(3), 243–259. https://doi.org/10.1016/j.ijfoodmicro.2011.11.025

Bekris, F., Vasileiadis, S., Papadopoulou, E., Samaras, A., Testempasis, S., Gkizi, D., ... & Karpouzas, D. G. (2021). Grapevine wood microbiome analysis identifies key fungal pathogens and potential interactions with the bacterial community implicated in grapevine trunk disease appearance. *Environmental Microbiome*, 16(1), 1-17. https://doi.org/10.1186/s40793-021-00390-1

Bertsch, C., Ramírez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Abou-Mansour, E., ... & Fontaine, F. (2013). Grapevine trunk diseases: complex and still poorly understood. *Plant Pathology*, 62(2), 243-265. https://doi.org/10.1111/j.1365-3059.2012.02674.x

Bettenfeld, P., Fontaine, F., Trouvelot, S., Fernandez, O., Courty, P.E. (2020). Woody plant declines. What's wrong with the microbiome? *Trends in Plant Science*, 25, 381-394. https://doi.org/10.1016/j.tplants.2019.12.024

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Bruez, E., Vallance, J., Gerbore, J., Lecomte, P., Da Costa, J. P., Guerin-Dubrana, L. (2014) Analyses of the temporal dynamics of fungal communities colonizing the healthy wood tissues of esca leaf-symptomatic and asymptomatic vines. *PLoS ONE*, *9*, e0095928. https://doi.org/10.1371/journal.pone.0095928.

Bruez, E., Haidar, R., Alou, M. T., Vallance, J., Bertsch, C., Mazet, F., ... & Rey, P. (2015). Bacteria in a wood fungal disease: characterization of bacterial communities in wood tissues of esca-foliar symptomatic and asymptomatic grapevines. *Frontiers in Microbiology*, 6, 1137. https://doi.org/10.3389/fmicb.2015.01137.

Bruno, G., & Sparapano, L. (2006a). Effects of three esca-associated fungi on Vitis vinifera L.: II. Characterization of biomolecules in xylem sap and leaves of healthy and diseased vines. Physiological and Molecular Plant Pathology, 69(4-6), 195-208. https://doi.org/10.1016/j.pmpp.2007.04.007

Bruno, G., & Sparapano, L. (2006b). Effects of three esca-associated fungi on Vitis vinifera L.: III. Enzymes produced by the pathogens and their role in fungus-to-plant or in fungus-to-fungus interactions. *Physiological and Molecular Plant Pathology*, 69(4-6), 182-194. https://doi.org/10.1016/j.pmpp.2007.04.006

Bruno, G., Sparapano, L., & Graniti, A. (2007). Effects of three esca-associated fungi on *Vitis vinifera* L.: IV. Diffusion through the xylem of metabolites produced by two tracheiphilous fungi in the woody tissue of grapevine leads to esca-like symptoms on leaves and berries. *Physiological and Molecular Plant Pathology*, 71(1-3), 106-124. https://doi.org/10.1016/j.pmpp.2007.12.004

Burruano, S., Giambra, S., Mondello, V., Greca, M. Della, Basso, S., Tuzi, A., & Andolfi, A. (2016). Naphthalenone polyketides produced by *Neofusicoccum parvum*, a fungus associated with grapevine Botryosphaeria dieback. *Phytopathologia Mediterranea*, 55(2), 197–206. https://doi.org/10.14601/Phytopathol_Mediterr-17393

Calvo, A. M., Wilson, R. A., Bok, J. W., & Keller, N. P. (2002). Relationship between secondary metabolism and fungal development. *Microbiology and Molecular Biology Reviews*, 66(3), 447-459. https://doi.org/10.1128/MMBR.66.3.447-459.2002

Ciferri, R., Ribaldi, M., & Corte, A. (1957). Revision of 23 strains of *Aureobasidium pullulans* (DE BY.) ARN.(~ *Pullularia pullulans*). *Istituto Botanico della r. Università. R. Laboratorio Crittogamico Pavia Atti*, 14, 78-90.

Claverie, M., Notaro, M., Fontaine, F., & Wery, J. (2020). Current knowledge on Grapevine Trunk Diseases with complex etiology: A systemic approach. *Phytopathologia Mediterranea*, 59, 29-53. https://doi.org/10.36253/phyto-11150

Cloete, M., Fischer, M., Mostert, L., & Halleen, F. (2014). A novel *Fomitiporia* species associated with esca on grapevine in South Africa. *Mycological Progress*, 13(2), 303-311. https://doi.org/10.1007/s11557-013-0915-5

Del Frari, G., Gobbi, A., Aggerbeck, M. R., Oliveira, H., Hansen, L.H., Ferreira, R. B. (2019). Characterization of the wood mycobiome of *Vitis vinifera* in a vineyard affected by esca. Spatial distribution of fungal communities and their putative relation with leaf symptoms. *Frontiers in Plant Science*, 10, 910. https://doi.org/10.3389/fpls.2019.00910

Dobra, A., Dussi, M. C., Diaz, K., & Sugar, D. (2014). First report of *Aureobasidium pullulans* as a postharvest disease of 'abate fetel' pears from an organic orchard in Rio Negro, Argentina. In XII International Pear Symposium 1094 (pp. 579-582).

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350-356. https://doi.org/10.1021/ac60111a017

Dygert, S., Li, L. H., Florida, D., & Thoma, J. A. (1965). Determination of reducing sugar with improved precision. *Analytical Biochemistry*, 13(3), 367-374. https://doi.org/10.1016/0003-2697(65)90327-1

Fischer, M. (2002). A new wood-decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (Hymenochaetales). *Mycological Progress*, 1(3), 315-324. https://doi.org/10.1007/s11557-006-0029-4

Fischer, M., & Peighami-Ashnaei, S. (2019). Grapevine, esca complex, and environment: The disease triangle. *Phytopathologia Mediterranea*, 58(1), 17-37. https://doi.org/10.14601/Phytopathol_Mediterr-25086

Forabosco, A., Bruno, G., Sparapano, L., Liut, G., Marino, D., & Delben, F. J. C. P. (2006). Pullulans produced by strains of *Cryphonectria parasitica*—I. Production and characterisation of the exopolysaccharides. *Carbohydrate Polymers*, 63(4), 535-544. https://doi.org/10.1016/j.carbpol.2005.10.005

Galli, V., Romboli, Y., Barbato, D., Mari, E., Venturi, M., Guerrini, S., & Granchi, L. (2021). Indigenous *Aureobasidium pullulans* strains as biocontrol agents of *Botrytis cinerea* on grape berries. *Sustainability*, 13(16), 9389. https://doi.org/10.3390/su13169389

Gams, W., & Crous, P. W. (2000). " *Phaeomoniella chlamydospora*" Gen. et Comb. Nov., a Causal organism of petri grapevine Decline and Esca. *Phytopathologia Mediterranea*, 39(1), 112-118. https://doi.org/10.14601/Phytopathol_Mediterr-1530

Gramaje, D., Urbez-Torres, J. R., & Sosnowski, M. R. (2018). Managing grapevine trunk diseases with respect to etiology and epidemiology: current strategies and future prospects. *Plant Disease*, 102(1), 12–39. https://doi.org/10.1094/PDIS-04-17-0512-FE

Graniti, A., Surico, G., & Mugnai, L. (2000a). Esca of grapevine: a disease complex or a complex of diseases? *Phytopathologia*

Mediterranea, 39(1), 16–20. https://doi.org/10.14601/Phytopathol_Mediterr-1539

Graniti, A., Bruno, G., &Sparapano, L. (2000b). Effects on plants of metabolites produced in culture by «*Phaeoacremonium chlamydosporum*», «*P. aleophilum*» and «*Fomitiporia punctata*». *Phytopathologia Mediterranea*, 39(1), 169-177. https://doi.org/10.14601/Phytopathol_Mediterr-1535

Guo, Q. Y., Cheng, L., Zhu, H. X., Wei, L. I., Wei, Y. H., Chen, H. Y., ... & Jian, W. A. N. G. (2020). Herbicidal activity of *Aureobasidium pullulans* PA-2 on weeds and optimization of its solid-state fermentation conditions. *Journal of Integrative Agriculture*, 19(1), 173-182. https://doi.org/10.1016/S2095-3119(19)62738-3

Haidar, R., Deschamps, A., Roudet, J., Calvo-Garrido, C., Bruez, E., Rey, P., & Fermaud, M. (2016). Multi-organ screening of efficient bacterial control agents against two major pathogens of grapevine. *Biological Control*, 92, 55-65.

Haidar, R., Yacoub, A., Vallance, J., Compant, S., Antonielli, L., Saad, A., ... & Rey, P. (2021). Bacteria associated with wood tissues of Esca-diseased grapevines: functional diversity and synergy with *Fomitiporia mediterranea* to degrade wood components. *Environmental Microbiology*, 23(10), 6104-6121. https://doi.org/10.1111/1462-2920.15676

Hofstetter, V., Buyck, B., Croll, D., Viret, O., Couloux, A., & Gindro, K. (2012). What if esca disease of grapevine were not a fungal disease? *Fungal Diversity*, 54(1), 51-67. https://doi.org/10.1007/s13225-012-0171-z

Kaur, S., Tiwari, V., Kumari, A., Chaudhary, E., Sharma, A., Ali, U., & Garg, M. (2022). Protective and defensive role of anthocyanins under plant abiotic and biotic stresses: An emerging application in sustainable agriculture. *Journal of Biotechnology*, 361, 12-29. https://doi.org/10.1016/j.jbiotec.2022.11.009

Kim, Y. K. (2014). First report of a new postharvest rot in sweet cherries caused by *Aureobasidium pullulans*. *Plant Disease*, 98(3), 424. https://doi.org/10.1094/PDIS-07-13-0740-PDN

Knapp, D. G., Lázár, A., Molnár, A., Vajna, B., Karácsony, Z., Váczy, K. Z., & Kovacs, G. M. (2021). Above-ground parts of white grapevine *Vitis vinifera* cv. Furmint share core members of the fungal microbiome. *Environmental Microbiology Reports*, 13(4), 509-520. https://doi.org/10.1111/1758-2229.12950

Lee, D. H., Cho, S. E., Park, J. H., Seo, S. T., Lee, S. H., & Lee, S. K. (2019). First report of *Aureobasidium pullulans* causing anthracnose on *Paeonia suffruticosa* in Korea. *Journal of Plant Pathology*, 101, 1255-1255. https://doi.org/10.1007/s42161-019-00315-5

Lorenzini, M., & Zapparoli, G. (2015). Occurrence and infection of *Cladosporium*, *Fusarium*, *Epicoccum* and *Aureobasidium* in withered rotten grapes during post-harvest dehydration. *Antonie Van Leeuwenhoek*, 108, 1171-1180. https://doi.org/10.1007/s10482-015-0570-8

Luini, E., Fleurat-Lessard, P., Rousseau, L., Roblin, G., & Berjeaud, J. M. (2010). Inhibitory effects of polypeptides secreted by the grapevine pathogens *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* on plant cell activities. *Physiological and Molecular Plant Pathology*, 74(5-6), 403-411. https://doi.org/10.1016/j.pmpp.2010.06.007

Martín, L., García-García, B., & Alguacil, M. D. M. (2022). Interactions of the Fungal Community in the Complex Patho-System of Esca, a Grapevine Trunk Disease. *International Journal of Molecular Sciences*, 23, 14726. https://doi.org/10.3390/ijms232314726

Martini, M., Musetti, R., Grisan, S., Polizzotto, R., Borselli, S., Pavan, F., & Osler, R. (2009). DNA-dependent detection of the grapevine fungal endophytes *Aureobasidium pullulans* and *Epicoccum nigrum*. *Plant Disease*, 93(10), 993-998. https://doi.org/10.1094/PDIS-93-10-0993

Miller, M. B., & Bassler, B. L. (2001). Quorum sensing in bacteria. *Annual review of microbiology*, 55(1), 165-199. https://doi.org/10.1146/annurev.micro.55.1.165

Mondello, V., Songy, A., Battiston, E., Pinto, C., Coppin, C., Trotel-Aziz, P., Clément, C., Mugnai, L., & Fontaine, F. (2018). Grapevine trunkdiseases: a review of fifteen years of trials for their control with chemicals and biocontrol agents. *Plant Disease*, 7, 1189–1217. http://doi.org/10.1094/PDIS-08-17-1181-FE

Mondello, V., Lemaître-Guillier, C., Trotel-Aziz, P., Gougeon, R., Acedo, A., Schmitt-Kopplin, P., ... & Fontaine, F. (2022). Assessment of a new copper-based formulation to control esca disease in field and study of its impact on the vine microbiome, vine physiology and enological parameters of the juice. *Journal of Fungi*, 8(2), 151. https://doi.org/10.3390/jof8020151

Moretti, S., Pacetti, A., Pierron, R., Kassemeyer, H. H., Fischer, M., Péros, J. P., ... & Farine, S. (2021). *Fomitiporia mediterranea* M. Fisch., the historical Esca agent: a comprehensive review on the main grapevine wood rot agent in Europe. *Phytopathologia Mediterranea*, 60(2), 351-379. https://doi.org/10.36253/phyto-13021

Mugnai, L., Graniti, A., & Surico, G. (1999). Esca (black measles) and brown wood-streaking: two old and elusive diseases of grapevines. *Plant Disease*, 83(5), 404-418. https://doi.org/10.1094/PDIS.1999.83.5.404

Niem, J. M., Billones-Baaijens, R., Stodart, B., & Savocchia, S. (2020). Diversity profiling of grapevine microbial endosphere and antagonistic potential of endophytic *Pseudomonas* against grapevine trunk diseases. *Frontiers in Microbiology*, 11, 477. https://doi.org/10.3389/fmicb.2020.00477

Petit, A. N., Vaillant, N., Boulay, M., Clément, C., & Fontaine, F. (2006). Alteration of photosynthesis in grapevines affected by esca. *Phytopathology*, 96(10), 1060-1066. https://doi.org/10.1094/PHYTO-96-1060

Pinto, C., Custódio, V., Nunes, M., Songy, A., Rabenoelina, F., Courteaux, B., ... & Fontaine, F. (2018). Understand the potential role of *Aureobasidium pullulans*, a resident microorganism from grapevine, to prevent the infection caused by *Diplodia seriata*. *Frontiers in Microbiology*, 9, 3047. https://doi.org/10.3389/fmicb.2018.03047

Prashanthi, S. K., & Kulkarni, S. (2005). *Aureobasidium pullulans*, a potential mycoherbicide for biocontrol of eupatorium [*Chromolaena odorata* (L.) King and Robinson] weed. *Current Science*, 88(1), 18-21.

Prasongsuk, S., Lotrakul, P., Ali, I., Bankeeree, W., & Punnapayak, H. (2018). The current status of *Aureobasidium pullulans* in biotechnology. *Folia Microbiologica*, 63(2), 129-140. https://doi.org/10.1007/s12223-017-0561-4

Rathnayake, R. M. S. P., Savocchia, S., Schmidtke, L. M., & Steel, C. C. (2018). Characterisation of *Aureobasidium pullulans* isolates from *Vitis vinifera* and potential biocontrol activity for the management of bitter rot of grapes. *European Journal of Plant Pathology*, 151(3), 593-611. https://doi.org/10.1007/s10658-017-1397-0

Rusjan, D., Persic, M., Likar, M., Biniari, K., & Mikulic-Petkovsek, M. (2017). Phenolic responses to esca-associated fungi in differently decayed grapevine woods from different trunk parts of 'Cabernet-Sauvignon'. *Journal of Agricultural and Food Chemistry*, 65(31), 6615-6624. https://doi.org/10.1021/acs.jafc.7b02188

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682. https://doi.org/10.1038/nmeth.2019

Songy, A., Fernandez, O., Clément, C., Larignon, P., & Fontaine, F. (2019). Grapevine trunk diseases under thermal and water stresses. *Planta*, 249(6), 1655-1679. https://doi.org/10.1007/s00425-019-03111-8

Sparapano, L., Campanella, A., & Bruno, G. (2001). Interactions between three fungi associated with esca of grapevine, and their secondary metabolites. Interactions between Three Fungi Associated with Esca of Grapevine, and Their Secondary Metabolites, *Phytopathologia Mediterranea*, 40(3), 417-422. https://doi.org/10.14601/Phytopathol_Mediterr-1626

Surico, G., Mugnai, L. & Marchi, G. (2006). Older and more recent observations on esca: a critical overview. *Phytopathologia Mediterranea*, 45, 68-86. https://doi.org/10.14601/Phytopathol_Mediterr-1847

Surico, G. (2009). Towards a redefinition of the diseases within the esca complex of grapevine. *Phytopathologia Mediterranea*, 48(1), 5–10. https://doi.org/10.14601/Phytopathol_Mediterr-2870

Trotel-Aziz, P., Abou-Mansour, E., Courteaux, B., Rabenoelina, F., Clément, C., Aziz, A., & Fontaine, F. (2019). *Bacillus subtilis* PTA-271 counteracts Botryosphaeria dieback in grapevine, triggering immune responses and detoxification of fungal phytotoxins. *Frontiers in Plant Science*, 10, 25. https://doi.org/10.3389/fpls.2019.00025

Wu, J. B., Zhan, R. L., Liu, F., & Cang, J. M. (2017). First report of a stem and fruit spot of pitaya caused by *Aureobasidium pullulans* in China. *Plant Disease*, 101(1), 249. https://doi.org/10.1094/PDIS-05-16-0702-PDN

Xie, Y., Fang, L., Mao, X., Wu, J., Wang, L., & Wang, H. (2022). First report of *Aureobasidium pullulans* var. pullulans causing spot blight of pear (*Pyrus pyrifolia*) in Zhejiang province of China. *Plant Disease*, 106(8), 2259. https://doi.org/10.1094/PDIS-10-21-2367-PDN

Zou, P., Lu, X., Jing, C., Yuan, Y., Lu, Y., Zhang, C., ... & Li, Y. (2018). Low-molecular-weightt polysaccharides from *Pyropia yezoensis* enhance tolerance of wheat seedlings (*Triticum aestivum* L.) to salt stress. *Frontiers in Plant Science*, 9, 427. https://doi.org/10.3389/fpls.2018.00427.