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Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California

Doctor of Sciences
in Life Sciences

Analyzing Mission grapevine of Baja California, its association with biological control agents, and its role as reservoir for trunk diseases fungi

A dissertation
submitted in partial satisfaction of the requirements for the degree
Doctor in Sciences

By:

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Resumen de la tesis que presenta **Carmen Sanjuana Delgado Ramírez** como requisito parcial para la obtención del grado de Doctor en Ciencias en Ciencias de la Vida.

Analizando las vides Misión de Baja California, su asociación con agentes de control biológico y su papel como reservorio de hongos patógenos de madera

Resumen aprobado por:

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En Baja California la vid es uno de los cultivos perennes más importantes, se estima hay cerca de 4,702.93 ha de viñedos establecidos con más de 30 cultivares. En el estado las primeras vides fueron traídas por los españoles en 1669, el cultivar introducido fue 'Listán Prieto,' conocido como 'Misión.' Actualmente existe interés en aprovechar este cultivar, debido a las características de su vino. Sin embargo, en estas vides se han observado síntomas asociados con de enfermedades de la madera, lo cual podría afectar la reproducción generalizada. Además, dado que las vides 'Misión' se han mantenido establecidas en condiciones de aridez es probable que las plantas mantengan una interacción con microorganismos benéficos que esten influyendo en su capacidad para resistir las condiciones adversas en las que crecen. El objetivo de este trabajo fue investigar la presencia de hongos causantes de enfermedades de la madera asociados con la vid 'Misión' e identificar microorganismos endófitos con potencial para usarse como agentes de control biológico para controlar hongos causantes de enfermedades de la madera de vid en viñedos comerciales. Inicialmente se colectaron muestras de vides 'Misión' de viñedos de Baja California. A partir de las muestras analizadas se obtuvieron 78 hongos y 135 bacterias. De los aislados fúngicos se seleccionaron 13, los cuales se identificaron molecular y morfológicamente como *Diaporthe ampelina* y *Diplodia seriata,* siendo esta última la especie más prevalente. Todas las cepas tuvieron temperaturas óptimas de crecimiento entre 25 y 28°C. En cuanto a la patogenicidad, la cepa de *D. ampelina* presentó niveles de virulencia intermedia, mientras que las cepas de *D. seriata* mostraron baja virulencia. Para identificar agentes de biocontrol, se determinó la actividad antagónica cualitativa de aislados obtenidos de vides 'Misión', así como de 35 cepas de *Trichoderma* aisladas de vid sobre el hongo de madera *L. brasiliensis* MXBCL28. A partir de este ensayo se seleccionaron once aislados los cuales se identificaron molecularmente como pertenecientes a los géneros *Bacillus* y *Trichoderma*. Las cepas identificadas molecularmente mostraron la capacidad de inhibir al hongo por competencia por espacio, la producción de compuestos volátiles y no volátiles, y adicionalmente las cepas de *Trichoderma* mostraron actividad de micoparasitismo. Al evaluar la actividad de biocontrol de once cepas sobre *L. brasiliensis* MXBCL28 en ensayos de invernadero y campo se observó que las plantas inoculadas con aislados de *Bacillus* y T*richoderma* mantuvieron un efecto inhibitorio sobre el hongo; ya que, las plantas inoculadas con estos microorganismos benéficos presentaron lesiones de menor tamaño con respecto a las plantas inoculadas solo con el hongo patógeno. Este trabajo proporciona información importante sobre el papel de las vides 'Misión' como reservorio de hongos de madera de vid y de agentes de biocontrol, lo cual permitirá apoyar decisiones futuras sobre la reproducción, gestión de estas vides en la región y sobre el manejo de enfermedades de la madera en viñedos comerciales.

Palabras clave: Vides 'Misión', hongos de madera, agentes de control biológico, control biológico, enfermedades de madera de vid

Abstract of the thesis presented **by Carmen Sanjuana Delgado Ramírez** as a partial requirement to obtain the Doctor of Sciences degree in Life Sciences

Analyzing Mission grapevine of Baja California, its association with biological control agents, and its role as a reservoir for trunk diseases fungi

Abstract approved by:

PhD. Rufina Hernádez Martínez
Co-Supervisor

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Co-Supervisor

The grapevine is one of the most important perennial crops in Baja California. There are about 4,702.93 ha. of established vineyards with over 30 cultivars. The first vines in the state were brought by the Spanish in 1669. The cultivar introduced was 'Listán Prieto,' known as 'Mission.' There is interest in taking advantage of this cultivar due to its wine's characteristics. However, symptoms associated with grapevine trunk diseases have been observed in these grapevines, which could affect general reproduction. In addition, since the 'Mission' grapevines have remained established in arid conditions, it is likely that the plants maintain an interaction with beneficial microorganisms that are influencing their ability to resist the adverse conditions in which they grow. This work aimed to investigate the presence of grapevine trunk diseases fungi associated with 'Mission' grapevine and identified endophytic microorganisms with the potential to use as biological control agents for controlling grapevine trunk diseases fungi in commercial vineyards. Initially, samples of 'Mission' grapevines were collected from Baja California vineyards. From the analyzed samples, 78 fungi and 135 bacteria were obtained. From the fungal isolates, 13 were selected, which were molecularly and morphologically identified as *Diaporthe ampelina* and *Diplodia seriata*, the latter being the most isolated species. The strains had optimal growth temperatures between 25 and 28°C. Regarding pathogenicity, it was observed that the *D. ampelina* strain has intermediate virulence levels, and the *D. seriata* strains have low virulence. To identify biocontrol agents, the antagonistic activity of all isolates obtained from 'Mission' grapevines, as well as of 35 *Trichoderma* strains isolated from grapevines on the fungus *L. brasiliensis* MXBCL28, was qualitatively determined. Eleven isolates were selected from these tests, which were molecularly identified as belonging to *Bacillus* and *Trichoderma* genera. The identified strains showed the ability to inhibit the fungus *L. brasiliensis* MXBCL28 through competition for space, by producing volatile and non-volatile compounds, and the *Trichoderma* strains also showed mycoparasitic activity. When evaluating the biocontrol activity of eleven strains on *L. brasiliensis* MXBCL28 in greenhouse and field trials, it was observed that plants inoculated with *Bacillus* and *Trichoderma* isolates maintained an inhibitory effect on the fungus, since plants inoculated with these beneficial microorganisms presented smaller lesions with respect to plants inoculated only with the pathogenic fungus. This work provides important information on the role of 'Mission' grapevines as a reservoir of grapevine wood fungi and biocontrol agents, which will support future decisions on the reproduction and management of these grapevines in the region and on the management of grapevine trunk diseases.

Keywords: 'Mission' grapevine, grapevine trunk fungi, biological control agents, biological control, grapevine trunk diseases

Dedication

For Angel and Valentina

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Chapter 1. Introduction

The grapevine (*Vitis vinifera*) is one of the most important perennial crops worldwide. Its fruit has a high commercial value and can be consumed fresh, as raisins, or used to produce wine, juices, and musts (Gramaje et al., 2018). During 2022, 7.3 million hectares of vineyards were established worldwide, with 50% concentrated in five countries: Spain (13.1%), France (11.2%), China (10.8%), Italy (9.9%), and Turkey (5.6%) (OIV, 2021; OIV 2022).

Mexico ranks 26th in grape production worldwide. The country's surface area of vineyards is around 36,861.48 ha, with the majority dedicated to table grape production. Sonora has the largest share of vineyards for table grape production (90.4%), followed by Zacatecas (6.6%), Aguascalientes (2.0%), and Baja California (1.4%) (SIAP, 2022b; SIAP, 2022c). In terms of grape production for industrial use, Zacatecas leads with the highest yields (38.13%), followed by Baja California (32.91%) and Aguascalientes (11.45%) (SIAP, 2022a; SIAP, 2022b). Raisin grape production is mainly concentrated in two states, Zacatecas (97.1%) and Baja California (2.9%) (SIAP, 2022b; SIAP, 2022c). At the national level, vine production creates around 500,000 jobs in agricultural areas and an additional 11,000 jobs, both directly and indirectly, making it the second-highest job-generating crop (SIAP, 2022a).

Baja California is one of the leading wine producers nationwide. This state is characterized by its arid climate, with maximum temperatures of 26.5°C, minimums of 13.4°C, and an annual rainfall of 99.1 mm. However, these climatic conditions have not limited grape production (SIAP, 2022b; SIAP, 2023). Currently, the state has 4,702.93 ha planted with grapevines, distributed in six regions: Guadalupe Valley, Tule Region, Ojos Negros Valley, Ejido Uruapan, Santo Tomás Valley, and San Vicente Valley (Gónzalez-Andrade and Fuentes-Flores, 2013; SIAP, 2023). More than 30 grapevine varieties are established in this area, with 'Cabernet Sauvignon, ' 'Merlot, ' Tempranillo, ' 'Chenin Blanc, ' 'Chardonnay, ' and 'Sauvignon Blanc' comprising the largest cultivated areas. Other cultivated varieties include 'Mission' and 'Rosa del Perú, ' also known as patrimonials (SEFOA, 2011; SIAP, 2022b).

In Mexico, the Spanish introduced the grapevine in the 16th century. Jesuit and Dominican friars disseminated grapevine cultivation in the country (Crowley, 1989). The introduced variety was Listan Prieto, now known as Mission in Mexico (López Franco, 2012; Góngora Rosado, 2016). This is a red variety considered rustic and semi-erect. Its cluster is funnel-shaped and compact, while its berries are spherical, medium size, and blue-black epidermis coloration (Figure 1) (Rodríguez-Torres, 2017; Magoni, 2021).

In Baja, California, vineyards currently established with 'Mission' cultivar plants have grapevines over 40 years old, obtained from the first grapevines introduced in the state by the missionaries. Throughout the state, only 37.96 ha of vineyards are established with 'Mission' grapevines in rainfed conditions and have limited agricultural management. Even so, yields are approximately 3.5 tons per hectare. From the fruits harvested from this variety, natural or sparkling wines are produced, known as missionary wines (Magoni, 2009; González-Andrade and Fuentes-Flores, 2013; SEFOA, 2011; Magoni, 2021).

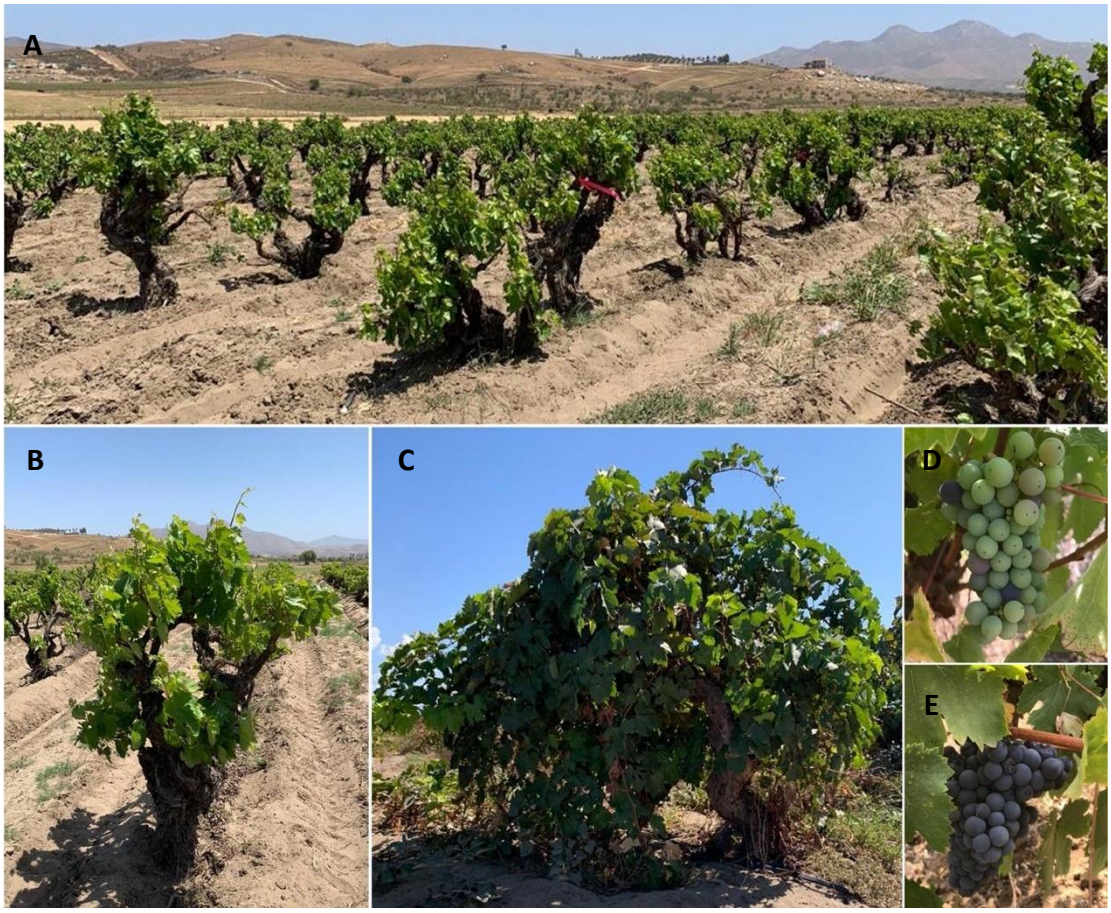

Figure 1. 'Mission' grapevines in Ejido Uruapan, Baja California. A) 'Mission' grapevines established under rainfed conditions. B-C) 'Mission' grapevine specimens showing a free-forming and unsupported pattern. D) Green grape cluster obtained from a 'Mission' plant. E) Mature grape cluster obtained from a 'Mission' grapevine.

Globally, grapevine trunk diseases (GTDs) constitute one of the most significant challenges in vineyards. GTDs are a complex that include black foot, Botryosphaeria dieback, Esca, Eutypa dieback, and Petri disease (Urbez-Torres et al., 2012). Currently, 133 fungal species distributed among 34 genera have been identified as causal agents of these diseases (Gramaje et al., 2018). Symptoms typically manifest several years after infection, including wood necrosis, cankers, foliar discoloration, cordon dieback, and plant death (Gramaje et al., 2018; Niem et al., 2020). The pathogens primarily enter the plant through wounds

made during the pruning season (Pollard-Flamand et al., 2022).

Botryosphaeria dieback is one of the most important GTDs. In total, 28 different fungal species are associated with this disease, belonging to the genera *Botryosphaeria, Diplodia, Dothiorella, Lasiodiplodia*, *Neofusicoccum, Neoscytalidium*, *Sphaeropsis,* and *Spencermartinsia* (Gramaje et al., 2018). These pathogens typically initiate infection by entering the plant, often through pruning wounds. The first symptoms of the disease, including leaf spots, fruit rots, dieback and necrosis in the shoots, wood discoloration, and canker formation, become noticeable usually years after the initial infection (Úrbez-Torres, 2012; Billions-Baaijens and Savocchia, 2019; Blundell and Eskalen, 2022).

Various species of the family Botryosphaeriaceae have been identified in the primary grapevine-producing regions of Mexico. In commercial vineyards of Baja California, the identified species are: *Botryosphaeria dothidea, Diplodia corticola, D. seriata, Lasiodiplodia brasiliensis*, *L. crassispora*, *L. exigua*, *L. gilanensis, L. theobromae*, *Neofusiccocum australe*, *N. parvum*, and *N. vitifusiforme* (Úrbez-Torres et al., 2009; Candolfi-Arballo, 2009: Rangel-Montoya et al., 2021; Rangel-Montoya et al., 2022). Regarding 'Mission' grapevines established in vineyards of Baja California, some plants have shown symptoms associated with grapevine trunk diseases but currently lack a clear identification of the fungi causing the observed symptoms. This lack of knowledge could be problematic, as these plants may serve as a reservoir of pathogenic microorganisms that infect other grapevine cultivars.

In general, the management of GTDs is complex, with no curative treatments available. Therefore, management strategies primarily revolve around implementing cultural and preventive measures, with each approach depending on the extent of damage to the plant, the specific pathogens involved, and the geographic region (Gramaje et al., 2018). These strategies commonly involve protecting pruning wounds by applying paints, pastes, fungicides, or beneficial microorganisms to prevent the entry of phytopathogenic fungi into the plant (Mondello et al., 2018; Gramaje et al., 2018; Billions-Baaijens et al., 2019).

While chemical fungicides can rapidly control many fungal diseases, their effectiveness in managing wood diseases in grapevines has been limited. Following the ban on sodium arsenite, products containing active ingredients from the carbamates or benzimidazoles group have demonstrated the highest efficacy in controlling pathogens from the *Botryosphaeria* and *Eutypa* genera (Andreolli et al., 2016; Gramaje et al., 2018; Mondello et al., 2018). As a result, the use of microorganisms as biocontrol agents has been considered a relevant alternative for managing these diseases. Microorganisms typically employ diverse

mechanisms to inhibit the growth of pathogens, promote plant growth, and colonize woody tissues, while consistently maintaining a broad-spectrum antagonistic activity (Mondello et al., 2018; Dini-Andreote, 2020; Jacob et al., 2020).

Various reports have shown that plants in arid conditions form associations with microorganisms, including bacteria and fungi, which can enhance stress resistance against the negative impacts of drought through various mechanisms. These mechanisms include improving the absorption of water and nutrients by inducing changes in root morphology, protecting against oxidative damage, regulating phytohormone levels, and suppressing phytopathogens, like those responsible of GTDs (Poudel et al., 2021; Riseh et al., 2021). Considering the time since their establishment and the complex climatic conditions in which the 'Mission' grapevines are found, characterized by a dry to very dry climate with an average annual rainfall of 99 mm per year (SIAP, 2023), it is probable that they maintain associations with beneficial microorganisms that allow them to survive in these adverse conditions.

Endophytic microorganisms from the genera *Bacillus, Paenibacillus*, *Pseudomonas*, *Streptomyces*, *Epicoccum,* and *Trichoderma* have been identified with antagonistic activity on *Diplodia seriata* (Almeida et al., 2020; Silva-Valderrama et al., 2021), *Phaeomoniella chlamydospora* (Haidar et al., 2016), *Phaeoacremonium aleophilum* (Del Frari et al., 2019), *Dactylonectria* sp*., Ilyonectria* sp*., Phaeoacremonium minimum* (Álvarez-Pérez et al., 2017), *N. parvum* (Silva-Valderrama et al., 2021) and *Lasiodiplodia theobromae* (Rezgui et al., 2016). Although these microorganisms have demonstrated antagonistic activity against trunk disease fungi under *in vitro* conditions, their biocontrol effectiveness does not always translate well when applied in the field.

Their success depends on various factors, including the ability of the microorganisms to survive and efficiently colonize plant tissues, the nature of the biocontrol agent, the specific pathogen, the method of application, and even the cultivar on which it is applied (Rabiey et al., 2019; Martínez-Diz et al, 2021; Niem et al., 2023).

Recently, 'Mission' grapevines have gained significance. This can be attributed to their heritage, the growing interest in wines produced from these heritage varieties, and their demonstrated adaptability to arid conditions with minimal agricultural management. Consequently, 'Mission' grapevines have become an intriguing subject of study in assessing their potential as reservoirs of pathogenic fungi and in the quest for microorganisms capable of controlling trunk diseases fungi. Considering this, this work aims to investigate the presence of grapevine trunk disease fungi associated with 'Mission' grapevines and identify

endophytic microorganisms with the potential to be used as biological control agents for controlling grapevine trunk disease fungi in commercial vineyards.

1.1 Justification

Due to the negative effect of climate change on productivity in grapevine cultivation in Baja, California, it is necessary to establish adapted cultivars. In this sense, 'Mission' grapevines have shown the ability to grow in arid conditions and with minimal agricultural management, which makes them a viable option for establishment in this wine region. Symptoms associated with grapevine trunk diseases, including dieback and formation of wedge-shaped necrotic lesions, have been observed in old grapevines of this cultivar. However, the pathogens responsible for causing these symptoms are unknown. Given the lack of knowledge about the pathogenic microorganisms associated with 'Mission' grapevines, it is essential to investigate these associations to establish management strategies and guarantee the health of the propagation material.

On the other hand, 'Mission' grapevines have shown the ability to maintain consistent production year after year, even when established in adverse conditions. In this case, the interaction between these plants and several beneficial microorganisms could be crucial in their ability to withstand such adversity. Given the lack of knowledge about microorganisms associated with 'Mission' grapevines, it is essential to investigate these associations.

This work aims to examine the presence of grapevine trunk disease fungi associated with 'Mission' grapevines and to identify endophytic microorganisms with the potential to be used as biological control agents to control grapevine trunk disease fungi in commercial vineyards.

1.2 Hypothesis

'Mission' grapevines from Baja California are reservoirs of grapevine trunk diseases fungi and beneficial microorganisms that can act as biocontrol agents.

1.3 Objectives

1.3.1 Main objective

To analyze the presence of grapevine trunk disease fungi associated with the 'Mission' grapevine and identify endophytic microorganisms with the potential to be used as biological control agents for controlling grapevine trunk disease fungi in commercial vineyards.

1.3.2 Specific objectives

1. To evaluate the presence of pathogenic fungi responsible for grapevine trunk diseases in 'Mission' grapevines.

2. To isolate and characterize biological control microorganisms associated with the 'Mission' grapevine with the potential as biological control agents against the pathogenic fungi causing grapevine trunk diseases.

Chapter 2. Analyzing the phytosanitary status of 'Mission' grapevines of Baja California, Mexico, regarding trunk diseases fungi

2.1 Abstract

In Baja, California, 'Mission' grapevines were introduced in 1869 and are now established under rainfed conditions with minimal agricultural management while maintaining production. These grapevines have remarkably adapted to drought and high temperatures, making them a viable option for cultivation in a region with less than 99.1 mm of annual precipitation. Like other grapevine varieties, 'Mission' grapevines frequently show symptoms of grapevine trunk diseases, but the specific pathogens responsible for these symptoms remain unidentified. Efforts are underway to utilize the 'Mission' variety for wine production, which requires the evaluation of its phytosanitary status to support widespread cultivation. This study aims to identify potential causal agents of trunk diseases in this variety. Initially, 'Mission' grapevine tissue samples were collected from eight vineyards established in Baja California. From the samples analyzed, 78 fungal strains were obtained, and 13 were morphologically and molecularly identified as trunk disease fungi, including *Diaporthe ampelina* and *Diplodia seriata*. The last was the most prevalent and was isolated from all the sampling sites. All evaluated strains demonstrated optimal growth temperatures between 25 and 28°C. Pathogenicity tests confirmed that all tested strains could induce lesions on grapevine stems of 'Mission,' 'Malvasia Blanca,' and 'Tempranillo,' thereby affirming their infective capacity and highlighting the role of 'Mission' grapevines as a reservoir for grapevine trunk fungi, albeit with lower virulence.

2.2 Introduction

Grapevine is a globally cultivated crop, with approximately 7.3 million ha of established vineyards worldwide. About 50% of the world's vineyard area is concentrated in five countries: Spain (13%), China (11%), France (11%), Italy (10%), and Turkey (6%) (OIV, 2021; OIV 2022). Mexico has around 36,587 ha of vineyards, ranking 26th worldwide (SIAP, 2023). The country produces a total of 452,125 tons of grapes annually (SIAP, 2023). The main grape-producing states in Mexico are Sonora, Zacatecas, Aguascalientes, Baja California, and Coahuila (SIAP, 2022a).

Globally, vineyards are affected by grapevine trunk diseases (GTDs). These complexes of diseases include Petri disease, Black foot, Esca, Eutypa dieback, and Botryosphaeria dieback (Úrbez-Torres, 2011; Mondello et al., 2018). Among these, Esca, Petri disease, and Botryosphaeria dieback are the most widespread (Hrycan et al., 2020; Kenfaoui et al., 2022). These diseases are caused by fungi that colonize the xylem and progress slowly, meaning that symptoms in infected plants may not become apparent until several years after infection (Hrycan et al., 2020). The main symptoms of GTDs include wood necrosis, cankers, foliar discoloration, cordon dieback, and plant death (Gramaje et al., 2018; Niem et al., 2020). Grapevine wood comprises lignocellulosic material that includes cellulose, hemicelluloses, aromatic lignin, tannins, proteins, and minor inorganic compounds (Čabalová et al., 2023). It has been proposed that the virulence of GTDs fungi could be influenced by the capability of each fungus to colonize and degrade grapevine wood. The degradation is achieved through the production of different hydrolytic enzymes such as carbohydrate-active enzymes (CAZymes), glycoside hydrolase, carbohydrate esterases and polysaccharide lyases, among others (Claverie et al., 2020; Belair et al., 2023). However, external factors have also been reported to influence the severity of the diseases, such as temperature, humidity, cultivar, vineyard age, management and pathogens (Claverie et al., 2020; Kenfaoui et al., 2022).

In the most important grapevine cultivation regions of Mexico, various species of GTDs fungi have been identified. In 1979, the presence of *Eutypa armeniacae* was identified in vineyards established in Aguascalientes, Coahuila and Sonora (Téliz, 1979). Subsequently, Úrbez-Torres et al., (2008) reported the presence of *Diplodia seriata* and *Lasiodiploda theobromae* in ten grapevine varieties established in vineyards located in Hermosillo, Sonora, and Ensenada, Baja California. Later, fungi associated with Esca belonging to *Phaeomoniella* and *Phaeoacremonium* were isolated in Baja California vineyards (Morales-Pedraza, 2010). Paolinelli-Alfonso et al., 2015 reported the presence of *Eutypella microteca* in 'Cabernet Sauvignon' grapevines established in Baja California. In 2020, Ávila Salazar et al., identified the presence of *Greeneria uvicola* in different vineyards located in Sonora. Later, Rangel-Montoya et al., (2021) identified *Lasiodiplodia gilanensis, L. brasiliensis, L. exigua, L. crassispora* in vineyards established in Baja California and Sonora. Other fungal species associated with Botryosphaeria dieback including *Botryosphaeria dothidea, Diplodia seriata, Neofusiccocum australe, N. parvum* and *N. vitifusiforme* were reported in Baja California and Coahuila (Rangel-Montoya, 2024). Recently, Rangel-Montoya and Hernández-Martínez (2024) identified three species of *Aspergillus*, *A. tubingensis*, *A. welwitschiae* and *A. niger*, as causal agents of cankers in grapevines established in vineyards in Baja California, Guanajuato and Sonora.

Baja California, Mexico faces adverse climatic conditions that have affected vineyard production. Changes in these conditions, including a longer dry season, high temperatures (ranging from 13.4 to 51.4°C) and an annual precipitation of only 99.1 mm (SIAP, 2023; CONAGUA, 2023), are hypothesized to increase the expression of symptoms associated with GTDs and fungal virulence. These harsh conditions are believed to affect the defense system of the plants, leading to increased susceptibility and promoting fungal infections (van Niekerk et al., 2011; Fontaine et al., 2016; Hrycan et al., 2020). To address this problem, it is necessary to find plant cultivars adapted to dry conditions. In this context, the 'Mission' grapevine is a promising option for expanding vineyard areas in Baja California, as it appears to be well-suited to arid and saline conditions (Andrade et al., 2013; Zurita-Silvar et al., 2021; Martain-Amozurrutia., 2021). The 'Mission' grapevine is a red cultivar known for its rustic and semi-erect growth habits. Currently, 'Mission' vineyards in the state consist of vines over 40 years old, obtained from the first grapevines introduced by missionaries. Throughout the state, only 37.96 ha of vineyards are planted with 'Mission' grapevines, which are grown under rainfed conditions with limited agricultural management. Despite these challenges, yields are approximately 3.5 tons per hectare (Magoni, 2009; SEFOA, 2011; Andrade et al., 2013; Magoni, 2021).

Despite their potential, 'Mission' grapevines in Baja California vineyards have displayed symptoms linked to trunk diseases fungi. Currently, the specific fungi responsible for these symptoms remain unidentified. This poses a concern as these plants could act as a reservoir of pathogenic microorganisms that may infect other grapevine cultivars. To address this issue, the objective of this study was to identify and characterize the grapevine fungi associated with trunk diseases affecting 'Mission' grapevines in Baja California vineyards.

2.3 Methodology

2.3.1 Isolation of microorganisms from 'Mission' grapevines

Samples were collected from eight vineyards in Baja California. Four of these samples were obtained from vineyards in the Guadalupe Valley region, while the remaining four came from vineyards in Ejido Uruapan. At each sampling site, five plants showing symptoms associated with GTDs, such as dieback and cankers, were selected for tissue sampling (Figure 2). For each chosen plant, three branches of semi-woody tissue, each measuring around 15 cm in length and located proximally to the symptomatic branch, were sampled.

The collected samples were then placed in plastic bags and stored at 4°C until processing. To isolate the fungi, bark was removed from each branch and cut into 1 cm fragments. The tissue was then surface sterilized by flaming and inoculated onto Potato Dextrose Agar (PDA) and water agar supplemented with chloramphenicol (25mg/mL), as described by Rangel-Montoya et al., (2021). The plates were then incubated at 28°C for a week, during which fungal colonies were recovered as they appeared and subsequently plated to isolate pure cultures. The fungal strains were preserved at 4°C in 20% glycerol.

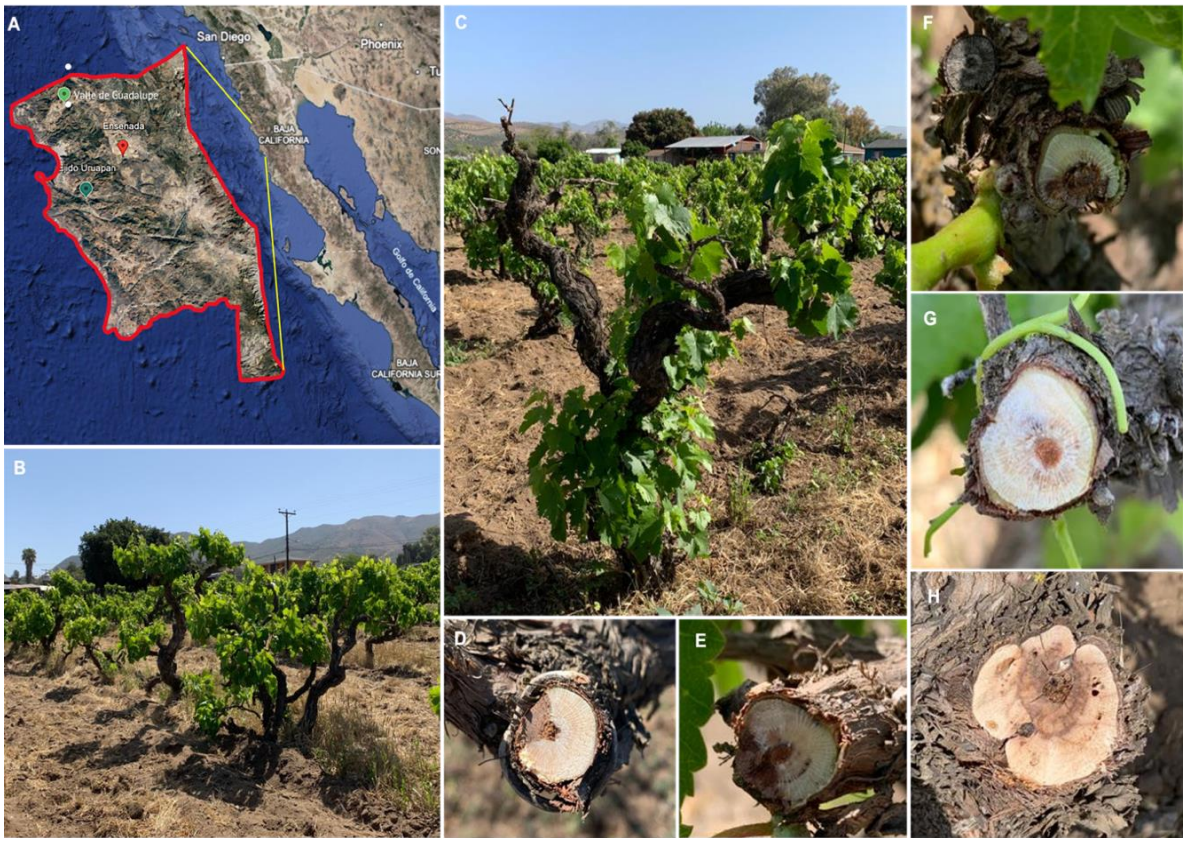

Figure 2.'Mission' grapevines from Baja California vineyards showing symptoms of grapevine trunk diseases. A) Map of Ensenada, Baja California, indicating the regions where 'Mission' grapevine tissue samples were collected. B) 'Mission' grapevines established in Baja California. C) 'Mission' grapevine with dieback symptoms. D-H) 'Mission' grapevine plants exhibiting necrosis in the vascular system, with wedge-shaped necrotic lesions visible.

2.3.2 Identification of fungi associated to GTDs

Morphological and molecular Identification were performed for those isolates exhibiting characteristics consistent with those reported by Wilcox et al., (2015) for fungi associated with GTDs. Morphological characterization was conducted by first growing each strain on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA). The plates were incubated for seven days at 28°C, and the colony characteristics were observed. To facilitate conidia production, the isolates were inoculated onto water agar supplemented with pine needles to promote the formation of pycnidia and conidia (Phillips et al., 2013). The plates were

incubated four weeks at room temperature under near-ultraviolet light with 12-hour light and 12-hour dark photoperiod. Once the pycnidia were obtained, they were resuspended in sterile water to obtain the conidia, which were observed under an optical microscope (AxioVert200 Zeiss). Length and width of 30 conidia were measured for each strain.

For molecular identification, all isolates were initially inoculated in Potato Dextrose Broth (PDB), and the cultures were incubated for five days at 28°C and with constant shaking at 110 rpm to promote mycelium growth. Total DNA extraction was then extracted following the CTAB protocol (Wagner et al., 1987). Amplification of the ITS region was performed using primers ITS1 (5´- TCCGTAGGTGAACCTGCGG-3´) and ITS4 (5´TCCTCCGCTTATTGATATGC3´) and the EF1-α region was amplified using primers EF1-728F (5' - CATCGAGAAGTTCGAGAAGG-3') and EF1-986R (5'- TACTTGAAGGAACCCTTA CC -3') (Carbone & Kohn 1967). PCR reactions were conducted in a final volume of 25 µL, containing 2.5 µL of 10X Buffer with 15 mM MgCl2, 0.5µL of 20 mM dNTPs, 1.0 µL of 10 µM of each primer, 0.125 µL of Taq DNA polymerase (DreamTaq DNA Polymerase, Thermo Scientific) at 5 units per µL, 1 µL of 30 ng·µL-1 DNA, and 18.75 µL of ultrapure water. Amplification was performed in a Bio-Rad T-100 thermal cycler under the following conditions: an initial cycle of 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and a final cycle at 72°C for 10 min.

PCR products were verified by electrophoresis in an agarose gel. Bands of approximately 650 bp for the ITS and 350 bp for the EF1-α region were purified and sequenced (Eton Bioscience, Inc., San Diego, USA). The resulting sequences were compared using Blast (https://blast.ncbi.nlm.nih.gov/) with the GenBank nucleotide database (http://www.ncbi.nlm.nih.gov) (Table 1). Phylogenetic trees were constructed using Maximum Likelihood and Maximum Parsimony methods with MEGA XI (Koichiro et al., 2021). The K2+G+I model was used with Maximum Likelihood analysis, with parameters set to the bootstrap method using 1000 replicates.

Table 1. List of GenBank and culture accession numbers for fungal species used in this study for phylogenetic analyses. Bold entries were isolated in this work.

Species	**Isolate**	**Host**	**Origin**	**GenBank accession number**	
				ITS	***tef1*-α**
Diaporthe ampelina	CBS 111888	*Vitis vinifera*	USA	KC343016	KC3437421
D. ampelina	CBS 143354	*Vitis vinifera*	Hungary	MG281127	MG281648
D. ampelina	CPC 29674	*Vitis vinifera*	Spain	MG280993	MG281514
D. ampelina	**HMVOLL20BCMX**	***Vitis vinifera***	**Mexico**	**PQ152474**	**PQ177957**
D. baccae	CBS136972	*Vaccinium corymbosum*	Italy	KJ160565	KJ160597

D. baccae	CBS143343	*Vitis vinifera*	France	MG281001	MG281522
D. bohemiae	CBS 143347	*Vitis vinifera*	Czech Republic	MG281015	MG281536
D. bohemiae	CBS 143348	*Vitis vinifera*	Czech Republic	MG281016	MG281537
D. celeris	CBS 143349	*Vitis vinifera*	United Kingdom	MG281017	MG281538
D. celeris	CPC 28267	*Vitis vinifera*	United Kingdom	MG281019	MG281540
D. eres	CBS 138594	*Ulmus laevis*	Germany	KJ210529	OM752197
D. eres	CBS 109767	*Acer campestre*	Austria	KC343075	KC343801
D. foeniculacea	CBS 111553	*Foeniculum vulgare*	Spain	KC343101	KC343827
D. foeniculacea	CBS 18727	*Camellia sinensis*	Italy	KC343107	KC343833
D. rudis	CAA987	*Foeniculum vulgare*	Portugal	MT073314	MT051934
D. rudis	CAA582	*Eucalyptus globulus*	Portugal	MW546041	MW768126
Diaporthella corylina	CBS 121124	*Corylus* sp.	China	KC343004	KC343730
Diplodia corticola	CBS 112549	*Quercus suber*	Portugal	AY259100	AY573227
D. corticola	CBS 112547	*Quercus suber*	Portugal	AY259110	DQ458872
Diplodia cupressi	CBS 16887	*Cupressus sempervirens*	Qualifiers	NR_111219	DQ458878
D. cupressi	CBS 121027	*Cupressus sempervirens*	-	MT587340	MT592045
D. mutila	CBS 112553	*Vitis vinifera*	Portugal	AY259093	AY573219
D. mutila	CBS 23030	*Phoenix dactylifera*	USA	DQ458886	DQ458869
D. pseudoseriata	UY788	*Blepharocalyx salicifolius*	Uruguay	EU080927	EU863181
D. pseudoseriata	UY671	*Blepharocalyx salicifolius*	Uruguay	EU080922	EU863179
D. sapinea	CBS39384	*Pinus nigra*	Netherlands	DQ458895	DQ458880
D. sapinea	CBS109725	*Pinus patula*	South Africa	DQ458896	DQ458881
D. seriata	CBS 112555	*Vitis vinifera*	Portugal	KF766161	AY573220
D. seriata	CPC 30978	*Tilia platyphyllos*	Germany	MT587393	MT592104
D. seriata	AA3	*Prunus dulcis*	USA	MZ079025	MZ093433
D. seriata	AS8	*Prunus dulcis*	USA	MZ079029	MZ093437
D. seriata	**EF14BCMX**	***Vitis vinifera***	**Mexico**	**PQ152462**	**PQ177945**
D. seriata	**HMV01BCMX**	***Vitis vinifera***	**Mexico**	**PQ152463**	**PQ177946**
D. seriata	**HMV04BCMX**	***Vitis vinifera***	**Mexico**	**PQ152464**	**PQ177947**
D. seriata	**HMV06BCMX**	***Vitis vinifera***	**Mexico**	**PQ152465**	**PQ177948**
D. seriata	**HMV10BCMX**	***Vitis vinifera***	**Mexico**	**PQ152466**	**PQ177949**
D. seriata	**HMV12BCMX**	***Vitis vinifera***	**Mexico**	**PQ152467**	**PQ177950**
D. seriata	**RP05BCMX**	***Vitis vinifera***	**Mexico**	**PQ152468**	**PQ177951**
D. seriata	**R2HG01BCMX**	***Vitis vinifera***	**Mexico**	**PQ152469**	**PQ177952**
D. seriata	**VEUL1M03CMX**	***Vitis vinifera***	**Mexico**	**PQ152470**	**PQ177953**
D. seriata	**VEUL1M07CMX**	***Vitis vinifera***	**Mexico**	**PQ152471**	**PQ177954**
D. seriata	**VEUL1M10CMX**	***Vitis vinifera***	**Mexico**	**PQ152472**	**PQ177955**
D. seriata	**VEUL2M12CMX**	***Vitis vinifera***	**Mexico**	**PQ152473**	**PQ177956**
Lasiodiplodia theobromae	CBS 16496	Fruit along coral	PNG	AY640255	AY640258

2.3.3 Determination of optimal growth temperature

To investigate the effect of temperature on the growth of the fungal isolates associated with 'Mission' grapevines, each strain was inoculated at the edge of PDA plates, using a 7 mm diameter mycelial plug. The plates were incubated at temperatures of 25°C, 28°C, 30°C, 35°C, 37°C and 40 °C (Rangel-Montoya et al., 2021) for five days. Each strain was evaluated in triplicate. The diameter of the colony was measured daily. Afterwards, the following formula was used to determine the growth rate:

$$\mathrm{GR} = \frac{\mathrm{R_f} - \mathrm{R_i}}{\mathrm{T_f} - \mathrm{T_i}} \quad (1)$$

where: GR= growth rate, R_f = Final colony radio (mm), R_i = Initial colony radio (mm), T_f = Final time when colony measured (d) and T_i = Initial time (day 1). The temperature where the fungi had the highest growth rate was considered the optimum growth temperature.

2.3.4 Pathogenicity assay of identified strains

Pathogenicity assays were performed using thirteen isolates. For this purpose, 25 cm long dormant canes with three internodes from 'Mission,' 'Tempranillo,' and 'Malvasia Blanca' cultivars were used. The fungi inoculum was obtained from cultures grown on PDA four days at 25°C. The canes were first superficially sterilized, and a 3.9 mm mechanical wound was created using a drill in the middle internode. A 2.5 mm diameter disk of mycelium was then placed inside the wound, which was covered with Parafilm® (Carlucci et al., 2015). In negative controls, canes were inoculated with a disk of PDA medium. To compare pathogenicity with fungi isolated from commercial vineyards, two strains were included as positive controls: *L. brasiliensis* MXBCL28 and *D. seriata* BY06BCMX (Rangel-Montoya et al., 2021; Rangel-Montoya et al., 2024). Ten repetitions per treatment were established. After inoculation, the detached canes were placed in plastic containers with just enough water to keep them hydrated without covering the wound. The canes were kept for 50 days in a growth chamber at 25°C under a photoperiod of 16 light-8 dark hours with 50% relative humidity. At the end of the assay, the length of the lesion was evaluated, and efforts were made to recover the inoculated microorganisms following the protocol described above. Data were analyzed using one-way ANOVA with post-HDS analysis for homogeneous group comparisons, with a significance level set at $\alpha < 0.05$. Statistical analysis was conducted using STATISTICA 8.0.

2.3.5 Evaluation of grapevine trunk diseases fungi growth on different carbon sources

Three strains from the Valle de Guadalupe region and two from Ejido Uruapan were used to assess the utilization of wood components as a carbon source. The strains were grown in Minimal Medium 9 (MM9) (3.0 K_2HPO_4 g/L, 3.0 KH_2PO_4 g/L, 0.5 NaCl g/L, 1.0 NH_4Cl g/L, 15.0 agar g/L) supplemented with one of the following carbon sources: glucose 1%, xylose 1%, lignin 0.1%, arabinose 1%, starch 1%, cellulose 1%, xylene 1%, glycerol 1%, pectin 1%, glycogen 1%, galactose 1%, tannic acid 0.1% or grapevine wood (Rangel-Montoya et al., 2023). A 7 mm mycelium disc of each fungus was inoculated at the edge of a Petri dish, and plates were then incubated at 28°C for six days. The mycelial growth rate was calculated after five days using the same formula for determining optimal growth temperature.

2.3.6 Histological analysis of grapevine canes

Histological analyses were conducted on grapevine canes used for the pathogenicity assay to observe the ability of *D. seriata* HMV12BCMX to degrade starch, cellulose, and lignin in two grapevine cultivars. Five canes from each 'Mission' and 'Tempranillo' cultivars inoculated with this fungus were selected. Additionally, canes inoculated with *L. brasiliensis* MXBCL28 were included to compare the cell wall degradation activity with control plants.

Half of the cane samples from the pathogenicity assay were fixed in 20 mL of FAA solution (Formalin-Aceto-Alcohol, 5:5:9) and stored at 4°C for 24 h. The samples were rinsed and preserved in 80% ethanol at 4°C until used. For histological observations, 70 µm thick sections of each sample were obtained using a manual microtome. The sections were stained with an iodine-potassium iodide solution (5% iodine, 10% potassium iodide) to visualize starch in black (Rangel-Montoya et al., 2023). Lignin was detected using 0.1% phloroglucinol-HCl (Nakano and Meshitsuka, 1992), and cellulose was observed with a 0.02% Calcofluor M2R White solution (Ruzin, 1999). For each staining procedure, five stained tissue slices were examined using an AXioVert200 microscope with a RisingCam U3CMOS camera. For Calcofluor M2R White staining, epifluorescence microscopy was performed with an AXioVert200 microscope equipped with an HBO100 100W mercury lamp and a DAPI filter (excitation at 330-380 nm, emission at 420 nm). Images were captured using Rising View software and analyzed with ImageJ 1.49v.

2.4 Results

2.4.1 Isolation and identification of GTDs fungi associated with 'Mission' grapevines

From the analyzed grapevine tissues, 78 fungal strains were obtained and identified morphologically as belonging to genera *Alternaria, Aspergillus, Chaetomium, Fusarium, Trichoderma, Sordaria, Diaporthe,* and *Diplodia.* Only strains with morphological characteristics similar to those reported for grapevine trunk fungi were processed for molecular identification.

According to the phylogenetic trees, strain HMV20BCMX was identified as *D. ampelina*, and the twelve *Diplodia* strains were identified as *Diplodia seriata*. The morphological characterization of mycelium and spores was performed for twelve *Diplodia* strains and one *Diaporthe* strain. In PDA medium *D. ampelina* HMVOLL20BCMX showed a white mycelium colony, concentric rings, irregular margins and absent of aerial mycelium. While in MEA medium the colony presented a light beige color, the formation of concentric rings, irregular edges and the absence of aerial mycelium (Figure 3). Two kinds of conidia were observed: α conidia are aseptate, hyalin, cylindrical, tapering towards the apex, and straight (11.1) 10.1 – 14.6 x (3.1) 2.1 – 3.6 (Figure 3 and Table 2). ß conidia are aseptate, hyaline, filiform, slightly curved and tapering towards the apex, with (24.2) 18.1 – 28.8 x (1.4) 1.1 – 1.9 µm (Figure 3 and Table 2).

Diplodia seriata isolates exhibited smoke-gray colonies with minimal aerial mycelium formation on PDA and MEA medium. The colonies displayed little or no aerial mycelium and had a dark brown color (Figure 4). The conidia are ovoid with a rounded apex and a truncate base, initially hyaline, that later develop a brown coloration and some of them have septa (Figure 4 and Table 2). Conidial sizes range from 18.1 to 25.7 µm in length and 9.6 to 13.36 µm in width. Specifically, *D. seriata* strains RPB05BCMX and VEUL2M12BCMX had the shortest conidia, while the VEUL1M10BCMX strain had the longest. Additionally, the presence of septa was observed in the spores of 11 of the isolated strains, only in the spores of the *D. seriata* strain HMV10 BCMX the presence of septa was not observed.

Table 2. Dimensions of conidia from grapevine trunk fungi associated with 'Mission' grapevines.

Isolate	Conidium size (µm)[1]	Mean ± SD[2]

***Diaporthe ampelina* HMVOLL20 (α conidia)**	(11.1) 10.1 – 14.6 x (3.1) 2.1 – 3.6	24.2 ± 2.6 x 1.5 ± 0.2
***Diaporthe ampelina* HMVOLL20 (ß conidia)**	(24.2) 18.1 – 28.8 x (1.4) 1.1 – 1.9	24.2 ± 2.6 x 1.5 ± 0.2
***Diplodia seriata* EF14BCMX**	(30.1) 25.3 – 30.3 x (11.5) 9.6 – 14.8	28.3 ± 1.5 x 12.0 ± 1.4
***D.seriata* HMV01BCMX**	(26.5) 21.3 – 29.1 x (10.9) 9.0 – 12.4	26.5 ± 2.2 x 10.9 ± 1.0
***D.seriata* HMV04CMX**	(29.0) 24.0 – 29.9 x (11.3) 10.8 – 14.0	27.7 ± 1.4 x 12.3 ± 0.9
***D.seriata* HMVOLL06BCMX**	(23.7) 21.6 – 25.8 x (12.2) 9.1 - 12.6	24.0 ± 1.1 x 11.1 ± 1.0
***D.seriata* HMV10 BCMX**	(23.5) 21.3 – 24.7 x (10.7) 9.1 – 12.1	23.0 ± 1.2 x 11.0 ± 0.7
***D.seriata* HMV12BCMX**	(26.7) 24.9 – 29.2 x (10.8) 9.4 – 11.6	26.7 ± 1.7 x 10.9 ± 0.7
***D.seriata* RP05BCMX**	(23.7) 19.4 – 24.7 x (10.6) 10.0 –12.8	22.6 ± 1.5 x 11.3 ± 0.8
***D.seriata* R2HG01BCMX**	(28.1) 23.5 – 29.1 x (10.1) 9.4 – 12.5	26.5 ± 1.8 x 18.8 ± 0.8
***D.seriata* VEUL1M03**	(25.1) 22.2 – 30.3 x (13.1) 10.1 – 15.0	25.3 ± 1.9 x 12.8 ± .10
***D.seriata* VEUL1M07**	(25.8) 22.9 – 29.3 x (11.8) 9.1 – 12.9	26.6 ± 1.7 x 11.3 ± 1.1
***D.seriata* VEUL1M10**	(27.1) 23.0 – 32.1 x (13.9) 11.0 – 17.1	27.8 ± 2.3 x 14.1 ± 1.8
***D.seriata* VEUL2M12**	(25.1) 19.3 – 27.6 x (12.1) 9.3 – 12.7	24.2 ± 2.2 x 11.1 ± 1.0

[1] Most repetitive value, minimum size, and maximum size for length and width of 30 conidia.
[2]SD = standard deviation.

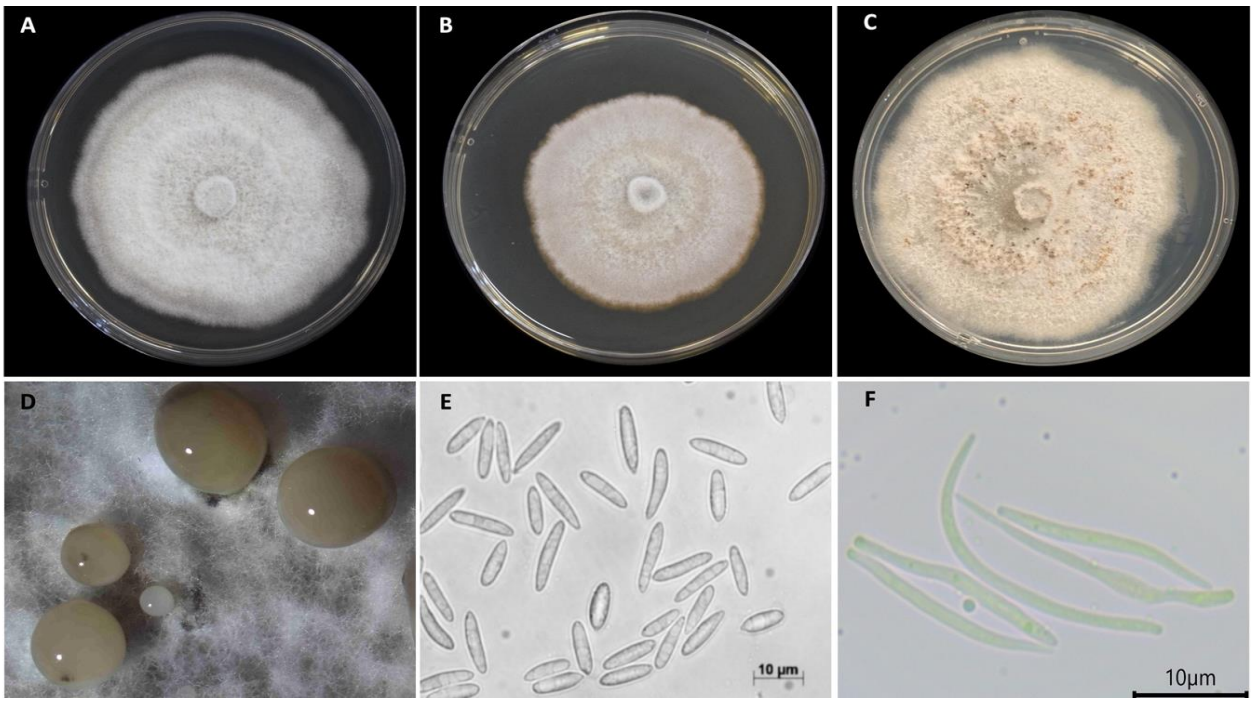

Figure 3. *Diaporthe ampelina* HMV20BCMX recovered from 'Mission' grapevine. A) *D. ampelina* growth in PDA medium. B) *D. ampelina* growth in MEA medium. C) Colony of *D. ampelina* growth with pycnidia. D) Individual pycnidia. E) α conidia of *D. ampelina*. F) ß conidia of *D. ampelina.*

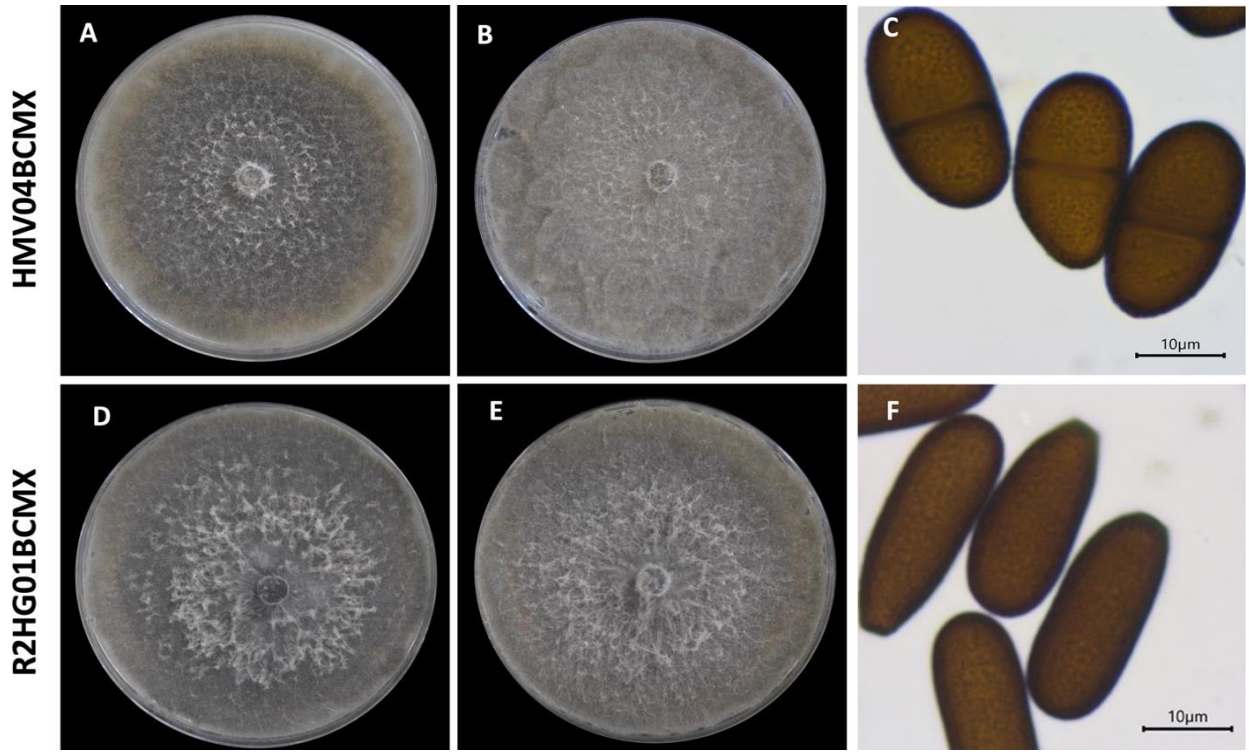

Figure 4. Strains of *D. seriata* associated with 'Mission' grapevine. A-B-C) *D. seriata* HMV01BCMX growth on PDA and MEA at 25°C for seven days showing brown conidia. D, E, F) HMV04BCMX growth on PDA and MEA at 25°C for seven days showing brown conidia.

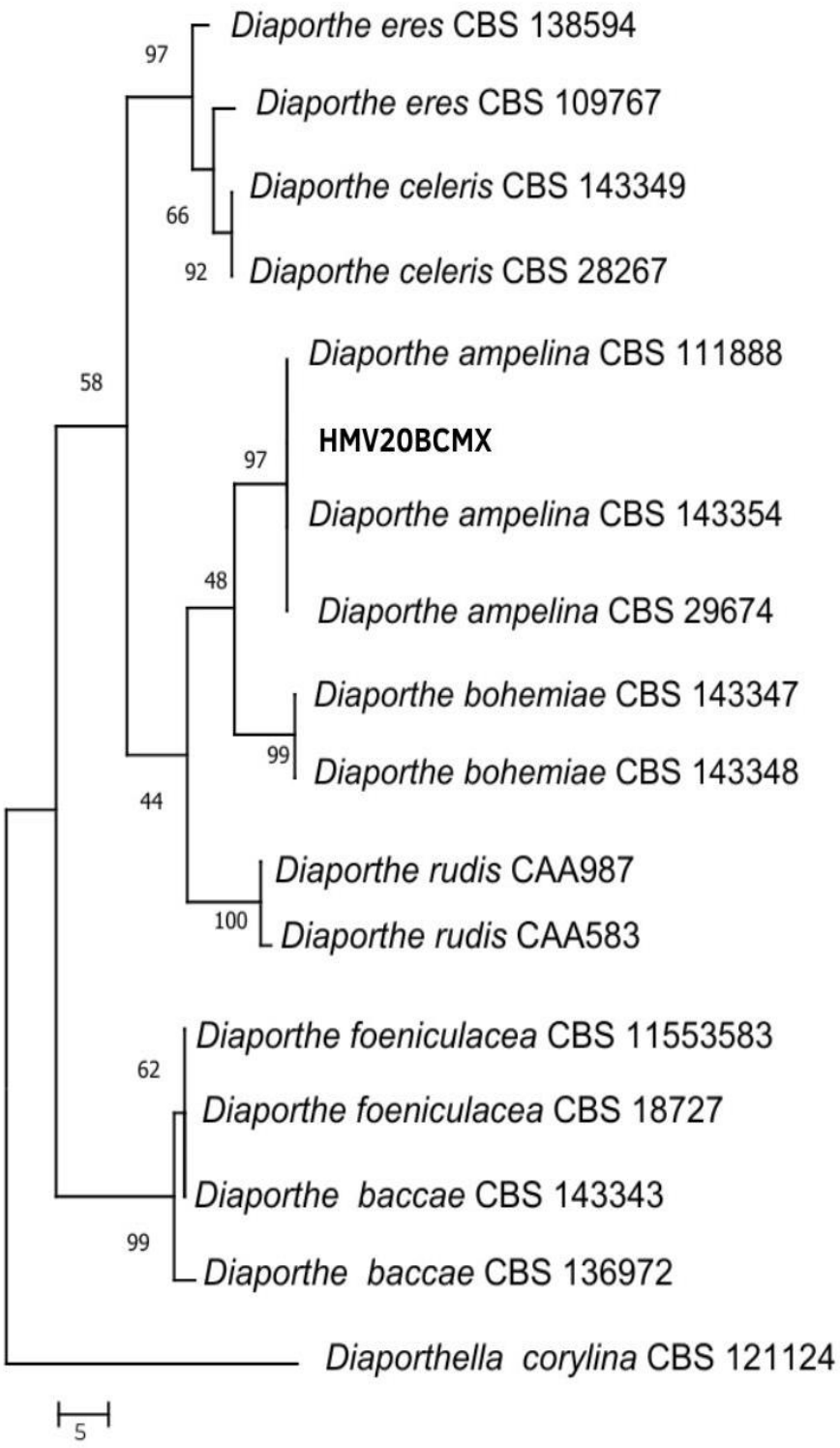

Figure 5. Most Parsimonious trees of *Diaporthe* isolates, based on a concatenated database of ITS and *tef-1α* sequences. The isolate described in the present study is indicated in bold font. Numbers above nodes represent bootstrap values obtained from 1000 replicates. The tree is rooted with *Diaporthe corylina* CBS121124.

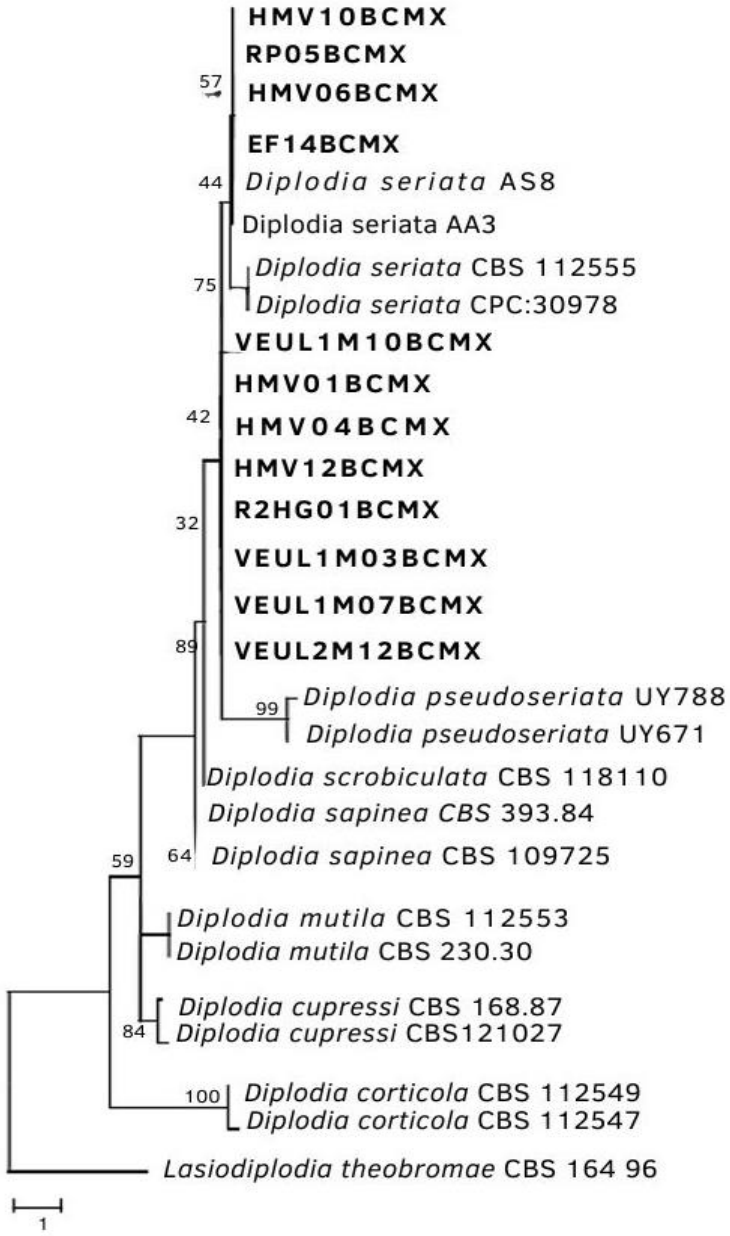

Figure 6. Most Parsimonious tree of *Diplodia* isolates, based on a concatenated database of ITS and *tef-1α* sequences. Isolates described in the present study are indicated in bold font. Numbers above nodes represent bootstrap values obtained from 1000 replicates. The tree is rooted with *Lasiodiplodia theobromae* CBS 16496.

2.4.2 Optimal growth temperature of grapevine trunk diseases fungi associated to 'Mission'

The thirteen evaluated strains exhibited an optimal growth temperature between 25 and 28°C. Specifically, *D. ampelina* HMV20BCMX had an optimal growth temperature of 25°C, with a growth rate of 3.42 mm per day (Table 3). This strain showed no growth when incubated at 30, 37, or 40°C, but it resumed

growth after being transferred back to room temperature. On the other hand*, Diplodia* strains exhibited an average growth rate ranging from 4.7 to 17.1 mm per day. *Diplodia seriata* VEUL1M03BCMX displayed the lowest growth rate at 25°C, while *D. seriata* HMV04BCMX exhibited the highest growth rate. Ten *Diplodia* strains grew at 37°C, with HMV06BCMX and VEUL1M03BCMX being the only exceptions (Table 3). None of the *D. seriata* strains grew at 40°C; however, they resumed growth after three days at room temperature.

Table 3. Mean growth rate (mm/day ± SD) of selected fungi isolated from 'Mission' grapevines at five temperatures after five days of incubation.

Isolate	Temperature					
	25 °C	**28 °C**	**30 °C**	**35 °C**	**37 °C**	**40 °C**
***D. ampelina* HMV20BCMX**	3.42 ± 0.8	3.0 ± 0.3	1.1 ± 0.2	0	0	0
***D. seriata* EF14BCMX**	16.3 ± 0.6	17.2 ± 0.4	13.8 ± 1.1	9.0 ± 0.4	0.6 ± 0.1	0
***D. seriata* HMV01BCMX**	16.3 ± 0.3	14.1 ± 2.1	12.1 ± 1.8	9.3 ± 1.0	0.5 ± 0.1	0
***D. seriata* HMV04CMX**	17.1 ± 0.2	14.9 ± 2.1	14.7 ± 0.4	9.4 ± 2.1	0.7 ± 0.1	0
***D. seriata* HMV06BCMX**	13.9 ± 2.1	13.2 ± 0.7	9.2 ± 0.2	5.1 ± 0.6	0	0
***D. seriata* HMV10BCMX**	15.6 ± 0.1	13.2 ± 3.2	14.4 ± 0.5	6.3 ± 1.4	0.5 ± 0.1	0
***D. seriata* HMV12BCMX**	15.5 ± 0.2	16.6 ± 2.4	13.7 ± 2.1	8.5 ± 0.9	0.5 ± 0.1	0
***D. seriata* RP05BCMX**	16.3 ± 0.1	21.7 ± 0.4	13.9 ± 0.2	6.0 ± 0.5	0.5 ± 0.2	0
***D. seriata* R2HG01BCMX**	16.4 ± 0.2	16.8 ± 0.3	12.4 ± 0.5	2.4 ± 0.2	0.6 ± 0.1	0
***D. seriata* VEUL1M03BCMX**	4.7± 1.1	5.8 ± 0.5	2.8 ± 0.2	0.5 ± 0.2	0	0
***D. seriata* VEUL1M07BCMX**	16.5 ± 0.4	16.3 ± 2.1	11.9 ± 2.2	3.8 ± 0.5	0.8 ± 0.1	0
***D. seriata* VEUL1M10BCMX**	16.6 ± 0.3	13.2 ± 0.4	11.8 ± 0.6	6.8 ± 0.5	0.9 ± 0.1	0
***D. seriata* VEUL2M12BCMX**	16.5 ± 0.3	12.04 ± 0.1	14.1 ± 0.1	6.5 ± 1.0	0.3 ± 0.1	0

Media ± average standard deviation.

2.4.3 Pathogenicity assays of grapevine trunk fungi isolated from 'Mission' grapevines on three cultivars

Among the different strains of grapevine trunk disease fungi evaluated, the isolate *L. brasiliensis* BCMXL28, that was used as a positive control, produced the most extensive lesions on detached canes of all three cultivars, with sizes ranging from 6.3 ± 1.00 cm to 7.35 ± 0.74 cm (Figure 7). The *D. ampelina* HMV20BCMX strain exhibited intermediate virulence, causing necrotic lesions of 3.86 ± 1.06 cm in 'Mission' and 'Malvasia Blanca' cultivars, while in 'Tempranillo,' lesions were only 1.90 ± 0.87 cm (Table 4, Figure 6).

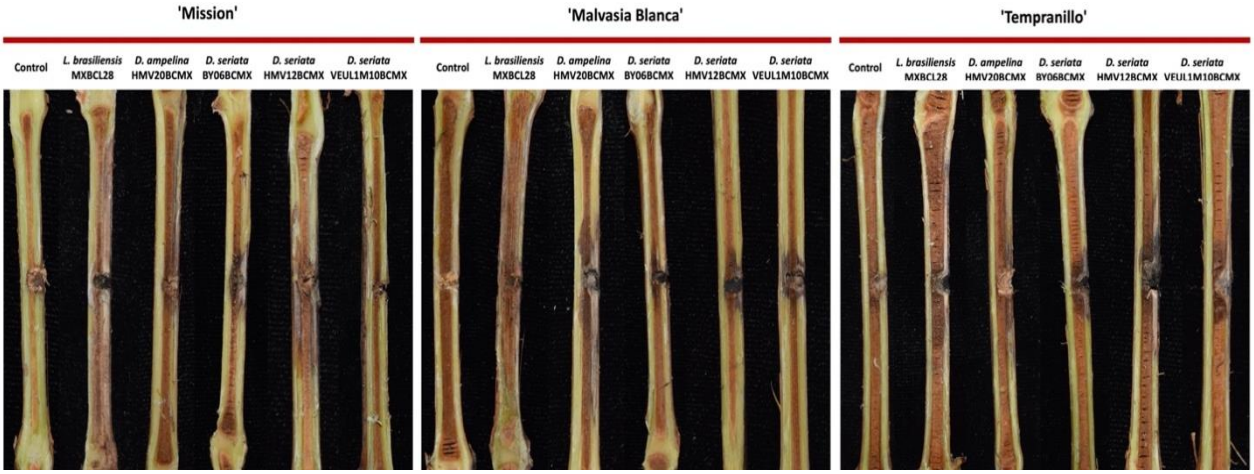

Figure 7. Representative images of canes from three grapevine cultivars, 50 days after inoculation with grapevine trunk diseases fungi of the genera *Lasiodiplodia* (MXBCL28), *Diaporthe* (HMVOLL20BCMX), and *Diplodia* (BY06BCMX, HMV12BCMX, VEUL1M10BCMX).

Table 4. Mean internal necrotic lesion length in three grapevine varieties caused by grapevine trunk diseases fungi.

Treatment	Lesion length (cm)		
	'Mission'	**'Malvasia Blanca'**	**'Tempranillo'**
Control	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**
***L. brasiliensis* BCMXL28**	7.35 ± 0.74 **i**	6.5 ± 1.81 **h**	6.3 ± 1.00 **f**
***D. seriata* BY06BCMX**	1.16 ± 0.44 **b**	2.48 ± 1.23 **f**	1.90 ± 0.87 **bcd**
***D. ampelina* HMV20BCMX**	3.86 ± 1.06 **h**	3.9 ± 0.45 **g**	2.48 ± 0.64 **de**
***D. seriata* EF14BCMX**	1.64 ± 0.68 **bcdef**	0.94 ± 0.26 **abc**	1.19 ± 0.45 **bc**
***D. seriata* HMV01BCMX**	1.5 ± 0.67 **bcd**	1.35 ± 0.16 **cde**	1.17 ± 0.37 **bcd**
***D. seriata* HMV04BCMX**	2.33 ± 0.63 **cdefg**	1.36 ± 0.47 **cde**	1.77 ± 0.73 **bc**
***D. seriata* HMV06BCMX**	1.09 ± 0.40 **b**	1.06 ± 0.18 **bc**	1.11 ± 0.45 **bc**
***D. seriata* HMV10BCMX**	2.70 ± 0.67 **g**	2.32 ± 0.66 **ef**	1.86 ± 0.41 **d**
***D. seriata* HMV12BCMX**	2.62 ± 1.03 **efg**	2.29 ± 0.98 **ef**	1.07 ± 0.34 **bc**
***D. seriata* RP05BCMX**	1.29 ± 0.40 **bc**	2.18 ± 0.73 **def**	1.47 ± 0.57 **bcd**
***D. seriata* R2HG01BCMX**	0.98 ± 0.53 **ab**	1.23 ± 0.26 **bcd**	2.71 ± 0.88 **e**
***D. seriata* VEUL1M03BCMX**	2.69 ± 1.03 **fg**	0.21 ± 0.09 **ab**	1.38 ± 0.45 **bc**
***D. seriata* VEUL1M07BCMX**	1.18 ± 0.51 **b**	1.23 ± 0.32 **bc**	0.85 ± 0.25 **ab**
***D. seriata* VEUL1M10BCMX**	1.57 ± 0.50 **bcde**	1.23 ± 0.27 **bcd**	1.24 ± 0.46 **bc**
***D. seriata* VEUL2M12BCMX**	2.55 ± 0.98 **defg**	0.8 ± 0.38 **ab**c	1.92 ± 0.53 **cd**

Average ± standard deviation. Different letters indicated statistical differences (α<0.05). Significance letters were grouped based on HDS analysis (P< 0.05).

All *D. seriata* strains evaluated exhibited low levels of virulence compared with *L. brasiliensis* BCMXL28; however, they varied in behavior. Some strains caused necrotic lesions of similar lengths across the cultivars. For example, *D. seriata* HMV06BCMX produced lesions of 1.09 ± 0.40 cm in 'Mission,' 1.06 ± 0.18 cm in 'Malvasia Blanca,' and 1.11 ± 0.45 cm in 'Tempranillo.' Other strains caused larger lesions in a single cultivar. For instance, *D. seriata* HMV12BCMX generated lesions of 2.62 ± 1.03 cm in 'Mission,' 2.29 ± 0.98 cm in 'Malvasia Blanca,' and 1.07 ± 0.34 cm in 'Tempranillo' (Table 4).

Additionally, some strains of *D. seriata,* such as EF14BCMX, HMV04BCMX, VEUL1M03BCMX, VEUL2M12BCMX, and HMV10BCMX, produced larger necrotic lesions in 'Mission' than in the other two cultivars. *Diplodia seriata* BY06BCMX caused lesions measuring 2.48 ± 1.23 cm in 'Malvasia Blanca,' which were longer than those produced by this strain in the other two cultivars. In 'Tempranillo,' *D. seriata* R2HG01BCMX caused lesions measuring 2.71 ± 0.88 cm, which were longer than those caused by this same strain in 'Mission' (0.98 ± 0.53) and 'Malvasia Blanca' (1.23 ± 0.26) (Table 4). At the end of the experiment, all inoculated fungi were recovered from the infected canes, and their species identities were verified based on their morphological characteristics.

2.4.4 Determination of the growth rate of grapevine trunk fungi associated with 'Mission' grapevines in different carbon sources

The five grapevine trunk fungi evaluated in this assay exhibited the highest growth rates when cultivated in MM9 supplemented with grapevine wood. All strains displayed sparse growth and a light gray mycelium in this medium (Table 5). Specifically, *D. ampelina* HVM20BCMX showed a slow growth rate, ranging from 0.9 mm per day on glucose to 3.2 mm on grapevine wood. No significant differences in growth were observed for this strain when inoculated in MM9 or with twelve different carbon sources (Table 5).

The *D. seriata* HMV12BCMX strains exhibited significant growth in MM9 supplemented with grapevine wood. However, no differences in growth were observed when the strains were cultured in MM9 or with the other twelve carbon sources (Table 5 and Figure 8). On the other hand, the *D. ampelina* HMV20BCMX, *D. seriata* EF14BCMX, *D. seriata* HMV12BCMX*, D. seriata* VEUL1M03 and *D. seriata* VEUL2M07BCMX demonstrated faster growth in wood-supplemented MM9. The lowest growth rates were recorded when all fungal strains growth in medium supplemented with pectin (1.9 mm/day).

D. seriata VEUL2M07BCMX displayed the highest growth rate in MM9 supplemented with grapevine wood (13.2 mm/day). In contrast, significantly lower growth rates were observed in media supplemented with xylose (1.5 mm/day) and starch (1.5 mm/day).

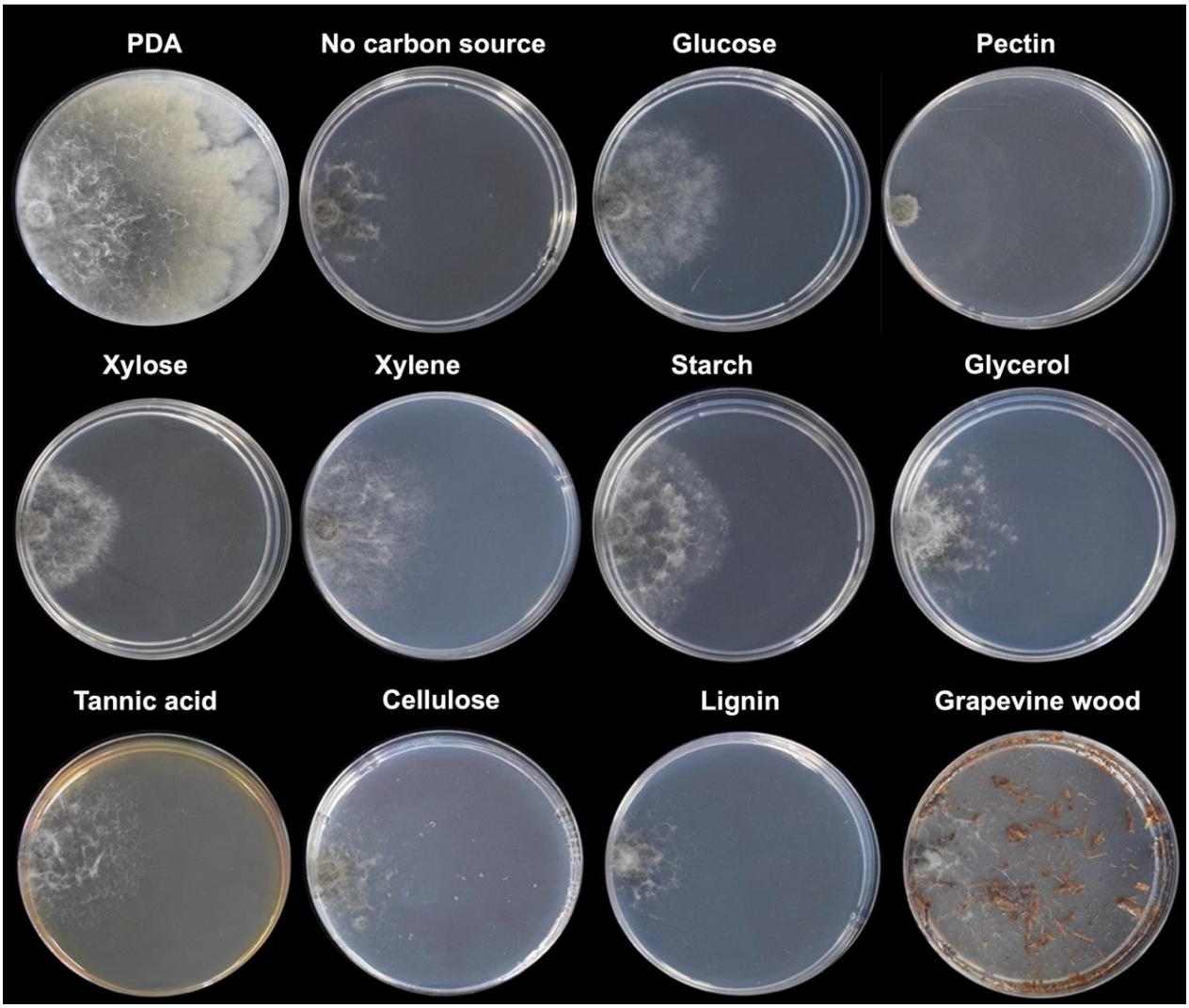

Figure 8. Representative images of *D. seriata* HMV12BCMX growth in Potato dextrose Agar (PDA), minimal medium 9 (MM9) without carbon source, or MM9 supplemented with different carbon sources.

Table 5. Growth rates of five grapevine trunk fungi associated with 'Mission' grapevine in different carbon sources

Carbon source	Growth rate (mm/day)				
	D. ampelina **HMV20BCMX**	***D. seriata*** **EF14BCMX**	***D. seriata*** **HVM12BCMX**	***D. seriata*** **VEUL1M03BCMX**	***D. seriata*** **VEUL2M07BCMX**
No carbon source	1.2 ± 0.2 **b**	2.2 ± 0.3 **b**	6.3 ± 0.4 b	5.2 ± 0.6 **b**	4.1 ± 0.4 **b**
Glucose	0.9 ± 0.2 **b**	1.8 ± 0.2 **b**	6.2 ± 0.4 **b**	5.2 ± 0.4 **b**	2.1 ± 0.4 **c**

Cellulose	1.2 ± 0.3 **b**	2.9 ± 0.2 **b**	5.8 ± 0.55 **b**	3.7 ± 0.6 **bc**	3.8 ± 0.3 **b**
Xylose	1.0 ± 0.2 **b**	1.9 ± 0.1 **b**	5.8 ± 0.5 **b**	2.7 ± 0.1 **c**	1.2 ± 0.5 **c**
Pectin	1.8 ± 0.1 **b**	3.1 ± 0.4 **b**	5.8 ± 0 **b**	1.9 ± 0.3 **c**	4.4 ± 0.9 **b**
Starch	1.4 ± 0.3 **b**	1.2 ± 0.23 **b**	5.8 ± 0.7 **b**	4.4 ± 0.5 **bc**	1.5 ± 0.5 **c**
Tannic acid	1.4 ± 0.1 **b**	2.2 ± 0.2 **b**	6.0 ± 0.5 **b**	4.0 ± 0 4 **bc**	4.4 ± 0.7 **b**
Lignin	0.8 ± 0.3 **b**	3.8 ± 0.4 **b**	5.4 ± 0.27 **b**	4.3 ± 0.5 **b**	4.4 ± 0.6 **b**
Xylene	1.0 ± 0.2 **b**	3.3 ± 0.2 **b**	6.0 ± 0.3 **b**	4.9 ± 0.7 **b**	4.9 ± 0.5 **b**
Glycerol	1.2 ± 0.4 **b**	3.4 ± 0.5 **b**	5.6 ± 0.2 **b**	4.4 ± 0.5 **bc**	3.5 ± 0.1 **bc**
Arabinose	1.3 ± 0.4 **b**	2.1 ± 0.3 **b**	6.1 ± 0.5 **b**	4.0 ± 0.4 **bc**	4.1 ± 0.2 **b**
Galactose	1.1 ± 0.1 **b**	2.5 ± 0.1 **b**	5.8 ± 0.2 **b**	5.5 ± 0.3 **b**	3.8 ± 1.0 **bc**
Glycogen	1.0 ± 0.2 **b**	1.4 ± 0.3 **b**	5.8 ± 0.2 **b**	5.3 ± 0.5 **b**	3.6 ± 0.2 **bc**
Grapevine wood	3.2 ± 0.2 **a**	13.6 ± 0 **a**	11.9 ± 0.1 **a**	11.4 ± 0.4 **a**	13.2 ± 0.2 **a**

Average ± standard deviation. Different letters indicated statistical differences (α<0.05). Significance letters were grouped based on HDS analysis (P< 0.05).

2.4.5 *Diplodia seriata* HMV12BCMX and *L. brasiliensis* L28BCMX as grapevine cell wall-degrading pathogens

For the histological analysis, canes inoculated with water (control), *D. seriata* HMV12BCMX, and *L. brasiliensis* MXBCL28 in both 'Mission' and 'Tempranillo' cultivars were selected from the pathogenicity test. Canes from the control treatment exhibited no necrotic lesions, whereas those inoculated with *D. seriata* HMV12BCMX showed lesions up to 2.62 ± 1.03 cm, and those inoculated *with L. brasiliensis* MXBCL28 displayed a lesion length of 7.35 ± 0.74 cm.

In both 'Mission' and 'Tempranillo' cultivars, the control canes dyed with potassium iodide solution showed the presence of starch stained in black, primarily in the xylem rays (Figure 9). In contrast, canes inoculated with *D. seriata* HMV12BCMX and *L. brasiliensis* MXBCL28 displayed unstained xylem rays, phloem, and pith, suggesting starch depletion in these tissues. Notably, among the two pathogenic strains evaluated; the canes inoculated with *L. brasiliensis* MXBCL28 exhibited a greater area of starch depletion (Figure 9).

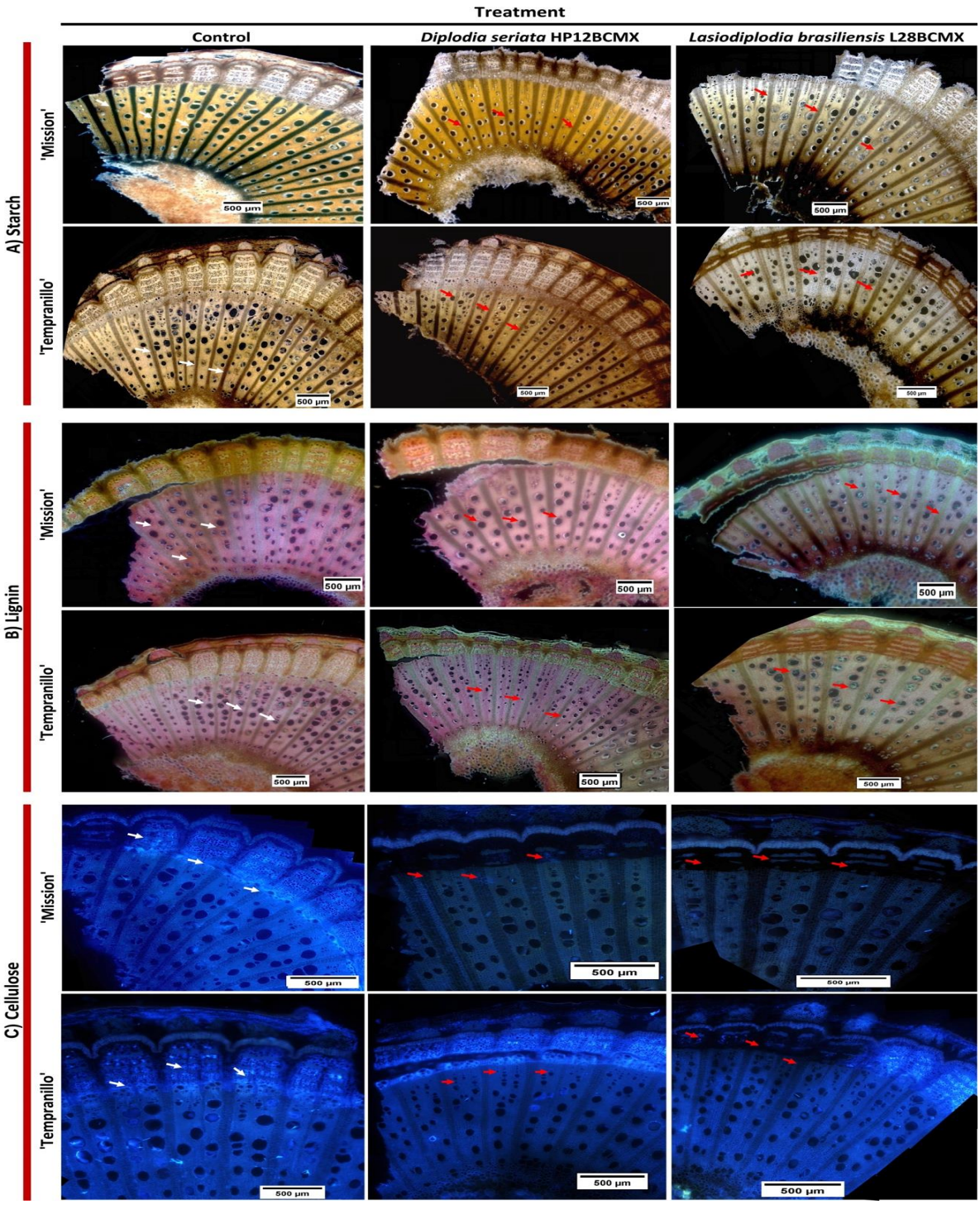

Figure 9. Representative images of 'Mission' and 'Tempranillo' grapevines canes with starch, lignin, and cellulose degradation by *D. seriata* HMV12BCMX and *L. brasiliensis* MXBCL28. A) In grapevine tissue, starch is observed in the control treatment as dark areas in the rays of the parenchyma (white arrows). In contrast, in the infected tissue, areas without darkening are observed (red arrows), which indicates the absence of starch in the tissue. B) The presence of lignin in the grapevine tissue was observed by staining with 0.1% phloroglucinol-HCl. In the control treatment, the tissue shows a purple-pink color (white arrow), indicating lignin presence. The infected grapevine tissue is light pink due to the degradation of lignin (red arrow). C) The presence of cellulose in the grapevine tissue stained with calcofluor was observed with epifluorescence. The control treatment tissue presented a blue color (white arrows). The tissues infected by the pathogenic strain it had a brown color due to the lack of cellulose (red arrows).

In the control plants of both cultivars, sections dyed with phloroglucinol-HCl to determine the presence of lignin, resulted in a purple-pink coloration of the tissue (Figure 9). In contrast, tissue inoculated with *D. seriata* HMV12BCMX displayed a lighter pink hue, indicating a lower lignin content. Grapevine tissue from both cultivars inoculated with *L. brasiliensis* MXBCL28 showed fewer light pink areas, predominantly surrounding the xylem vessels and near the wound site (Figure 9).

In the control plants of both cultivars, cellulose in the grapevine tissue, as observed using Calcofluor M2R White, displayed uniform fluorescence throughout the section (Figure 9). However, brown areas were observed in the tissue inoculated with *D. seriata* HMV12BCMX and *L. brasiliensis* MXBCL28, indicating a lack of cellulose in the samples. A comparison between samples inoculated with the pathogenic fungi in the two cultivars revealed that the 'Mission' cultivar exhibited a larger area with brown coloration. Conversely, in the 'Tempranillo' cultivar, the absence of cellulose was observed primarily in the region adjacent to the inoculation site (Figure 9).

Although disease progression caused by *D. seriata* HMV12BCMX may be lower, overall, no differences were observed between the cultivars regarding how both pathogenic fungi degraded components of the cell wall and non-structural carbohydrates. This suggests that these fungi exhibit similar behavior during their grapevine colonization process, irrespective of the cultivar.

2.5 Discussion

In this work, the role of 'Mission' grapevines as a reservoir of grapevine trunk diseases fungi was studied for the first time. Two species of grapevine trunk fungi, *D. ampelina,* and *D. seriata*, were identified in association with 'Mission' grapevines. In Mexico, *D. seriata* was first reported in vineyards in Sonora and Baja California in 2008 (Úrbez-Torres et al., 2008). Subsequently, this fungus was identified again in vineyards in Baja California and Coahuila (Rangel-Montoya et al., 2024). As for the *D. ampelina,* this strain had not been previously recorded in the country; this study represents the first documentation of this fungus associated with grapevines in Mexico.

In commercial vineyards in Mexico, fungi from the genera *Botryosphaeria*, *Eutypa*, *Eutypella, Lasiodiplodia*, *Neofusicoccum*, *Phaeomoniella,* and *Phacremonium* have been isolated (Telliz et al., 1978; Úrbez-Torres et al., 2008; Úrbez-Torres et al., 2008; Candolfi-Carballo, 2009; Morales-Pedraza, 2010; Paolinelli-Alfonso et al., 2015; Rangel-Montoya et al., 2021; Rangel-Montoya et al., 2024). Among these, *L. brasiliensis*, *L. gilanesis* and *N. parvum* are noted for their high virulence (Rangel-Montoya et al., 2021). Interestingly, the

fungal richness found in 'Mission' grapevines was limited to only two species. This limited richness could be attributed to several factors. One possibility is that other trunk disease fungi introduced more recently through plant material from different regions, have not yet established themselves in these older vines. Another possibility is that the microbiota present on the 'Mission' grapevines might be inhibiting the colonization of additional pathogens. Additionally, the selective dry environment could also be a key factor in the observed limited fungal richness.

Fungal species of the *Diaporthe* genus have been identified as causal agents of Phomopsis dieback, with *D. ampelina* being one of the primary causal agents of this disease. This strain is noted for its high virulence and ability to infect any green tissue of the plant (Úrbez-Torres et al., 2013; Gramaje et al., 2018). *Diaporthe* strains have been primarily reported in vineyards in Europe and the USA (Úrbez-Torres et al., 2013; Guarnaccia et al., 2018). The strain of *D. ampelina* identified in this study exhibited a white colony with concentric rings, which forms pycnidia from which two types of conidia are released, appearing sack-like. Notably, the alpha conidia were observed to be longer than previously reported, while beta conidia dimensions were consistent with earlier findings (Gomes et al., 2013; Wilcox et al., 2016).

Diplodia seriata was the most abundant fungi isolated from symptomatic plant tissue. This fungal species has been identified in vineyards across various countries, including Australia (Savocchia et al., 2007), Mexico (Úrbez-Torres et al., 2008; Rangel-Montoya et al., 2024), USA (Úrbez-Torres and Gubler, 2009), Iran (Mohammadi et al., 2013), Spain (Elena et al., 2015), Hungary (Kovács et al., 2017), Tunisia (Chebil et al., 2017), Czech Republic (Spetik et al., 2023) among others. All strains of *D. seriata* isolated from 'Mission' grapevine exhibited dark gray colonies with low production of aerial mycelium when cultured on PDA medium. The conidia were cylindrical, measuring 22.5–26 × 9.5–14 µm, initially hyaline and turning brown as they mature, with some exhibiting septa. The conidial dimensions of *D. seriata* strains obtained are consistent with those reported for this species in vineyards in Mexico (Úrbez-Torres et al., 2011; Rangel-Montoya et al., 2024).

Temperature is a critical factor in the life cycle of fungi, as it typically affects their growth, spore production, and infection rate. Furthermore, fungal strains that adapt quickly to increased temperatures often exhibit greater virulence (Hunjan et al., 2020; Nnadi et al., 2021; Seidel et al., 2024). For instance, *L. brasiliensies* MXBCL28 and *L. exigua* MXVS5 are highly virulent and able to grow even at 37°C. (Rangel-Montoya et al., 2021). Grapevine trunk disease fungi isolated from 'Mission' showed optimal growth temperatures between 25 and 28°C. Specifically, *D. ampelina* did not exhibit good growth at temperatures exceeding 30°C. In contrast, *D. seriata* strains showed minimal growth at temperatures above 37°C. Other

strains of *D. seriata* isolated from irrigated commercial vineyards of Mexico have the same optimal growth temperature as those from 'Mission' grapevines (Rangel-Montoya et al., 2024). Understanding the relationship between temperature and fungal growth could help managing trunk diseases fungi.

In the pathogenicity test, all evaluated strains caused necrotic lesions in 'Mission,' 'Tempranillo,' and 'Malvasia Blanca.' Different strains of *D. seriata* isolated from 'Mission' plants caused small necrotic lesions. This species is reported to be a low-virulence pathogen (Elena et al., 2015). In canes of 'Tempranillo,' *D. seriata* obtained from different regions in Spain caused necrotic lesions of 0.23 to 2.02 cm (Elena et al., 2015). Úrbez-Torres et al., (2008) reported the pathogenicity of five strains of *D. seriata* isolated in Mexican vineyards; these isolates caused necrotic lesions in one-year-old cuttings and green shoots of the 'Chardonnay' and 'Thompson Seedless.' In another study, Rangel-Montoya et al., (2021) indicated that one strain of *D. seriata* RFP05MX can cause lesions smaller than 1.5 cm in green and woody tissue, while strains of *D. seriata* isolated in Chilean vineyards produced vascular lesions ranging from 2.6 and 13.3 cm in 'Cabernet Sauvignon' (Larach et al., 2023).

On the other side, *D. ampelina* HP20BCMX exhibited greater virulence than the other strains associated with 'Mission' grapevines. This fungal species is recognized as having high virulence (Van Niekerk et al., 2005). In 'Cardinal' grapevines, the pathogenicity of the *D. ampelina* MH143Trs was confirmed; after two months, this fungus caused necrotic lesions up to 2.72 ± 0.6 cm (Akgül and Ahioğlu 2019). In the Western

Cape province of South Africa, three isolates of *D. ampelina* were identified that, when inoculated in 'Bukettraube' shoots, caused necrotic lesions ranging from 4.88 to 6.56 cm (Lesuthu et al., 2019). The strain *D. ampelina* AFP282, isolated from 'Sultana' rootstock caused necrotic lesions of 0.97 to 1.32 cm when inoculated in 'Paulsen' rootstocks (Akgül et al., 2023). The observed variability in virulence among different strains of *D. seriata* and *D. ampelina* may be attributed to factors such as the grapevine varieties used in the pathogenicity test, inoculation conditions, type of inoculum, incubation conditions, and the infective capacity of the strains.

The virulence of trunk disease fungi is linked to their ability to degrade cell wood components (cellulose, hemicellulose, lignin) and plant defense compounds, as well as their capacity to colonize the plant tissues (Cruz-Lopes et al., 2014; Claverie et al., 2020). Generally, fungi from the Botryosphaeriaceae produce various cell wall-degrading enzymes. The most crucial enzymes target pectin, hemicellulose, and xylan, which enable the fungus to penetrate and invade tissues, as well as to extract nutrients for growth (Stempien et al., 2017; Nazar et al., 2022; Belair et al., 2023). The evaluation of the ability of *D. ampelina*

and *D. seriata* isolates to utilize different carbon sources associated with plant structural and storage components, the strains showed that the strains presented low growth rates on the various inoculated sources and minimal aerial mycelium formation. In contrast, when inoculated in grapevine wood, all strains showed the highest levels of growth. This differs from findings for *L. brasiliensis* MLXBCL28, which demonstrated a high growth rate when inoculated in pectin or grapevine wood, along with the formation of dense, dark gray aerial mycelium on all carbon sources (Rangel-Montoya et al., 2023). Strains of *Diplodia* have shown low levels of cell wall degrading enzymes, which may be related to their low virulence (Belair et al., 2023). In contrast, strains *Diaporthe* produce cell wall-degrading enzymes as peroxidases, and oxidoreductases, among other enzymes (Morales-Cruz et al., 2015; Hilário and Gonçalves, 2023).

In the histology analysis carried out in this study, it was observed that *D. seriata* HMV12BCMX depletes starch, lignin, and cellulose from some areas of the 'Mission' and 'Tempranillo' grapevine tissue. However, this effect is less pronounced than that observed with *L. brasiliensis* MLXBCL28, both in our study and as reported by Rangel-Montoya et al., (2023).

The establishment and progression of fungi that cause grapevine trunk diseases are usually slow, so plant symptoms are often observed years after infection (Hrycan et al., 2020). This delay may be related to the fact that these fungi can initially exist as endophytes and only become aggressive pathogens when the plant is exposed to environmental stress conditions, such as high temperatures or low water availability (Calvo-Garrido et al., 2021; Fernandez et al., 2023). Fungi described as grapevine-associated endophytes include strains of *B. dothidea*, *D. ampelina* and *D. seriata*, among others (Hrycan et al., 2020; Belair et al., 2023; Hilário and Gonçalves, 2023). Considering the low levels of virulence observed in the pathogenicity assay of *D. ampelina* and *D. seriata* strains found in 'Mission' grapevines, as well as the plant´s subsistence without disease management, it is likely that these fungi persist as endophytes for a significant portion of the plant's life. Although these grapevine trunk diseases fungi associated with 'Mission' grapevines do not represent a serious problem due to their low virulence, it is necessary to establish management strategies to prevent the spread of these pathogens and ensure the health of the plant reproductive material.

In Baja California, arid climatic conditions predominate, these conditions directly affect grapevine crop yield. Given the tendency for arid conditions to increase in the state, it is essential to establish grapevine cultivars that are adapted to them. 'Mission' grapevines are a viable option as they have demonstrated the ability to maintain sustained production for many years, even under non-irrigated conditions. This work provides essential information on grapevine trunk diseases fungi associated with 'Mission' grapevines, which will aid in developing management to preserve this cultivar.

2.6. Bibliography

Akgül, D., & Ahioğlu, M. (2019). Fungal Pathogens Associated with Young Grapevine Decline in the Southern Turkey Vineyards. *BIO Web Conference, 15*, 10–27. https://doi.org/10.1051/bioconf/20191501027

Akgül, D. S., Güngör, Savaş N., Yildiz, M., Bülbül, İ., & Özarslandan, M. (2023). Current status of grapevine trunk disease pathogens on asymptomatic nursery-produced grapevines in Türkiye. *Phytopathologia Mediterranea, 62*(2), 151-163. https://doi.org/10.36253/phyto-14148

Aliquó, G., Torres, R., Lacombe, T., Boursiquot, J. M., Laucou, V., Gualpa, J., Fanzone, M., Sari, S., Perez Peña, J., & Prieto, J. A. (2017). Identity and parentage of some South American grapevine cultivars present in Argentina. *Australian Journal of Grape and Wine Research*, *23*, 452–460. https://doi.org/10.1111/ajgw.12282

Andrade, S. G., Arón N. & Flores, F. (2013). Matriz de insumo producto vitivinícola de Baja California México. *Revista de Economía*, *30*(81), 57–57. https://doi.org/10.33937/reveco.2013.41

Ávila Salazar, J. M., Moreno Salazar, S. F., Ávila-Luzanía, M. A.,Guzmán Ortíz, J. M., Navarro Nava, F. H., & Rentería Martínez M. E. (2020). *Greeneria uvicola* associated with die-back in vineyards of Sonora, Mexico. *Phytopathologia Mediterranea, 59*(3), 605-613. https://doi.org/10.14601/Phyto-11977

Belair, M., Restrepo-Leal, J.D., Praz, C., Fontaine, F., Rémond, C., Fernández, O., & Besaury, L. (2023). Botryosphaeriaceae gene machinery: Correlation between diversity and virulence. *Fungal Biology, 127* (5), 1010-1031. https://doi.org/10.1016/j.funbio.2023.03.004

Bettenfeld, P., Cadena, J. C., Jacquens, L., Fernandez, O., Fontaine, F., van Schaik, E., Courty, P. E., & Trouvelot, S. (2021). The microbiota of the grapevine holobiont: A key component of plant health. *Journal of Advanced Research*, *40*, 1-15. https://doi.org/10.1016/j.jare.2021.12.008

Blundell, R. & Eskalen, A. (2022). Biological and chemical pruning wound protectants reduce infection of grapevine trunk disease pathogens. *Califronia Agriculture*, *75*(3), 128-134. https://doi.org/10.3733/ca.2021a0018

Čabalová, I., Krilek, J., Kačík, F., Lagaňa, R., & Jurczyková, T. (2023) Valorization of Wood-Based Waste from Grapevine. *Forests,* 2023, *14*, 442. https://doi.org/10.3390/f14030442

Calvo-Garrido, C., Songy, A., Marmol, A., Roda, R., Clément, C., & Fontaine, F. (2021). Description of the relationship between trunk disease expression and meteorological conditions, irrigation and physiological response in Chardonnay grapevines. *OENO One*, *55*(2), 97–113. https://doi.org/10.20870/oeno-one.2021.55.2.4548

Candolfi-Arballo, O. (2009). Caracterización de anamorfos de Botryosphaeriaceae asociados a deterioro progresivo en vid (Vitis vinifera L.) aislados de la región vitivinícola de Baja California [Tesis de Maestría, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California]

Carlucci, A., Cibelli, F., Lops, F., & Raimondo, M. L. (2015). Characterization of *Botryosphaeriaceae* Species as Causal Agents of Trunk Diseases on Grapevines. *Plant Disease*, *99*(12), 1678–1688. https://doi.org/10.1094/PDIS-03-15-0286-RE

Claverie, M., Notaro, M., Fontaine, F., & Wéry, J. (2020). Current knowledge on Grapevine Trunk Diseases with complex etiology: a systemic approach. *Phytopathologia Mediterranea, 59*(1), 29-53. https://doi.org/10.36253/phyto-11150

Cruz-Lopes, L., Prozil, S., Evtuguin, D. V., Arshanitsa, A., Solodovnik, V., & Telysheva, G. M. (2014). Evaluation of grape stalks as a feedstock for pellets production. In *Proceedings of the 13th European Workshop on Lignocellulosics and Pulp*, Seville, Spain. https://www.researchgate.net/publication/263602517_Evaluation_of_grape_stalks_as_a_feedstock_for_pellets_production

Chebil S., Fersi R., Bouzid M., Quaglino F., Chenenaoui S., Melki, I., Durante G., Zacchi, E., Bahri, B. A., Bianco, P. A., & Rhouma, A. (2017). Fungi from the Diaporthaceae and Botryosphaeriaceae families associated with grapevine decline in Tunisia. *Ciencia e investigación agraria: revista latinoamericana de ciencias de la agricultura, 44*(2), 127-130. http://dx.doi.org/10.7764/rcia.v44i2.1625.

Comisión Nacional del Agua (CONAGUA) (2023). Reporte anual del clima en México 2023. Coordinación General del Servicio Meteorologico Nacional, *13* (13). https://smn.conagua.gob.mx/tools/DATA/Climatolog%C3%ADa/Diagn%C3%B3stico%20Atmosf%C3%A9rico/Reporte%20del%20Clima%20en%20M%C3%A9xico/Anual2023.pdf

Elena, G., Garcia-Figueres, F., Reigada S., & Luque J. (2015). Intraspecific variation in *Diplodia seriata* isolates occurring on grapevines in Spain. *Plant Pathology, 64*, 680-689. https://doi.org/10.1111/ppa.12296

Fernandez O., Lemaître-Guillier C., Songy, A., Robert-Siegwald G.; Lebrun, M. H., Schmitt-Kopplin, P., Larignon, P., Adrian, M. & Fontaine, F. (2023). The Combination of Both Heat and Water Stresses May Worsen Botryosphaeria Dieback Symptoms in Grapevine. *Plant, 12*, 753. https://doi.org/10.3390/plants12040753

Gomes, R. R., Glienke, C., Videira, S. I., Lombard, L., Groenewald, J. Z., & Crous, P. W. (2013). *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia, 31*, 1-41. 10.3767/003158513X666844

Góngora-Rosado, M. A. (2016). Propuestas de prácticas sustentables en la industria vitivinícola de Baja California [Tesis de Maestría en Administración Integral del Ambiente. Colegio de la Frontera Norte, A. C. y el Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California]

Guarnaccia, V., Groenewald, J. Z., Woodhall, J., Armengol, J., Cinelli, T., Eichmeier, A., Ezra, D., Fontaine, F., Gramaje, D., Gutierrez-Aguirregabiria, A., Kaliterna, J., Kiss, L., Larignon, P., Luque, J., Mugnai, L., Naor, V., Raposo, R., Sándor, E., Váczy, K. Z., & Crous, PW. (2018). *Diaporthe* diversity and pathogenicity revealed from a broad survey of grapevine diseases in Europe. *Persoonia, 40*, 135-153. https://doi.org/10.3767/persoonia.2018.40.06

Gutiérrez-Gamboa, G., Liu, S., & Pszczólkowski, P. (2019). Resurgence of minority and autochthonous grapevine varieties in South America: A review about their oenological potential. *Journal of the Science of Food and Agriculture, 100*(2) 465-482. 10.1002/jsfa.10003

Gramaje D., Úrbez-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*, 12–39. https://doi.org/10.1094/PDIS-04-17-0512-FE

Hilário, S., & Gonçalves, M. F. M. (2023). Mechanisms Underlying the Pathogenic and Endophytic Lifestyles in *Diaporthe*: An Omics-Based Approach. *Horticulturae, 9*, 423. https://doi.org/10.3390/horticulturae9040423

Hrycan, J., Hart, M., Bowen, P., Forge, T., & Úrbez-Torres, J.R. (2020). Grapevine trunk disease fungi: their roles as latent pathogens and stress factors that favour disease development and symptom expression. *Phytopathologia Mediterranea, 59*(3), 395-424. https://doi.org/10.14601/Phyto-11275

Hunjan, M. S., & Lore, J. S. (2020). Climate Change: Impact on Plant Pathogens, Diseases, and Their Management. In: Jabran, K., Florentine, S., Chauhan, B. (eds) *Crop Protection Under Changing Climate* (pp. 99-139). Springer.

International Organization of Vine and Wine (OIV). (2018). *Distribution of the world's grapevine varieties. Focus OIV 2017*. https://www.oiv.int/public/medias/5888/en-distribution-of-the-worlds-grapevine-varieties.pdf

International Organization of Vine and Wine (OIV). *Annual assessment of the world vine and wine sector in 2021*. Intergobemental organization. https://www.oiv.int/sites/default/files/documents/OIV_Annual_Assessment_of_the_World_Vine_and_Wine_Sector_in_2021.pdf

International Organization of Vine and Wine (OIV). (2022). *State of the world vine and wine sector in 2022.* https://www.oiv.int/sites/default/files/documents/OIV_State_of_the_world_Vine_and_Wine_sector_in_2022_2.pdf

Kenfaoui, J., Radouane, N., Mennani, M., Tahiri, A., El Ghadraoui, L., Belabess, Z., Fontaine, F., El Hamss, H., Amiri, S., Lahlali, R., & Ait Barka, E. (2022). A Panoramic View on Grapevine Trunk Diseases Threats: Case of Eutypa Dieback, Botryosphaeria Dieback, and Esca Disease. *Journal of Fungi,* 2022, *8*, 595. https://doi.org/10.3390/jof8060595

Kovács C., Balling P., Bihari Z., Nagy A., & Sándor E. (2017). Incidence of grapevine trunk diseases is influenced by soil, topology and vineyard age, but not by *Diplodia seriata* infection rate in the Tokaj Wine Region, Hungary. *Phytoparasitica, 45*, 21–32. https://doi.org/10.1007/s12600-017-0570-5

Larach, A., Vega-Celedón, P., Salgado, E., Salinas, A., Riquelme, N., Castillo-Novales, D., Sanhueza, P., Seeger, M., & Besoain, X. (2023). Higher Virulence of *Diplodia seriata* Isolates on Vines of cv. Cabernet Sauvignon Associated with 10-Year-Old Wood Compared to Young Tissue. *Plants, 12*, 2984. https://doi.org/10.3390/plants12162984

Lesuthu, P., Mostert, L., Spies, C. F. J., Moyo, P., Regnier, T., & Halleen, F. (2019). *Diaporthe nebulae* sp. nov. and First Report of *D. cynaroidis***,** *D. novem*, and *D. serafiniae* on Grapevines in South Africa. *Plant Disease, 103*(5): 808-817. https://doi.org/10.1094/PDIS-03-18-0433-RE

Liu, D., & Howell, K. (2021). Community succession of the grapevine fungal microbiome in the annual growth cycle. *Enviromental Microbiology*, *23*, 1842-1857. https://doi.org/10.1111/1462-2920.15172

López-Franco, F. (2012). Los vitivinicultores de la región de Baja California: un acercamiento a su comportamiento informativo. *Biblioteca Universitaria*, *15*(1), 49-61. Doi: https://doi.org/10.22201/dgb.0187750xp.2012.1.41

Magoni, C. (2009). *Historia de la vid y el vino en la Península de la Baja California*. 1ra ed.Universidad Iberoamericana, Tijuana (pp: 283)

Magoni, C. (2021). Recuento histórico de la vitivinicultura en México, con énfasis en Baja California. *En Vinos y variedades patrimoniales. Resumen de las primeras jornadas Latinoamericanas* (pp: 21-22). Prieto, J.A. (Ed.). INTA Ediciones.

Martain-Amozurrutia., F. (2021). Experiencias comerciales: México. *En Vinos y variedades patrimoniales. Resumen de las primeras jornadas Latinoamericanas* (pp 47-46). Prieto, J.A. (Ed) INTA Ediciones.

Martínez-Diz, M. D. P., Díaz-Losada, E., Andrés-Sodupe, M., Bujanda, R., Maldonado-González, M. M., Ojeda, S., Yacoub, A., Rey, P., & Gramaje, D. (2021). Field evaluation of biocontrol agents against black-foot and Petri-diseases of grapevine. *Pest Management Science*, *77*, 697–708. https://doi.org/10.1002/ps.6064

Mondello, V., Larignon, P., Armengol, J., Kortekamp, A., Vaczy K., Serrano, E., Rego, C., Mugnai, L., & Fontaine, F. (2018). Management of grapevine trunk diseases: Knowledge transfer, current strategies and innovative strategies adopted in Europe. *Phytopathologia Mediterranea, 57*(3): 369–383. https://doi.org/10.14601/Phytopathol_Mediterr-23942

Morales-Cruz, A., Amrine, K. C. H., Blanco-Ulate, B., Lawrence, D. P., Travadon, R., Rolshausen P. E., Baumgartner K., & Cantu, D. (2015). Distinctive expansion of gene families associated with plant cell wall degradation, secondary metabolism, and nutrient uptake in the genomes of grapevine trunk pathogens. *BMC Genomics, 16*, 469. https://doi.org/10.1186/s12864-015-1624-z

Morales-Pedraza, L. G. (2010). Caracterización de hongos asociados a la enfermedad de Petri y Esca en el valle de Guadalupe, Baja California [Tesis de Maestría en Ciencias, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California]

Nakano J., & Meshitsuka G. (1992). The detection of lignin. In: *Methods in lignin chemistry* (pp 23-32). S.Y. Lin, C.W. Dence, (Eds.) Springer. https://doi.org/10.1007/978-3-642-74065-7_2ç

Nazar, P. F., Pedrosa, B., Oliveira, M., Fidalgo, C., Devreese, B., Driessche, G. V., Félix, C., Rosa, N., Alves, A., Duarte, A. S., & Esteves, A. C. (2022). Unveiling the Secretome of the Fungal Plant Pathogen *Neofusicoccum parvum* Induced by *In Vitro* Host Mimicry. *Journal of Fungi*, *8,* 971. https://doi.org/10.3390/jof8090971

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

Niem, J. M., Billones-Baaijens, R., Stodart, B. J., Reveglia, P., & Savocchia, S. (2023). Biocontrol Potential of an Endophytic *Pseudomonas poae* strain against the Grapevine Trunk Disease Pathogen *Neofusicoccum luteum* and Its Mechanism of Action. *Plants, 12*, 2132. https://doi.org/10.3390/plants12112132

Nnadi, N. E., & Carter, D. A. (2021). Climate change and the emergence of fungal pathogens. *PLoS, pathogens, 17* (4). https://doi.org/10.1371/journal.ppat.1009503

Paolinelli-Alfonso, M., Serrano-Gómez, C., & Hernández-Martínez, R. (2015). Occurrence of Eutypella microtheca in grapevine cankers in Mexico. *Phytopathologia Mediterranea*, *54*(1), 86–93. https://doi.org/10.14601/Phytopathol_Mediterr-14998

Papadopoulou, E., Bekris, F., Vasileiadis, S., Papadopoulou, K. K., & Karpouzas, D.G. (2022). Different factors are operative in shaping the epiphytic grapevine microbiome across different geographical scales: biogeography, cultivar, or vintage. *Journal of Sustainable Agriculture and Environment*, *1*, 287-301. https://doi.org/10.1002/sae2.12030

Phillips, A. J. L., Alves, A., Abdollahzadeh, J., Slippers, B., Wingfeld, M. J., Groenewald, J. Z., & Crous, P. W. (2013). The *Botryosphaeriaceae*: genera and species known from culture. *Studies in Mycology*, *76*, 51–167. https://doi.org/10.3114/sim0021

Rabiey, M., Hailey, L. E., Roy, S. R., Grenz, K., Al-Zadjali, M. A. S., Barrett, G. A., & Jackson, R. W. (2019). Endophytes vs. tree pathogens and pests: Can they be used as biological control agents to improve tree health? *European Journal of Plant Pathology*, *155,* 711–729. https://doi.org/10.1007/s10658-019-01814-y

Rangel-Montoya, E. A., Paolinelli-Alfonso, M., Rolshausen, P. E., Valenzuela-Solano, C., & Hernandez-Martinez, R., (2021). Characterization of *Lasiodiplodia* species associated with grapevines in Mexico. *Phytopathologia Mediterranea, 60*(2), 237–251. https://doi.org/10.36253/phyto-12576

Rangel-Montoya, E.A., Rolshausen, P.E., & Hernández-MartÍnez. R. (2023). Unravelling the colonization mechanism of *Lasiodiplodia brasiliensis* in grapevine plants. *Phytopathologia Mediterranea, 62*(2) 135-149. https://doi.org/10.36253/phyto-14198

Rangel-Montoya, E. A., Candolfi-Arballo, O., Obrador-Sánchez, J. A., Valenzuela-Solano, C., & Hernández-Martínez, R. (2024). Enhancing epidemiological knowledge of Botryosphaeriaceae in Mexican vineyards. *Phytopathologia Mediterranea*, 191–206. https://doi.org/10.36253/phyto-15292

Rangel-Montoya, E. A. & Hernandez-Martinez, R. (2024). Identification and pathogenicity of *Aspergillus* species associated with vine canker in Mexico. *Journal of Plant Pathology,* 1-14, https://doi.org/10.1007/s42161-024-01686-0

Rodríguez-Torres, I. (2017). *Variedades de vid cultivadas en Canarias: Descriptores morfólogicos, caracterización morfológica molecular, agrónomica y enológica* (2da ed). ICIA.

Ruzin S.E. (1999). *Plant microtechnique and microscopy* (Vol. 198). New York: Oxford University Press.

Savocchia S., Steel C. C., Stodart, B.J., & Somers, A. (2007). Pathogenicity of Botryosphaeria species from declining grapevines in subtropical regions of Eastern Australia. *Vitis*, *46*(1), 27-32.

Schilling, M., Farine, S., Péros, J. P., Bertsch, C., & Gelhaye, E. (2021). Wood Degradation in Grapevine Diseases. In *Advances in Botanical Research*; Elsevier: Amsterdam, The Netherlands *99*, 175–207. 10.1016/bs.abr.2021.05.007

Secretaria de Fomento Agropecuario del Gobierno del Estado (SEFOA). (2011). Estudio estadístico sobre producción de uva en Baja California, GobBC, OEIDRUS, SAGARPA. https://www.nacionmulticultural.unam.mx/empresasindigenas/docs/1873.pdf

Seidel, D., Wurster, S., Jenks, J. D., Sati, H., Gangneux, J. P., Egger, M., Alastruey-Izquierdo, A., Ford, N. P., Chowdhary, A., Sprute, R., Cornely, O., Thompson, G. R. 3rd, Hoenigl, M., & Kontoyiannis, D. P. Impact of climate change and natural disasters on fungal infections. *Lancet Microbe online ahead of issue publication, 5*(6), e594-e605. 10.1016/S2666-5247(24)00039-9

Servicio de Información Agroalimentaria y Pesquera (SIAP). (2022a). Producción de uva en México. https://www.gob.mx/cms/uploads/attachment/file/771603/Producci_n_Uva_en_M_xico.pdf

Servicio de Información Agroalimentaria y Pesquera (SIAP). (2022b). Baja California Infografía agroalimentaria. https://nube.siap.gob.mx/avance_agricola/

Servicio de Información Agroalimentaria y Pesquera (SIAP). (2022c). Avance de siembras y cosechas. https://nube.siap.gob.mx/infografias_siap/pag/2022/Baja-California-Infografia-Agroalimentaria-2022

Servicio de Información Agroalimentaria y Pesquera (SIAP). (2023). Avance de siembras y cosechas. https://nube.siap.gob.mx/avance_agricola/

Sosnowski M.R., Ayres M.R., & Scott E.S. (2021). The Influence of Water Deficit Stress on the Grapevine Trunk Diseases Pathogens *Eutypa lata* and *Diplodia seriata. Plant Disease, 105*(8), 2217-2221. https://doi.org/10.1094/PDIS-07-20-1538-RE

Spetik, M., Tekielska D. A., Berraf-Tebbal A., Pecenka J., Stuskova K., Mahamedi, A. E., & Eichmeier, A. (2023). Diversity of *Botryosphaeriaceae* Species Associated with Grapevine Trunk Diseases in the Czech Republic. *Diversity, 15*(7), 800. https://doi.org/10.3390/d15070800

Stempien, E., Goddard, M. L., Wilhelm, K., Tarnus, C., Bertsch, C., & Chong J. (2017). Grapevine Botryosphaeria dieback fungi have specific aggressiveness factor repertory involved in wood decay and stilbene metabolization. *PLoS ONE, 12*(12), e0188766. https://doi.org/10.1371/journal.pone.0188766

Swift, J. F., Hall, M. E., Harris, Z. N., Kwasniewski, M. T., & Miller, A. J. (2021). Grapevine Microbiota Reflect Diversity among Compartments and Complex Interactions within and among Root and Shoot Systems. *Microorganisms*, *9*, 92. 10.3390/microorganisms9010092

Téliz, D. & Valle, P. (1979). Eutypa dieback in Mexican vineyards. *Plant Diseases, 63*, 312-314.

This, P., Lacombe, T., & Thomas, M. (2006). Historical origins and genetic diversity of wine grapes*. Trends in Genetics*, *22*(9), 511–519. https://doi.org/10.1016/j.tig.2006.07.008

Úrbez-Torres, J. R., Leavitt, G. M., Guerrero, J. C., Guevara, J., & Gubler, W. D. (2008). Identification and Pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata*, the Causal Agents of bot Canker Disease of Grapevines in Mexico. *Plant Disease, 92*(4), 519-529. https://doi.org/10.1094/PDIS-92-4-0519

Úrbez-Torres J.R. & Gubler, W.D. (2009). Pathogenicity of Botryosphaeriaceae species isolated from grapevine cankers in California. *Plant Disease, 93*(6), 584–592. https://doi.org/10.1094/PDIS-93-6-0584

Úrbez-Torres, J.R. (2011). The status of Botryosphaeriaceae species infecting grapevines. *Phytopathologia Mediterranea*, *50*(4), 5–45. https://doi.org/10.14601/Phytopathol_Mediterr-9316

Úrbez-Torres J. R., Peduto F., Smith R. J., & Gubler, W. D. (2013). Phomopsis dieback: A grapevine trunk disease caused by *Phomopsis viticola* in California. *Plant Disease, 97*, 1571-1579. https://doi.org/10.1094/PDIS-11-12-1072-RE

van Niekerk, J. M., Groenewald J. Z., Farr, D. F., Fourie P. H., Halleer, F., & Crous, P. W. (2005). Reassessment of Phomopsis species on grapevines. *Australasian Plant Pathology*, *34*, 27-39. https://doi.org/10.1071/AP04072

Wagner, D. B., Furnier, G. R., Saghai-Maroof, M. A., Williams, S. M., Dancik, B. P., & Allard, R. W. (1987). Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. Proceedings of the National Academy of Sciences, *84*, 2097–2100.

Wilcox W. F., Gubler W. D. & Uyemoto J. K. (Eds.). (2016). PART I: Diseases Caused by Biotic Factors. *Compendium of Grape Diseases, Disorders, and Pests.* (2nd) (pp: 17–146). American Phytopathological Society.

Zurita-Silvar, A., Reyes, M., Valenzuela, C., Díaz, I., & Verdugo-Vásquez, N. (2021). Uso de genotipos criollos como medida de adaptación frente a estreses ambientales. *En Vinos y variedades patrimoniales. Resumen de las primeras jornadas Latinoamericanas* (pp 32-32). Prieto, J.A. (Ed). INTA Ediciones.

Chapter 3. Heritage grapevines as sources of biological control agents for Botryosphaeria dieback pathogens

3.1 Abstract

Grapevine trunk diseases cause severe damage in grapevines. Management strategies focus on protection of grapevine pruning wounds using chemical fungicides or biological control agents. Botryosphaeria dieback, caused mainly by *Lasiodiplodia* spp., is one of the main trunk diseases in northwest Mexico. This study obtained endophytic bacteria and fungi from the heritage grapevine *Vitis vinifera* cv. 'Mission' for potential biological control of Botryosphaeria dieback. A collection of 135 bacterial and 37 fungal isolates were obtained and initially tested for antagonistic activity against *Lasiodiplodia brasiliensis*. The most promising isolates belonging to *Trichoderma* and *Bacillus* spp. were selected and characterized to determine their modes of action. *Bacillus* isolates produced volatile organic compounds that inhibited growth of *Neofusicoccum parvum*, and diffusible organic compounds with antifungal effects against *L. brasiliensis* and *N. parvum*. *Trichoderma* isolates produced diffusible organic compounds and were mycoparasites. In greenhouse assays, plants inoculated with three *Trichoderma asperellum* isolates (T20BCMX, EF09BCMX and EF11BCMX), *B. amyloliquefaciens* (BEVP26BCMX) or *Bacillus* sp. (rbES015), applied preventively in soil, gave up to 50% smaller necrotic lesions when compared with the plants inoculated only with *L. brasiliensis*. In the field, plants inoculated with three *Bacillus* isolates (BEVP02BCMX, BEVP26BCMX, BEVP31BCMX) or five *Trichoderma* (T11BCMX, T15BCMX, T17BCMX, T20BCMX and EF11BCMX) had lesions up to four times smaller than control plants inoculated only with *L. brasiliensis*. This study has demonstrated the potential of heritage grapevines to provide biological control agents for Botryosphaeria dieback.

3.2 Introduction

Grapevine trunk diseases (GTDs) cause severe problems in vineyards. These are a complex of diseases that include black foot, Esca, Eutypa dieback, Petri disease, and Botryosphaeria dieback (Úrbez-Torres et al., 2012). Approx. 133 fungal species in 34 genera have been reported as causal agents of GTDs (Gramaje et al., 2018). Among them, Botryosphaeria dieback is considered to be the most important (Billones-Baaijens and Savocchia, 2019). Almost 30 species of *Botryosphaeria, Diplodia, Dothiorella, Lasiodiplodia,*

Neofusicoccum, Neoscytalidium, Phaeobotryosphaeria and *Spencermartinsia* have been identified as causal agents of Botryosphaeria dieback (Gramaje et al.,2018).

Among the fungi associated with Botryosphaeria dieback, *Lasiodiplodia* and *Neofusicoccum* contain the most virulent species identified in different countries, including the United States of America (Úrbez-Torres and Gubler*,* 2009), Iraq (Abdullah et al., 2012) and Mexico (Rangel-Montoya et al., 2021). *Lasiodiplodia* spp. have white colonies in culture, that later become dark gray with abundant mycelium, and their conidia can be aseptate hyaline or septate pigmented, with longitudinal striations. *Neofusicoccum* spp. have initially white colonies, and some species as *N. luteum* and *N. australe* produce yellow pigments. Their abundant aerial mycelium later turns gray, and conidia are hyaline, unicellular, and aseptate, with a subtruncate bases (Zhang et al., 2021).

Botryosphaeria dieback symptoms usually appear in grapevines several years after pathogen infections, and the symptoms include wedge-shaped perennial cankers, wood discolourations, brown streaking on the wood under the bark, and premature plant death (Bertsch et al., 2013; Spagnolo et al., 2014; Gramaje et al., 2018; Niem et al., 2020). The pathogen enters host plants through wounds, made mostly during pruning (Gramaje et al., 2018). Management of GTDs is complex, and no curative treatments known to date. Therefore, strategies commonly focus on implementation of cultural and preventive treatment measures, with each depending on plant damage, the pathogens involved, and the geographic region (Gramaje et al., 2018). These include the protection of pruning wounds with fungicides or biological control agents (BCAs), and curative surgery (Mondello et al., 2018).

The search for endophytic microorganisms for biological control of GTDs pathogens has increased, due to their potential to have antagonistic activities against different species of fungi and, in some cases, to also promote plant growth (Dini-Andreote, 2020; Jacob et al., 2020). Different endophytic microorganisms have been reported as antagonists of GTDs pathogens. These include fungi (*Aspergillus, Chaetomium, Clonostachys*, *Cladosporium, Epicoccum, Fusarium,* and *Trichoderma*)*,* which have shown antagonistic *in vitro* activity against *Diplodia* and *Neofusicoccum* spp. (Almeida et al*.,* 2020; Silva-Valderrama et al., 2021). As well, bacteria (*Bacillus*, *Burkholderia, Paenibacillus*, *Pseudomonas,* and *Streptomyces*) have been recognized as antagonists of *Diplodia, Lasiodiplodia,* and *Neofusiccocum* (Haidar et al., 2016). Among the organisms with potential as BCAs, *Bacillus* and *Trichoderma* show *in vitro* antagonistic activity through different mechanisms of action, including competition for space, production of volatile and non-volatile compounds, or mycoparasitism. In greenhouse and field trials, these organisms have shown efficacy for control of GTD fungi when applied to grapevine pruning wounds (Almeida *et al.,* 2020).

In Mexico, the Spanish introduced grapevine (*Vitis vinifera*) in the 16th century, and Jesuit and Dominican friars disseminated its cultivation (Crowley, 1989). 'Listan Prieto,' now known as 'Mission,' was introduced in Mexico and in the United States of America (Walker et al*.,* 2019). In the Baja California peninsula, the first grapevines were established in the San Francisco Javier mission, located in Loreto (Magoni, 2009). From then on, grapevine cultivation continued to expand. Currently in Baja California, 'Mission' grapevines are over 40 years old, and have been propagated from the first vines introduced in the state by the Jesuit missionaries, and these plants are considered to be heritage grapevines. Approx. 38 ha of this variety are grown with minimal management in Baja California, with yields of approx. 3.5 tons ha^{-1} (SEFOA, 2011; Andrade et al., 2013). Different reports have shown that plants growing in arid conditions associate with microorganisms (bacteria and fungi). These can enhance plant drought resistance through various mechanisms, including improved water and nutrient absorption by inducing changes in root morphology, protecting against oxidative damage, regulating phytohormone levels, and suppressing phytopathogens such as those responsible of GTDs (Poudel et al., 2021; Riseh et al., 2021). Considering the time since they were established and the complex climatic conditions in which the 'Mission' grapevines have been planted (dry to very dry climate with average rainfall of 200 mm p.a. (INEGI, 2017), it is likely that these plants maintain associations with beneficial microorganisms which allow them to survive in the adverse conditions and resist plant pathogens. Therefore, the objective of the present study was to obtain endophytic bacteria and fungi from the cultivar 'Mission' with the potential as BCAs of Botryosphaeria dieback fungi, thus providing sustainable alternatives for the control of this disease in commercial vineyards of Baja California.

3.3 Methodology

3.3.1 Sampling and Isolation of microorganisms from heritage grapevines

Microorganisms were isolated from lignified 1-year-old branches of heritage grapevines cultivar 'Mission,' growing in local vineyards in the Guadalupe valley (31.994722, -116.683896) and Ejido Uruapan (31.628436, -116-434295), Baja California. Small tissue fragments were cut from each branch sample, and after bark removal, these were surface sterilized by flaming (Rangel-Montoya et al., 2021). Subsequently, for bacteria isolations, branch fragments were transferred to plates containing LB Agar (ATCC media No.1065), YPD Agar (1245) or King's medium B Agar (1213), or PY medium (tryptone 5.0 g, yeast extract 3.0 g, $CaCl_2$ 0.9 g, pH 6.8), supplemented with cycloheximide (final concentration100 µg mL^{-1}). For fungal

isolations, tissue fragments were inserted in plates containing Potato Dextrose Agar (PDA), or water agar supplemented with chloramphenicol (final concentration 25 µg mL^{-1}). The isolation plates were incubated at 30°C until microorganism growth was observed, and the resulting bacterial and fungal colonies were recovered and subcultured to obtain pure cultures. Fungal strains were preserved at 4°C in 20% glycerol, and bacteria strains in 35% glycerol solution at −20°C. Additionally, *Bacillus* sp. rbES015 and 35 strains of *Trichoderma* were obtained from the collection of the Phytopathology Laboratory of CICESE.

3.3.2 Screening for antifungal activity

The GTD fungi *Lasiodiplodia brasiliensis* MXBCL28 (Rangel-Montoya et al., 2021) and *Neofusicoccum parvum* 14P4MX (Rangel-Montoya, 2021) were used to test the biological control potential of the obtained fungal and bacterial isolates. Using a flame-sterilized 7 mm cork borer, an agar plug with mycelium was obtained from a 4-day-old culture, from each fungus grown on PDA. The plug was then placed on the center of a fresh PDA plate, and incubated at 25°C. When the fungus colony reached 1 cm diam. 5 µL of four different potential BCA bacterial cultures or one mycelium plug from potential fungal BCAs, were inoculated at the edges, as described by Guevara-Avendaño et al., (2018). Plates with only GTD fungi were used as experimental controls. These assays were each carried out in triplicate. After 7 days of incubation at 30°C, the inhibition of radial growth of *L. brasiliensis* mycelium was assessed. In total, 135 bacterial and 39 fungal isolates were screened in these assays. After discarding isolates with low or no inhibition activity, quantitative fungal inhibition assays were carried out for 58 strains (21 bacteria and 37 fungi), as described for the qualitative assays (above). For each of these quantitative assays, the *L. brasiliensis* mycelium plug was placed at the edge of the Petri plate, and only one bacterial or fungi strain was inoculated directly opposed to it. The following formula (Méndez-Bravo et al., 2018) was used to calculate the percentage of inhibition of mycelial growth:

$$\text{Percentage of inhibition} = \frac{(\mathrm{R} - \mathrm{r})}{\mathrm{R}} \text{ x } 100 \qquad (2)$$

where R is the colony radius of the pathogenic fungus growing alone in the control plates, and r is the colony radius of the fungus growing in the plate in confrontation with a tested isolate.

All in vitro antagonistic assays were performed in triplicate. These procedures allowed selection of the most promising BCAs for used in the experiments described below.

3.3.3 Evaluation of antagonistic effects by volatile organic compounds

Antagonistic effects of volatile organic compounds produced by eleven selected strains (four bacteria and seven fungi) were evaluated against *L. brasiliensis* and *N. parvum*, using the two sealed base plate method of Rangel-Montoya et al., (2022). For each potential fungal BCA isolate, a mycelial plug was placed in the centre of a PDA plate, and for each bacterium, 20 µL of culture was spread in a PY plate. The lid of each plate was the replaced by a second PDA plate with a mycelial plug of the pathogen at the centre. The two plates were sealed with tape and incubated at 30°C for 4 d, with mycelium growth assessed every 24 h. As experimental controls, non-inoculated PDA or PY plates were used as the covering plate.

3.3.4 Evaluation of the antagonistic effects by diffusible organic compounds

The antagonistic activity of diffusible organic compounds produced by eleven selected BCAs strains was evaluated against *L. brasiliensis* and *N. parvum.* For bacteria, 5 mL of liquid PY medium was inoculated with a single colony, and then incubated at 30°C and 110 rpm in a shaker incubator. After 7 d, cultures were each centrifuged at 10,000 rpm for 20 min., and the resulting supernatant was filter-sterilized using a 20 µm syringe filter. PDA plates containing 15% (v/v) of sterile bacterial supernatant were then prepared, and a mycelial plug of each pathogen was placed in the centre of each test plate (Salvatierra-Martinez et al., 2018). The plates were then incubated at 30°C for 4 d, registering mycelium growth every 24 h. For evaluation of fungal isolates, cellulose membrane assays were used (Mayo-Prieto et al*.,* 2020). A mycelial plug disc of each fungus was inoculated in the centre of each PDA plate. The plug was then covered with a sterile cellulose membrane, and the plates were incubated at 30°C for 48 h. The membrane with the mycelial growth was then removed, and a mycelial plug of the pathogen was placed in the centre of the plate. Plates were incubated at 30°C for 4 d, and mycelium growth was assessed every 24 h.

3.3.5 Evaluation of mycoparasitism activity

Mycoparasitism activity of seven selected *Trichoderma* isolates was assessed against *L. brasiliensis* and *N.*

parvum, using the pre-colonized plate method described by Bailey et al., (2008). A mycelial plug of each pathogenic fungus was inoculated at the edge of a PDA plate, and after 5 d incubation at 30°C, an agar strip (4.0 × 0.5 cm) from a colony of a *Trichoderma* isolate was placed at the opposite side. The plates were incubated for 28 d at 30°C in darkness. Ten mycelial plugs were then collected from each plate in a straight line beginning near the agar strip and extending towards the opposite edge of the plate. The mycelial plugs were then inoculated into PDA plates and incubated for 24 h at 30°C in darkness, followed by 5 d incubation under white light at room temperature. As experimental controls, cultures of *L. brasiliensis* and *N. parvum* were used, grown without *Trichoderma* and maintained under the same conditions. Mycoparasitism was determined by assessing the presence of *Trichoderma* and the phytopathogenic fungi in the ten mycelial plugs collected. Micoparasitic activity was also assessed under a microscope. For each of these assessments, a dual culture assay was performed in water agar plates, and after 3 d incubation at 30°C, a fragment of agar was cut from the centre of the plate. The obtained samples were observed with inverted microscope (Zeiss Axiovert 200), and the obtained images were analyzed using Zeiss AxioVision SE64, Rel. 4.9.1 software.

3.3.6 In vitro screening for plant growth promoting traits

Coluorimetric tests were carried out to determine plant growth promotion by the potential BCA bacteria and fungi strains. For these assays, the strains were recovered from glycerol stock cultures, and bacteria were inoculated into PY liquid medium, and fungi onto PDA medium. Bacterial cultures were incubated for 2 d at 30°C and 100 rpm in a shaker incubator. Fungi were incubated at 30°C for 7 d, Coluorimetric tests were carried out in triplicate, in 35 mm diam. Petri dishes for solid media or 10 mL capacity tubes for liquid media, as described in the sections below. Petri plates or tubes with the corresponding media but without BCAs were used as experimental controls.

3.3.6.1 Mineral solubilization assays

Some microorganisms are capable of hydrolyzing organic and inorganic insoluble mineral compounds to soluble forms, that can be assimilated by plants, acting as biofertilizers or plant grow-promoters. Phosphate, potassium, and zinc solubilization assays were performed for the potential BCAs. In each case, 5 µL of bacterium culture or a 7 mm diam. mycelial plug of fungus were inoculated at the centre of each assay plate. Inorganic phosphate solubilization was evaluated on modified Pikovskaya agar (0.5 g L^{-1} yeast

extract, 10 g L^{-1} glucose, 5 g L^{-1} $Ca_3(PO_4)_2$, 0.5 g L^{-1} $(NH_4)_2SO_4$, 0.2 g L^{-1} KCl, 0.1 g L^{-1} $MgSO_4$, 0.1 mg L^{-1} $MnSO_4$, 0.1 mg L^{-1} $FeSO_4$, 10 mg L^{-1} of bromocresol purple, 15 g L^{-1} agar, pH 7.2). After inoculation, Petri dishes were incubated for 72 h at 30°C. Colour change from purple to yellow indicated a positive phosphate solubilizing strain (Gupta *et al.,* 1994; Zheng *et al.,* 2018).

Inorganic potassium solubilization was determined on modified Pikovskaya agar (Pikovskaya, 1948), using KNO_3 and bromocresol green. After inoculation, Petri plates were incubated for 72 h at 30°C. Colour change from blue to yellow indicated a positive potassium solubilizing strain. Zinc solubilization was assessed using zinc-supplemented Pikovskaya medium complemented with 1.2 g L^{-1} ZnO and bromothymol blue. Inoculated Petri dishes were incubated in the dark for 72 h at 30°C. Colour change from blue to yellow indicated a positive zinc solubilizing strain (Bapiri *et al.,* 2012).

3.3.6.2 Indole Acetic Acid (AIA) production assays

Indole acetic acid (IAA) is one of the most physiologically active auxins. It induces production of long roots and root hairs, and lateral roots, which are involved in nutrient uptake by plants (Datta and Basu, 2000). For determination of IAA production by bacterial strains, 5 µL of 1-d-old cultures were reinoculated in 96 well microplates, with 200 µL PY liquid medium supplemented with tryptophan to a final concentration of 500 µg mL^{-1}. Microplates were incubated for 48 h at 30°C and 110 rpm in a shaker incubator. Subsequently, 100 µL of Salkowski reagent (50 mL, 35% $HClO_4$, 1 mL 0.5 M $FeCl_3$) (Ahmad et al., 2008) were then added per well, and the microplates were each covered with aluminum foil and incubated for 30 min. For each fungal strain, three 7 mm diam. mycelial plugs were inoculated into 5 mL of PDB medium, and these cultures were incubated for 7 d at 30°C and 110 rpm in a shaker incubator. Two hundred µL of the culture were then placed in 96 well microplates, and 100 µL of Salkowski reagent were immediately added. Microplates were each covered with aluminum foil and incubated for 30 min. For the bacteria and fungi, change of colour to pink indicated a positive result.

3.3.6.3 Hydrogen cyanide production assays

Hydrogen cyanide (HCN) is produced by some BCAs, and its toxicity to phytopathogens makes the BCAs suitable for biocontrol. After inoculating the bacteria onto solid PY and the fungi onto PDA in Petri plates, a filter paper moistened with a solution of 0.5% sodium carbonate in 0.5% picric acid (Ahmad et al., 2008)

was fixed to each Petri plate cover. The plates were subsequently sealed with parafilm and incubated in dark at 30°C for 4 d. Development of orange-red colour indicated positive hydrogen cyanide producer strains.

3.3.6.4 Siderophore production assays

Siderophores are competitive traits used for BCAs to sequester iron, depriving pathogens of this element required for their growth and pathogenesis. To test for siderophore production, chrome azurol S agar (CAS) medium (Schwyn and Neilands, 1987) was prepared as described by Lynne *et al.* (2011). In the centre of each assay plate was inoculated 5 µL of bacterial culture or a 7 mm diam. mycelial plug of fungus. The Petri dishes were incubated in the dark for 96 h at 30°C. Colour changes from blue to yellow indicated siderophore producing strains.

3.3.6.5 Chitinase production assays

Chitin is an important component of the cell walls of fungi, and chitinolytic microorganisms are likely to act as biocontrol agents and pathogen antagonists. Chitinase determination basal medium (0.3 g L^{-1} $MgSO_4 \cdot 7H_2O$, 3 g L^{-1} NH_4 (SO_4), 2 g L^{-1} KH_2PO_4), 1 g L^{-1} citric acid, 15 g L^{-1} agar, 0.2 g L^{-1} Tween-80, pH 4.7) was supplemented with 4.5 g L^{-1} colloidal chitin and 0.15 g L^{-1} bromocresol purple (Agrawal and Kotasthane, 2012). After inoculation, the Petri dishes were incubated for 48 h at 30°C. Colour changes from yellow to purple indicated chitinase producer strains.

3.3.7 Molecular identification of selected isolates

Bacterial isolates were identified by sequencing of the 16S rRNA genes. Genomic DNA was purified using the Gentra Puregen kit (Qiagen). The 16S rRNA gene was amplified using the 27F and 1492R primers (Frank *et al.,* 2008). PCR reactions were each prepared in a final volume of 25 µL, containing 1 µL of genomic DNA (25 ng μL^{-1}), 2.5 µL of Taq Buffer 10×, 0.5 µL of dNTP mix (10 mM), 0.5 µL of primer 27F (10mM), 0.5 µL of primer 1492R (10mM), 0.2 µL of Taq DNA polymerase (5 units μL^{-1}) (Thermo Fisher), and 19.8 µL of ultrapure water to complete the volume. Amplifications were each carried out in a MiniAmp Plus Thermal

Cycler (Thermofisher), under the following conditions: a 3 min initial denaturation step of 95°C, followed by 30 cycles of 95°C for 30 sec, 48°C for 30 sec, 72°C for 1 min, and a final cycle 72°C for 10 min.

Table 6. GenBank and culture accession numbers of bacterium species used in the present study for phylogenetic analyses. Isolates from this study are highlighted in bold font.

Species	Isolate	Isolation source	Origin	GeneBank accession number
				16S rRNA
Bacillus amyloliquefaciens	NBRC 15535	Soil	Japan	NR_112685
B. amyloliquefaciens	W9	Marine water sample	India	MH188056
B. amyloliquefaciens	AB-525	Rice cake	China	KJ879953
B. amyloliquefaciens	BsA3MX	Strawberry rhizosphere	Mexico	MW651769
B. amyloliquefaciens	BsC11MX	Strawberry rhizosphere	Mexico	MW651770
B. amyloliquefaciens	**BEVP26BCMX**	**Grapevine**	**Mexico**	**OQ073757**
B. amyloliquefaciens	**BEVP31BCMX**	**Grapevine**	**Mexico**	**OQ073762**
B. axarquiensis	CIP 108772	River-mouth sediments	Spain	DQ993670
B. axarquiensis	**BEVP02BCMX**	**Grapevine**	**Mexico**	**OQ073758**
B. cereus	ATCC 14579	Unknown	Unknown	AE016877
B. circulans	IAMI 12462	Soil	Unknown	D78312
B. coagulans	NBRC 12583	Evaporated milk	Unknown	AB271752
B. licheniformis	ATCC 14580	Unknown	Unknown	CP000002
B. mojavensis	IFO 15718	Soil	USA	AB021191
B. mojavensis	**BEVP01BCMX**	**Grapevine**	**Mexico**	**OQ073759**
B. mycoides	ATCC 6462	Soil	Unknown	AB021192
B. siamensis	PD-A10	Poo-dong	Thailand	GQ281299
B. siamensis	RET2912	Landfill soil	India	MN530054
B. siamensis	LFS1715	Landfill soil	India	MN519261
B. subtilis	DSM10	Unknown	Unknown	AJ276351
B. subtilis subsp. *spizizenii*	NBRL B-23049	Tunisian desert	Tunisia	AF074970
B. thuringiensis	IAM 12077	Mediterranean flour moth	Unknown	D16281
B. vallismortis	DSM 11031	Soil	USA	AB021198
B. velezensis	CR-502	Brackish water	Spain	AY603658
Alicyclobacillus acidocaldarius	DSM 446	Acid hot spring	USA	AJ496806

Fungal isolates were identified by sequencing of the elongation factor *tef-1α* gene. Total genomic DNA was extracted from mycelia using cetyltrimethylammonium bromide (CTAB), as described by Wagner *et al.* (1987). The *tef-1α* gene was amplified using EF1and EF2 primers (O'Donnell *et al.,* 1998). PCR reactions were each prepared in a final volume of 25 µL, containing 1 µL of genomic DNA (25 ng μL^{-1}), 2.5 µL of Taq Buffer 10×, 0.5 µL of dNTP mix (10 mM), 0.62 µL of primer EF1 (10 mM), 0.62 µL of primer EF2 (10 mM), 0.125 µL of Taq DNA polymerase (5 units μL^{-1}) (Thermo Fisher) and 19.6 µL of ultrapure water to complete

the volume. Amplification was carried out in a MJ Mini Gradient Thermal Cycler (BioRad) under the following conditions: a 3 min initial denaturation step of 95°C, followed by 35 cycles of 95°C for 1 min, 57°C for 1 min, 72°C for 1 min, and a final cycle 72°C for 10 min.

Table 7. List of GenBank and culture accession numbers of fungal species used in the present study for phylogenetic analyses. Isolates from this study are highlighted in bold font.

Species	Isolate	Isolate source	Origin	GenBank accession number
				tef-1α
Trichoderma asperellum	Th047	Soil	Colombia	AB568381.1
T. asperellum	cds	No available	Brazil	KP696459.1
T. asperellum	ST1	No available	Spain	KJ677260.1
T. asperellum	**T11BCMX**	**Carnation**	**Mexico**	**OQ161180**
T. asperellum	**T15BCMX**	**Grapevine**	**Mexico**	**OQ161181**
T. asperellum	**T20BCMX**	**Grapevine**	**Mexico**	**OQ161182**
T. asperellum	**EF09BCMX**	**Grapevine**	**Mexico**	**OQ161183**
T. asperellum	**EF11BCMX**	**Grapevine**	**Mexico**	**OQ161184**
T*richoderma atroviride*	DAOM 238037	No available	Thailand	KJ871093
T. atroviride	PARC1011	No available	Italy	MT454114
Trichoderma guizhouense	DAOM 231412	No available	No available	AY605764
T. guizhouense	DAOM 231435	No available	No available	EF191321
T. guizhouense	PARC1022	*Pronus persica*	Italy	MT454125
Trichoderma harzianum	DAOM 233986	No available	No available	EF392749
T. harzianum	DAOM 242937	No available	No available	KX463434
T. harzianum	PARC1019	*Pronus persica*	Italy	MT454122
T. harzianum	**T06BCMX**	**Grapevine**	**Mexico**	**OQ161179**
Trichoderma koningiopsis	Arak-96	Soil	Iran	KP985652
T. koningiopsis	ITCC 7291	Soil	India	LN897322
T. koningiopsis	PARC1024	Pronus persica	Italy	MT454127
Trichoderma longibrachiatum	DAOM 234103	No available	No available	DQ125467
T. longibrachiatum	CIB T13	No available	Colombia	EU280033
T. longibrachiatum	PARC1015	No available	Italy	MT454118
T. longibrachiatum	**T17BCMX**	**Grapevine**	**Mexico**	**OQ161184**
Trichoderma paraviridescens	BMCC:LU786	No available	New Zealand	KJ871271
T. paraviridescens	KX098484	No available	New Zealand	KX098484
T. paraviridescens	PARC1016b	No available	Italy	MT454119

All obtained PCR products were verified by electrophoresis on 1% agarose gels, purified using the GenElute PCR Clean-Up Kit (Sigma-Aldrich), and sent to Eton Bioscience Inc. for sequencing. The resulting sequences were aligned using MEGA XI (Kumar et al., 2018), with the multiple alignment program MUSCLE. the bacteria sequences were blasted against the GenBank 16S Ribosomal RNA sequences database (Table 6), and the *Trichoderma* spp. sequences were compared with the GenBank elongation factor 1α gene sequences database (Table 7), and the closest matches were used to construct each alignment. A

Maximum-Parsimony method was used with Bootstrap values based on 1,000 replicates. New sequences were deposited in the GenBank (Tables 6 and 7).

3.3.8 Greenhouse biocontrol assays of *Lasiodiplodia brasiliensis*

Grapevine plants (*Vitis vinifera* 'Cabernet Sauvignon') obtained from 1-year-old cuttings were used to determine the biocontrol activity of selected bacterial and fungal strains and a rhizosphere strain rbES015 obtained in a previous study (Delgado-Ramírez et al*.,* 2021). Grapevine shoots were submerged in a 3 g L^{-1} solution of rooting agent ROOTEX (Cosmocel SA) and were then planted in tubs containing Cosmopeat substrate (Cosmocel SA). After 45 d, the plants were transplanted into 3.78 L plastic pots. Two weeks after transplanting, 50 mL of a solution (1×10^6 CFU) of each potential beneficial microorganism was applied at the base of the plant stem, followed by a second application 7 d later. Control treatments were inoculated with sterile water. For each tested isolate, ten replicates were used. Immediately after the second application of potential BCA, inoculations of the plants with *L. brasiliensis* were carried out through mechanical wounds in the woody tissues, each made with a drill bit (2 mm diam.), followed by insertion of a mycelium plug inside each hole. After inoculation, the wounds were each covered with parafilm. Plugs of sterile PDA were used as experimental control inoculations. The plants were then kept under greenhouse conditions for 60 d, and necrotic lesions generated in the stems were measured. Attempts were also made to recover the inoculated microorganisms.

3.3.9 Vineyard biocontrol assays of *Lasiodiplodia brasiliensis*

A field biocontrol trial was carried out in a 2-year-old 'Chenin Blanc' vineyard, in Ejido el Porvenir, Baja California. Fifty plants, which did not show symptoms associated with wood diseases, were chosen per row on five vineyard rows, leaving an interval of three to five plants between each selected vine. The experimental design was completely randomized with ten grapevines per treatment. Putative BCAs evaluated included five bacteria (BEVP01BCMX, BEVP02BCMX, BEVP26BCMX, BEVP31BCMX, and rbES015) and six fungi (T06BCMX, T11BCMX, T15BCMX, T17BCMX, T20BCMX, and EF11BCMX). In each selected plant, a pruning cut was made in a woody branch, and 10 µL of a 1×10^6 CFU suspension of the selected biocontrol organism were inoculated, and 20 plants were treated with each isolate. Five days later, a second inoculation of the biocontrol agent was made in the same wound. One hour later, 10 µL of a $1x10^5$ suspension of fragmented mycelium of *L. brasiliensis* was applied to ten of the plants. Negative controls

were inoculated only with sterile distilled water. The inoculated branches were each sealed with parafilm and then covered with a paper bag (Figure 18). One month later, the treated branches were cut, the length of the lesions produced by *L. brasiliensis* was measured, and a tissue fragment from each branch was inoculated onto PDA to assess if the pathogen and the inoculated BCA was present.

3.3.10 Statistical analyses

Data obtained from the greenhouse and vineyard biocontrol experiments were analyzed using one-way ANOVA, with post-LSD analysis, and an $\alpha < 0.05$ test for statistical significance, using the STATISTICA 8.0 package.

3.4 Results

3.4.1 Isolation, screening and molecular identification of microorganisms from heritage grapevines

A total of 135 isolates of bacteria from the heritage grapevine tissues were characterized by morphological characteristics as *Bacillus, Paenibacillus* and *Pseudomonas*. Isolates of fungi included two *Trichoderma* spp., and *Alternaria*, *Chaetomium, Sordaria* and *Diplodia* spp. Given the small number of potential beneficial fungal isolates recovered, 35 uncharacterized *Trichoderma* strains from our laboratory collection were included in this study.

From the 172 evaluated strains, 37 fungal and 21 bacterial isolates showed antagonistic activity in the qualitative antagonism assays (Figure 10). Quantitative dual culture assays and screening for plant growth promotion traits were performed only for those 58 isolates. Results showed that mean inhibition proportions for these BCAs against *L. brasiliensis* were between 3.4% to 52.8%, and that the isolates had different plant growth promotion characteristics (Table 11). Based on inhibition proportions, and possession of at least one growth promoting trait, four bacteria (BEVP01BCMX, BEVP02BCMX, BEVP26BCMX, and BEVP31BCMX) and seven fungi (EF09BCMX, EF11BCMX, T06BCMX, T11BCMX, T15BCMX, T17BCMX, and T20BCMX) were selected.

Table 8. Mean percent inhibition of *Lasiodiplodia brasiliensis* by different potential biocontrol microorganisms, and their respective production of plant growth promotion compounds, for selected *Bacillus* and *Trichoderma* isolates.

Isolate	Mean inhibition %	Production				Solubilization		
		SID	CHI	HCN	IAA	P	K	ZN
***B. mojavensis* BEVP01BCMX**	51.3	+	+	-	+	-	-	-
***B. axarquiensis* BEVP02BCMX**	17.1	-	+	-	+	+	+	-
***B. amyloliquefaciens* BEVP26BCMX**	38.0	+	+	-	+	+	-	-
***B. amyloliquefaciens* BEVP31BCMX**	50.6	-	-	-	+	-	-	-
***T. asperellum* EF09BCMX**	51.8	+	+	-	+	+	-	-
***T. asperellum* EF11BCMX**	51.7	+	+	-	+	+	-	-
***T. harzianum* T06BCMX**	41.0	+	+	-	+	-	-	-
***T. asperellum* T11BCMX**	29.1	+	+	-	+	-	-	-
***T. asperellum* T15BCMX**	25.1	+	+	-	+	-	-	-
***T. longibrachiatum* T17BCMX**	52.8	+	+	-	+	-	-	-
***T. asperellum* T20BCMX**	39.3	+	+	-	-	-	-	-

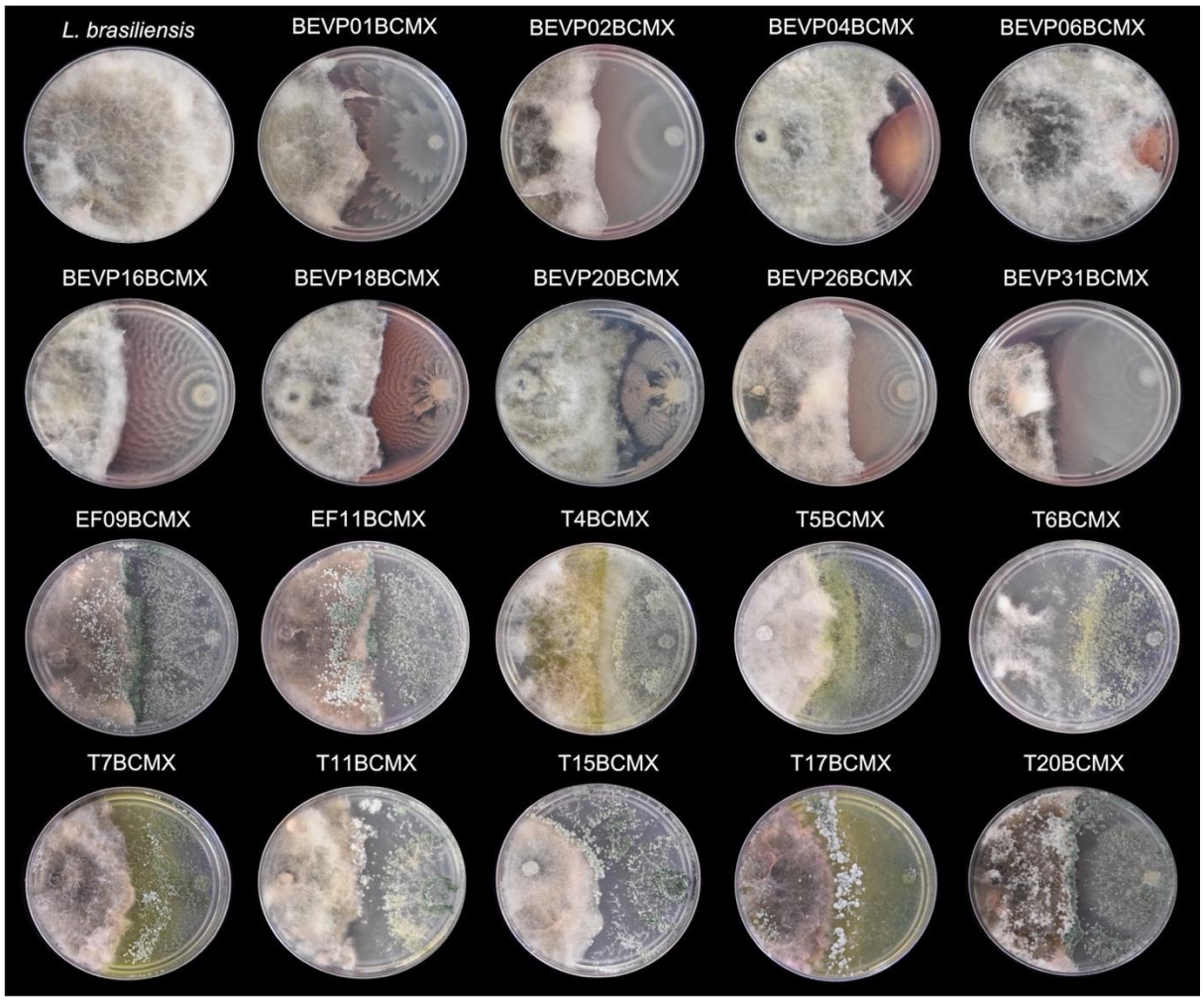

Figure 10. Representative images of dual culture assays of selected *Bacillus* and *Trichoderma* isolates against *Lasiodiplodia brasiliensis.*

Molecular identification of these isolates confirmed that all the bacteria were *Bacillus* spp. (Figure 19), and

all the fungi were *Trichoderma* spp. (Figure 20). Mean inhibition proportions ranged from 17.1% to 51.8%. Almost all the selected isolates (except *T. asperellum* T20BCMX) produced AIA, and (except *B. amyloliquefaciens* BEVP31BCMX) produced chitinase. Most of the isolates (except *B*. *axarquiensis* BEVP02BCMX and *B. amyloliquefaciens* BEVP31BCMX) produce siderophores. Four isolates (*B*. *axarquiensis* BEVP02BCMX*, B. amyloliquefaciens* BEVP26BCMX, *T. asperellum* EF09BCMX and *T. asperellum* EF11BCMX) solubilized phosphate, and one isolate (*B*. *axarquiensis* BEVP02BCMX) solubilized potassium. None of the isolates solubilized zinc or produced HCN (Table 8).

3.4.2 Evaluation of antifungal effect of volatile and diffusible organic compounds

Table 9. Mean percent inhibition of *Lasiodiplodia brasiliensis* and *Neofusicoccum parvum* from volatile organic compounds and diffusible organic compounds by different *Bacillus* and *Trichoderma* isolates.

Strain	Mean inhibition (%) from volatile organic compounds (%)		Mean inhibition (%) from diffusible organic compounds	
	L. brasiliensis	***N. parvum***	***L. brasiliensis***	***N. parvum***
***Bacillus mojavensis* BEVP01BCMX**	0	31.2	62.1	78.2
***B. axarquiensis* BEVP02BCMX**	0	23.6	31.6	76.4
***B. amyloliquefaciens* BEVP26BCMX**	0	26.2	40.4	73.8
***B. amyloliquefaciens* BEVP31BCMX**	0	34.0	49.3	66.0
***Trichoderma asperellum* EF09BCMX**	0	0	63.5	100
***T. asperellum* EF11BCMX**	0	0	61.2	98
***T. harzianum* T06BCMX**	0	0	0	0
***T. asperellum* T11BCMX**	0	0	67.1	100
***T. asperellum* T15BCMX**	0	0	81.1	100
***T. longibrachiatum* T17BCMX**	0	0	66.8	98
***T. asperellum* T20BCMX**	0	0	63.5	100

The eleven isolates were further screened for the antifungal activity from diffusible and volatile organic compounds. None of the assessed *Trichoderma* or *Bacillus* isolates produced volatile organic compounds with suppressive effects on *L. brasiliensis* (Figure 11; Table 9). However*,* all the *Bacillus* isolates affected growth of *N. parvum*, with mean inhibition percentages ranging from 22.6% to 34.0%. Isolate BEVP31BCMX gave the greatest inhibition (Figure 11; Table 9). In contrast, the 11 isolates affected the

growth of both pathogenic fungi by the production of diffusible organic compounds. The *Bacillus* isolates gave mean inhibition percentages from 40.4% to 62.1% against *L. brasiliensis*, and from 66% to 78% against *N. parvum,* while the *Trichoderma* strains gave 61.2% to 81.1% inhibition of *L. brasiliensis* and close to 100% inhibition of *N. parvum* (Figure 12; Table 9). While *T. harzianum* T06BCMX did not affect radial colony growth of either of the pathogenic fungi, this isolate caused a significant decrease in aerial mycelium (Figure 12).

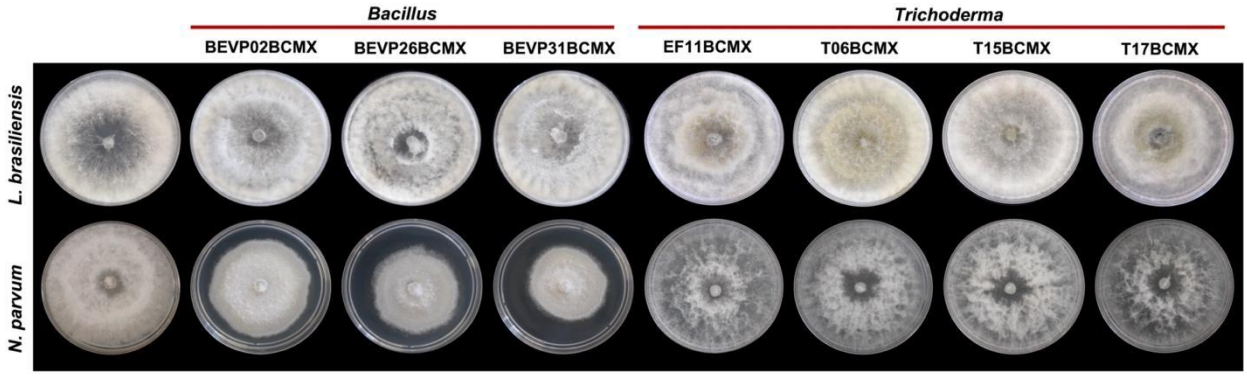

Figure 11. Representative images of the antifungal effects of volatile organic compounds produced by Bacillus and Trichoderma isolates against Lasiodiplodia brasiliensis and Neofusicoccum parvum.

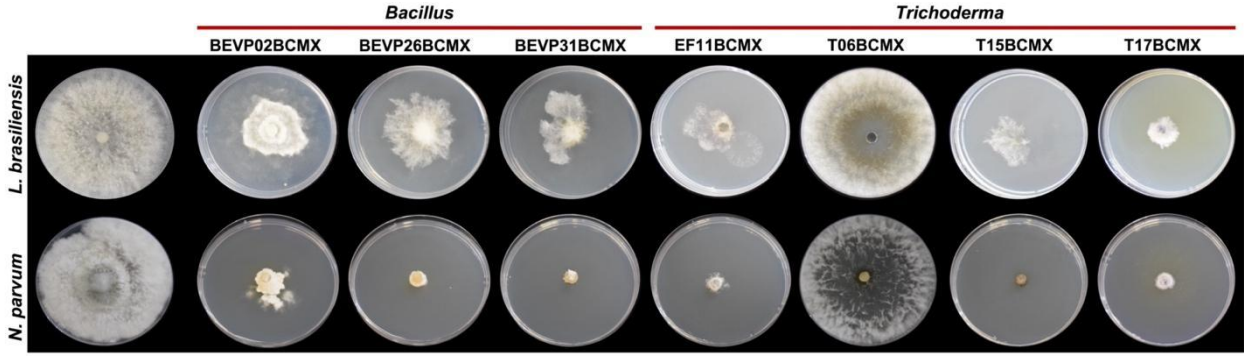

Figure 12. Representative images of the antifungal effect of diffusible organic compounds produced by *Bacillus* and *Trichoderma* isolates against *Lasiodiplodia brasiliensis* and *Neofusicoccum parvum.*

3.4.3 Characterization of micoparasitic activity of *Trichoderma* strains

The pre-colonized plate experiments showed that all the assessed *Trichoderma* isolates had vigorous microparasitic activity, with colonization percentages ranging from 70% to 100% (Table 10). When the colonization percentage was 100%, the inoculated phytopathogenic fungus could not be recovered, indicating total suppression. Microscope observations from dual culture assays indicated that all the *Trichoderma* isolates coiled around, and cause morphological deformations, of *L. brasiliensis* hyphae, while the isolates *T. asperellum* T15BCMX and *T. longibrachiatum* T17BCMX also induced lysis of mycelium

walls (Table 10; Figure 13).

Table 10. Mean colonization percentages from pre-colonized plate assays, and microscope observations, indicating mycoparasitism activity of six *Trichoderma* isolates against *Lasiodiplodia brasiliensis*.

Isolate	Mean colonization percentage (%)	Type of effect of mycoparasitism		
		Coiling	Hyphae deformation	Cell lysis
***T. asperellum* EF09CMX**	100	+	+	-
***T. asperellum* EF11BCMX**	70	+	+	-
***T. harzianum* T06BCMX**	80	+	+	-
***T. asperellum* T11BCMX**	90	+	+	-
***T. asperellum* T15BCMX**	100	+	+	+
***T. longibrachiatum* T17BCMX**	90	+	+	+
***T. asperellum* T20BCMX**	86	+	+	-

+ positive result, - negative result

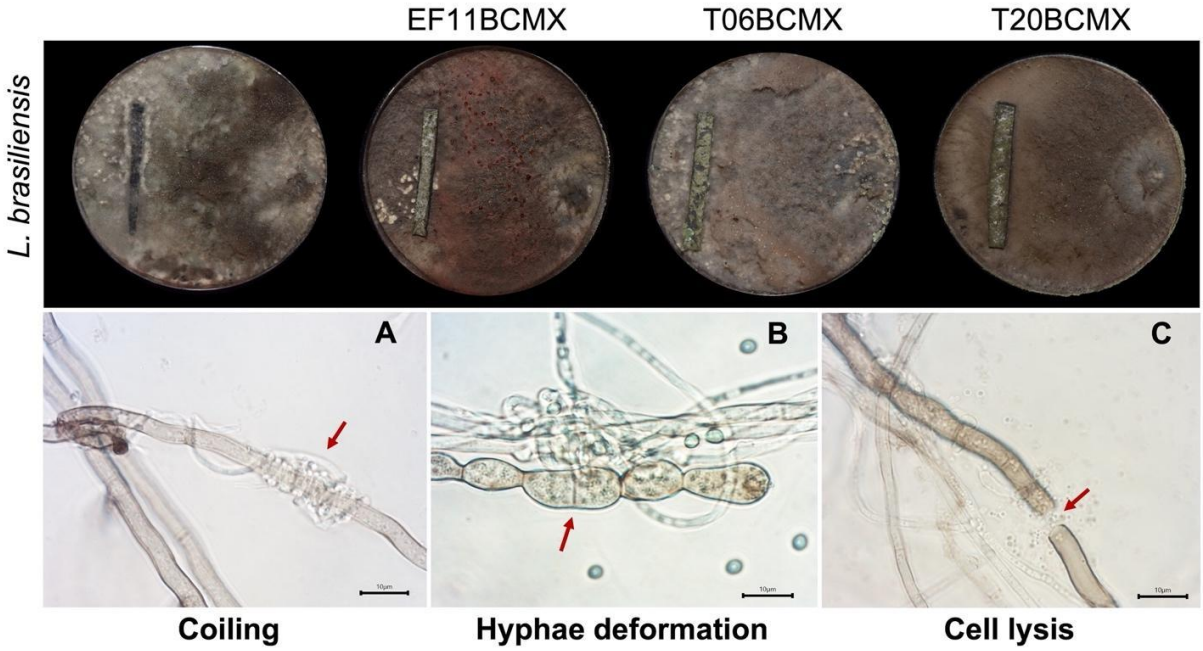

Figure 13. Representative microscope images (captured after 28 d incubation at 30°C) from pre-colonization assays of *Trichoderma* isolates against *Lasiodiplodia brasiliensis* showing mycoparasitism activity of the *Trichoderma* isolates (red arrows indicate effects caused by *Trichoderma*). A, Hyphal coiling of *T. asperellum* EF11BCMX against *L. brasiliensis*. B) *T. harzianum* T06BCMX causing deformation in *L. brasiliensis* hyphae. C) Lysis of the hyphal wall of *L. brasiliensis* induced by *T. longibrachiatum* T17BCMX.

3.4.4 Evaluation of biocontrol activity of selected bacterial and fungal isolates in greenhouse trials

The preventative application to soil of the *Trichoderma* and *Bacillus* isolates, for suppression of *L. brasiliensis* infection revealed the following. While the untreated grapevine plants inoculated with *L. brasiliensis* developed wounds of mean length up to 10.0 cm, the plants treated with three isolates of *T. asperellum* (T20BCMX, EF09BCMX, and EF11BCMX), *B. amyloliquefaciens* BEVP26BCMX and *Bacillus* sp. rbES015 showed significantly shorter necrotic lesions (Figures 14 and 15). Plants inoculated with *L. brasiliensis* and treated with *T. harzianum* T6BCMX developed larger lesions than plants inoculated only with *L. brasiliensis* (Figures 14 and 15). This isolate was the only *Trichoderma* isolate that gave no effect in the diffusible compounds assays. In the greenhouse tests, all the *Bacillus* and *Trichoderma* isolates were recovered from the root tissues of the inoculated plants, and *L. brasiliensis* was re-isolated from the stems of the plants.

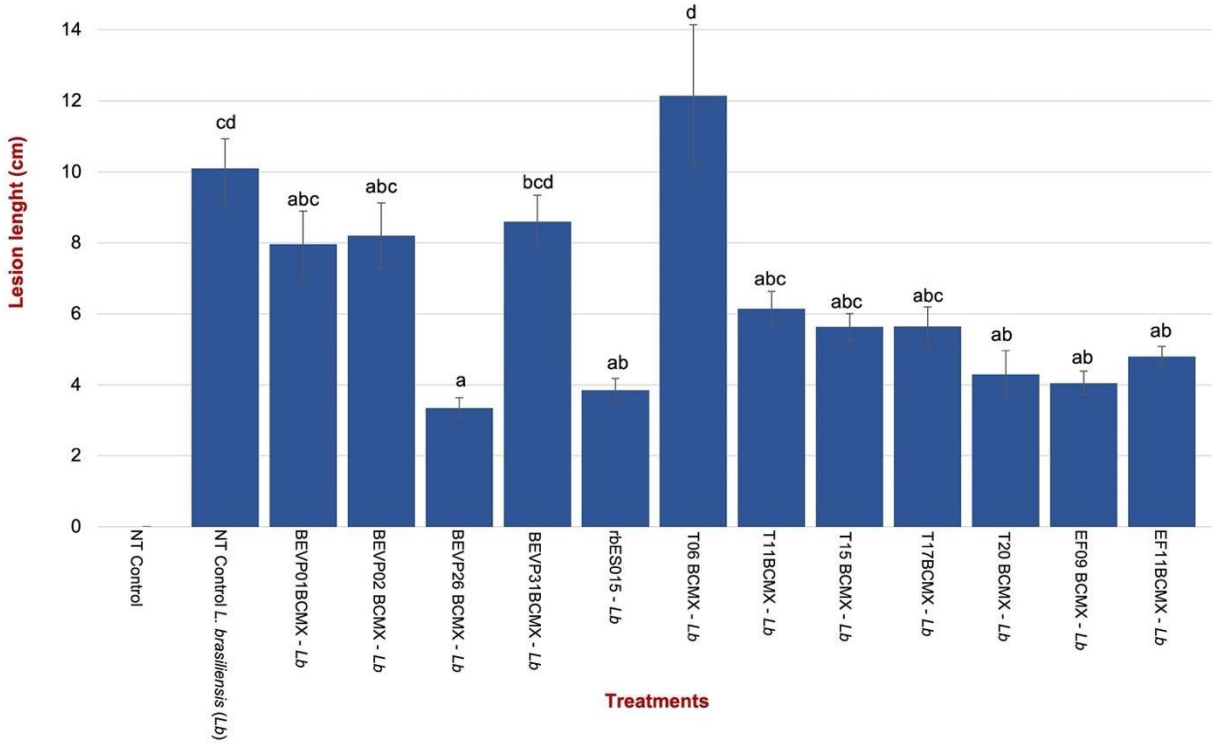

Figure 14. Mean internal necrotic lesion lengths caused by *Lasiodiplodia brasiliensis* (*Lb*) in grapevine plants, after treatments with different *Bacillus* (BEVP02BCMX, BEVP26BCMX and BEVP31BCMX) or *Trichoderma* isolates (T11BCMX, T15BCMX, T17BCMX, T20BCMX, and EF11BCMX). NT, Non-treated Control. Each bar represents the mean for ten (± SE). Different letters indicate differences ($P < 0.05$), according to LSD tests after ANOVA.

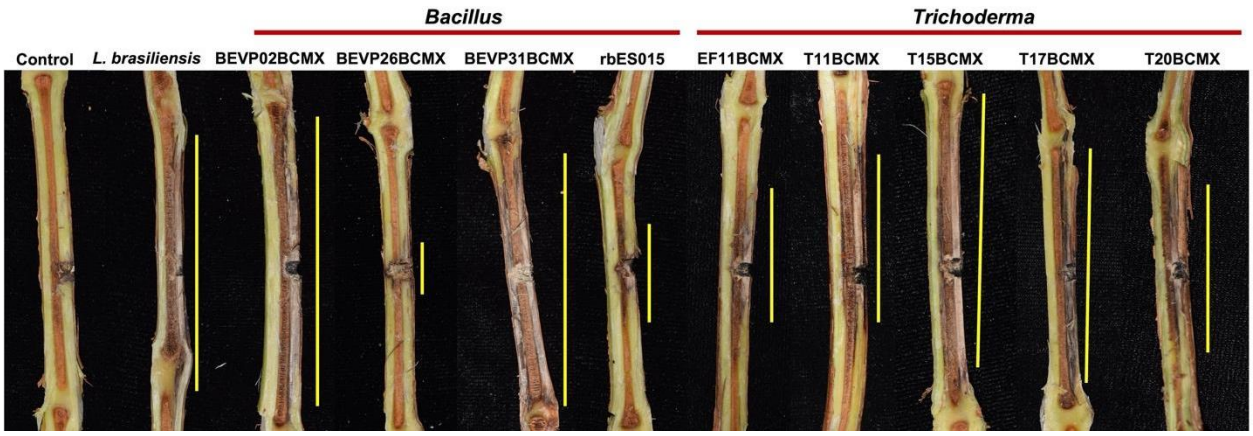

Figure 15. Images of grapevine stems after preventive soil inoculations with *Bacillus* or *Trichoderma* isolates and *Lasiodiplodia brasiliensis*. The yellow lines indicate the lengths of necrotic lesions caused by *L. brasiliensis* in 'Cabernet Sauvignon' stems.

3.4.5 Evaluation of biocontrol activity of selected isolates under vineyard conditions

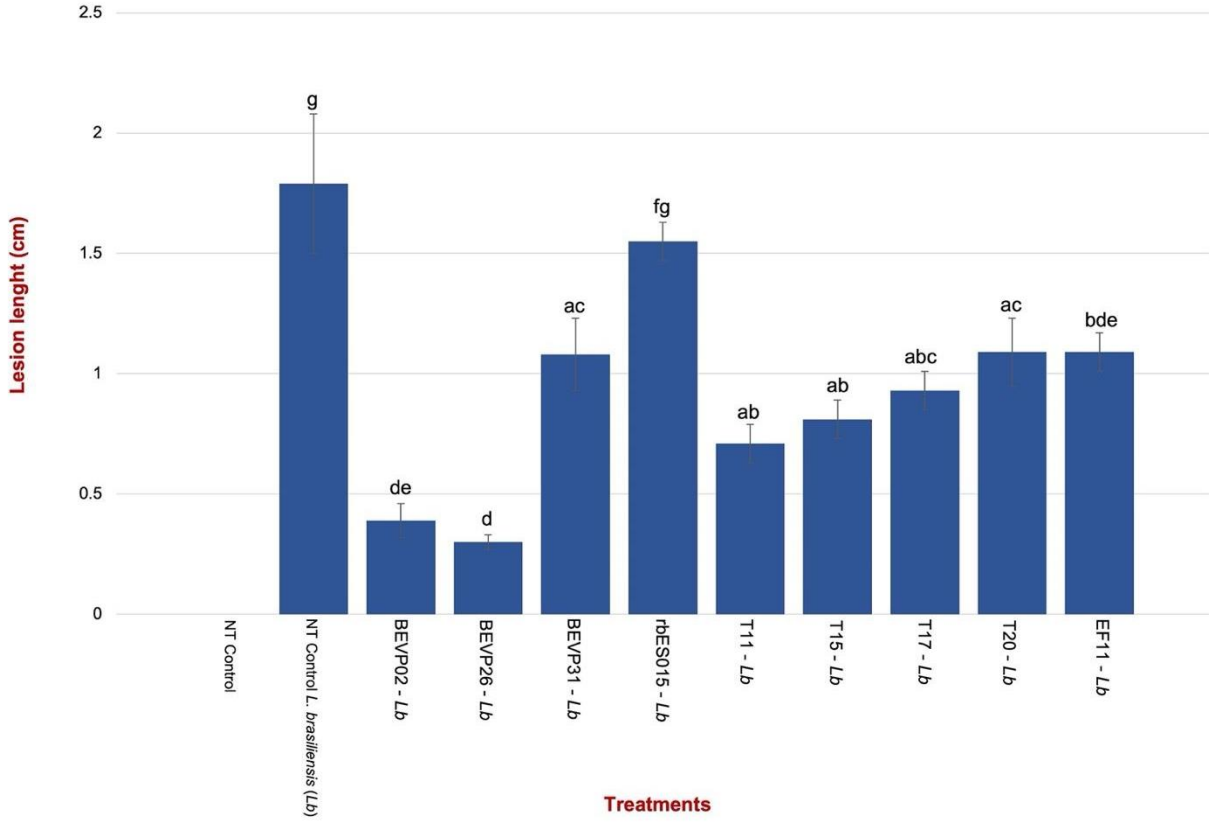

Figure 16. Mean internal necrotic lesion lengths in grapevines, caused by *Lasiodiplodia brasiliensis* after treatments with isolates of *Bacillus* (BEVP02BCMX, BEVP26BCMX, and BEVP31BCMX) or *Trichoderma* (T11BCMX, T15BCMX, T17BCMX, T20BCMX, and EF11BCMX). NT, Non-treated control. Each bar represents the mean for ten plants (± SE). Different letters over bars indicate differences ($P < 0.05$), according to LSD tests

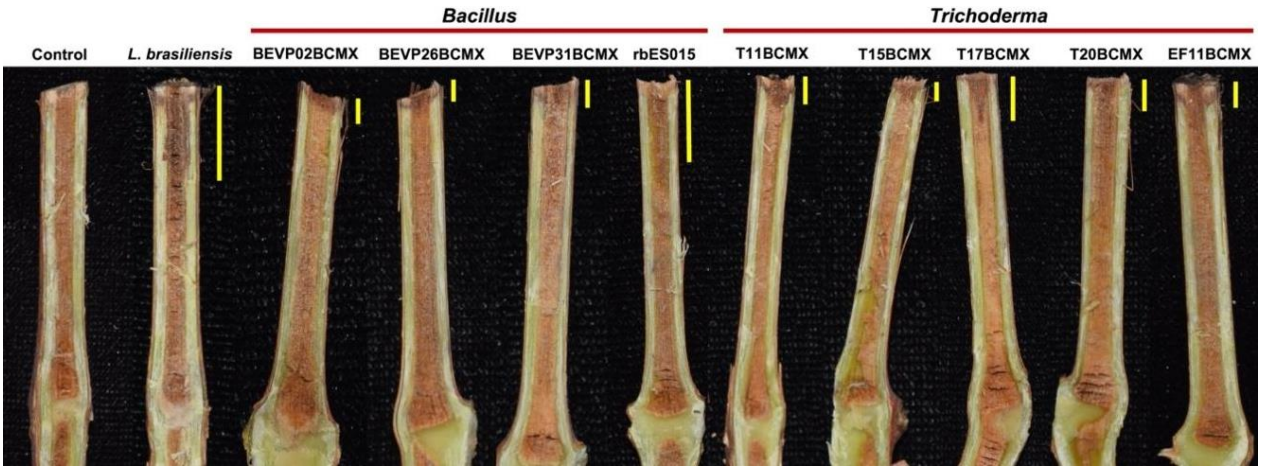

Figure 17. Images field-grown grapevine stems after treatments of pruning wounds with different *Bacillus* or *Trichoderma* isolates against *Lasiodiplodia brasiliensis* infections. The images are of ten replicates representative plants, taken of 30 d after *L. brasiliensis* inoculations.

In the field assessments, grapevine branches preventively treated with most of the evaluated *Bacillus* and *Trichoderma* isolates showed two to five times shorter lesions than the untreated branches inoculated only with *L. brasiliensis* (Figure 16). Between the *Trichoderma* isolates no statistically significant differences were detected, while among *Bacillus* isolates, *B. axarquiensis* BEVP02BCMX and *B. amyloliquefacines* BEVP26BCMX gave significant differences from *B. amyloliquefacines* BEVP31BCMX. Isolate *B. amyloliquefaciens* BEVP26BCMX gave the strongest effect, with a 5-fold reduction on the length of the necrotic lesions (Figures 16 and 17). All the beneficial organisms were re-isolated from the respective treated branches, while *L. brasiliensis* was only re-isolated from the experimental controls and the *Bacillus*-treated, vines but not from the *Trichoderma*-treated branches.

3.5 Discussion

This study has identified endophytic microorganisms associated with heritage grapevines that biocontrol *L. brasiliensis and N. parvum, which are* two of the most virulent fungi associated with Botryosphaeria dieback (Gramaje et al., 2018; Rangel-Montoya et al., 2021). The isolates used in this research were initially obtained from GTD symptomatic grapevines growing in the Guadalupe Valley in Baja California, Mexico (Rangel-Montoya et al*.,* 2021). Considering the conditions in which heritage grapevines grow in this region, with no irrigation and with little cultural management, the study has identified beneficial microorganisms that could be useful in commercial vineyards. This is the first study focusing on biological control potential of microorganisms associated with heritage grapevines.

Under *in vitro* conditions, several *Bacillus* isolates obtained from heritage grapevines inhibited *L. brasiliensis* and *N. parvum,* and showed additional characteristics associated with plant growth promotion, including production of siderophores and indole acetic acid, and solubilization of phosphate and potassium. In dual culture assays five *Bacillus strains* inhibited growth of *L. brasiliensis* by up to 51%, showing that these assays were useful for the initial screening and selection of strains with antagonistic activity. This method was previously shown to be useful for identifying two *Bacillus* strains with antagonistic effects against *Diaporthe ampelina, Diplodia seriata, Eutypa lata* and *N. parvum* (Blundell et al., 2021), and two strains of *Bacillus velezensis* against eight different fungi, including *L. theobromae, D. seriata* and *N. parvum* (Bustamante et al., 2022). While *Bacillus* spp. inhibited the growth of *N. parvum by* up to 34% through the production of organic volatile compounds, *L. brasiliensis was not affected.* However, a decrease in formation of aerial mycelium was observed, indicating a slight antagonistic effect on this fungus. However, diffusible compounds had strong antifungal effects against both pathogenic fungi, although these were less against *L. brasiliensis* (from 40% to 62%), indicating that diffusible compounds were the main antagonistic mechanisms of these *Bacillus* isolates. The production of volatile antifungal compounds, such as ketones, alcohols, esters, pyrazine, acids, hydrocarbons, heterocycles, aldehydes, phenols, thioalcohols, and thioesters, has been reported for *Bacillus* spp. For example, *B. amyloliquefaciens* CPA-8 produced 1,3 pentadiene, acetoin (3-hydroxy-2 butanone), and thiophene, that reduced *in vitro* mycelial growth of *Monilina laxa, M. fructicola,* and *Botrytis cinerea* (Gotor-Vila et al., 2017). Diffusible compounds with antifungal activity have also been identified from *Bacillus* spp., including iturin, fengycin, macrolactin, surfactin. *Bacillus* INECOL-6004, INECOL-6005, and INECOL-6006 showed antagonistic activity against *Fusarium kuroshium* by the production of iturin, fengycin, and surfactin (Guevara-Avedaño et al., 2020). Identifying the metabolites produced by the *Bacillus* isolates obtained in the present study, and their role in disease suppression, would be worthwhile.

All of the seven selected *Trichoderma* strains showed *in vitro* antagonistic activity. Additionally, they all produced siderophores, chitinase, and indole acetic acid. Several previous *studies have identified Trichoderma spp.* with activity against *GTD* pathogens. Úrbez-Torres et al., (2020) evaluated the antagonistic activities of sixteen *Trichoderma* strains against *D. seriata, E. lata* and *N. parvum, with T. atroviride* PARC1018 giving the greatest inhibition of *D*. *seriata and E. lata,* and *T. koningiopsis* against *N. parvum*. Similarly, Blundell et al*.,* (2021) reported that *T. asperellum* UC8360 inhibited *D. seriata, E. lata, N. parvum and D. ampelina,* with *D. ampelina* being the most inhibitory, while a *T. harzianum isolate gave* high rates of inhibition of *N. parvum (*Langa-Lomba et al., 2022). In the present study, three strains showed inhibition proportions greater that 50%. Two of these (*T. asperellum* EF09BCMX and *T. asperellum* EF011BCMX) were isolated from heritage grapevines, indicating that the heritage grapevine cv. ‘Mission’

contains beneficial microorganisms that can be used as BCAs with additional benefit of plant growth promotion. Different *Trichoderma* strains inhibit growth of phytopathogenic fungi through the production of volatile compounds (Zhao et al*.,* 2022), In the present study, however, the selected strains did not inhibit the mycelial growth of *L. brasiliensis* or *N. parvum* by the production of volatile compounds, although decreases in the formation of aerial mycelium were observed.

Mycoparasitism is considered to be an important biocontrol mechanism of *Trichoderma* (Sood et al., 2020). However, previous studies showed that this mode of action is not always present. For example, from 50 *Trichoderma isolates* evaluated against *Moniliophthora roreri,* mycoparasitism varied between 0% and 100%, with only nine isolates reaching 100% (Reyes-Figueroa et al., 2016). Leiva et al. (2020) also found that for 199 *Trichoderma* isolates*,* mycoparasitism rates varied from 32% to 100%. Isolates with this capacity parasitize and colonize phytopathogens, reducing the fungal inoculum and alleviating the intensity of the diseases they cause (Nusaibah and Musa, 2019, Mukherjee et al., 2022). The pre-colonized plate assays of the present study showed a colonization rates of *L. brasiliensis* from 70% to 100%. When *T. asperellum* EF09BCMX and *T. asperellum* T15BCMX were evaluated, *L. brasiliensis* was not recovered from the plates, indicating total elimination of the pathogen, as expected by the 100% colonization obtained. In contrast, the microscopical observations showed that hyphae from seven *Trichoderma* isolates coiled around hyphae of *L. brasiliensis*. This ability has been extensively reported. For example, *T. asperellum* UDEAGIEM-H01 formed coils around hyphae of *F. oxysporum* and *Macrophomina phaseolina* (Díaz-Gutiérrez et al*.,* 2021), *T. harzianum* KMISO2-2-19A around *Fusarium virguliforme* hyphae (Pimentel et al., 2020), and *T. koningiopsis around hyphe of Phytophthora xcambivora* (Frascella et al., 2022). Coiling around hyphae is the first step of *Trichoderma* mycoparasitic activity and is followed by production of hydrolytic enzymes that allow *Trichoderma* to penetrate the hosts and absorb their contents (Rocha-Ramirez et al., 2002). *Trichoderma isolates* produce extracellular cell wall degrading enzymes such as endochitinases, b-1,3- glucanases, and proteases, that lyse pathogen mycelium (Harman et al., 2004; Druzhinina *et al.,* 2011). Although the production of cell wall degrading enzymes was not evaluated in the present study, *T. asperellum* T15BCMX and *T. longibrachiatum* T17BCMX caused deformation and the cell wall lysis of *L. brasiliensis* hyphae, indicating production of enzymes that damaged cell walls, and potential as BCAs.

Although the *in vitro* tests provided information on the antagonistic potential of the evaluated strains, low inhibition proportions may not indicate that isolates will perform poorly when applied as biocontrol agents under field conditions. Effectiveness of biocontrol in the field often depends on capacity to colonize plant tissues, establish compatible interactions, prevail in the hosts, and tolerate abiotic factors (Finkel et al.,

2017; Afzal et al., 2019). For example, isolate *B. axarquiensis* BEVP02BCMX showed low inhibition in dual culture assays against *L. brasiliensis*, but when it was applied as a preventive pruning protectant, it reduced the size of the necrotic lesions. Effectiveness of this strain could be related to its ability to colonize grapevine tissues, which is possibly expected considering its endophytic nature. However, beneficial microorganisms do not always reduce damage caused by pathogens, as was observed here with *T. harzianum* T06BCMX, since plants showed longer necrotic lesions compared to those inoculated only with *L. brasiliensis*. This effect has been observed previously. Leal et al., (2021) reported that plants inoculated with *T. atroviride* SC1 and *B. subtilis* PTA-271, applied in soil against *N. parvum,* developed longer necrotic lesions than experimental controls. This is a good reason why potential biological control agents should be thoroughly assessed.

In plants, beneficial microorganisms have been evaluated using different methods, including preventative applications in substrates, or directly applied to pruning wounds (Haidar et al., 2016). In the present study, selected isolates were first evaluated by direct application to soil, and then in a vineyard by applying them to pruning wounds. In the greenhouse assay, two *Bacillus* isolates (*Bacillus* sp. rbES015 and *B. amyloliquefaciens* BEVP26BCMX) and three *T. asperellum* isolates (EF09BCMX, EF11BCMX, and T20BCMX) reduced necrotic lesion lengths caused by *L. brasiliensis.* Since the beneficial microorganisms were applied in soil without direct contact with the pathogen, the observed effect could be due to activation of host systemic response. Previous studies have indicated that non-pathogenic bacteria and fungi have capabilities to reduce damage caused by pathogens through activation of induced host systemic resistance. Haidar et al., (2016) and Zehra et al., (2021) identified different bacteria isolates with biocontrol activity against *P. chlamydospora*, when applied preventatively as drenches. Similarly, Haidar et al., (2021) identified different bacteria isolates that reduced necrotic lesions caused by *N. parvum* in grapevines when inoculated in soil. This opens the possibility for applying selected biocontrol isolates during irrigation or as drenches, diminishing the costs of biocontrol applications.

Pathogens causing GTDs enter grapevines mainly through pruning wounds (Gramaje et al., 2018), so control strategies should focus on wound protection. Few studies have been carried out in field conditions, and they generally used commercial formulations and specific strains. Martínez-Diz et al., (2020) evaluated the *Trichoderma isolates* SC1 and 1-1237 against *D. seriata* and *P. clamydospora*, observing low efficacy. The low effectiveness of commercial formulations based on biocontrol agents is common, and has been mainly attributed to microorganism failure to colonize plant tissues (Mutawila et al*.,* 2016). In the present study, eight isolates applied directly to grapevine wounds reduced necrotic lesions caused by *L. brasiliensis,* and only *Bacillus sp.* rbES015 failed. This isolate was obtained from soil so may be incapable

of colonizing grapevine tissues. The microorganisms obtained in the present study showed strong biocontrol activity, even though they were applied without the addition of carriers and protectants. Although comprehensive evaluation needs to be carried out, it may be possible to transfer the selected strains to a company or association to develop a formulation based on the strains to improve its usability and stability for grape producers.

In this research, a strain of *T. longibrachiatum* was shown to be a good candidate as a biological control agent. However, this species has been reported as an opportunistic human pathogen of immunosuppressed patients (Myoken et al., 2002; Lipový et al., 2021; Vasiliki et al., *2021*). Therefore, the use of *T. longibrachiatum* T17BCMX as biological control agent should be restricted, though it showed excellent antagonistic activity in assays carried out under *in vitro* and *in planta* conditions. Most of the other identified *Trichoderma* strains were *T. asperellum*, which is widely used as a biological control agent, without reported human risks.

The antagonistic activity of the *Bacillus* isolates was mainly due to production of diffusible compounds, while in the *Trichoderma* spp. it resulted from production of diffusible compounds and the mycoparasitism. However, volatile compounds may also have contributed to the biological control activity of the isolates, since all the selected strains produced these compounds that inhibited *Neofusicoccum in vitro*.

In the present study strains with antagonistic activity were tested separately. Combination of isolates of *Bacillus* and *Trichoderma* have been previously shown to be successful. For example, *B. subtilis* PTA-271 and *T. atroviride* SC1 were evaluated individually and together against *N. parvum* Bt67 in two grapevine varieties (‘Tempranillo’ and ‘Chardonnay’). ‘Tempranillo’ plants inoculated with either *T. atroviride* SC1 or the consortium had fewer internal lesions caused by *N. parvum* (Leal et al., 2021). In the future, compatibility among the isolates identified in the present study could be determined for the development of a consortium that takes advantage of the strengths of different isolates.

In conclusion, this study has identified *Bacillus* and *Trichoderma* isolates with biocontrol activity against *L. brasiliensis* when applied preventatively to soil and to the pruning wounds. Therefore, heritage grapevines of Baja California have been shown to be a reservoir of beneficial microorganisms, which can be potentially utilized in commercial grapevine varieties to help reduce damage caused by grapevine trunk disease fungi.

3.6 Acknowledgments

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3.7 Bibliography

Abdullah, H. A. S., Mohanad, K. M. A., Muhammed, A. H. A. B., & Zulfiqar, A. (2012). First report of grapevine dieback caused by *Lasiodiplodia theobromae* and *Neoscytalidium dimidiatum in* Basrah, Southern Iraq. *African Journal of Biotechnology, 11*(95), 16165–16171. 10.5897/AJB12.010

Afzal, I., Shinwari, Z.K., Sikandar, S., & Shahzad, S. (2019). Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. *Microbiological Research, 221,* 36–49. https://doi.org/10.1016/j.micres.2019.02.001

Agrawal, T., & Kotasthane, A. S. K. (2012). Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in central India. *Springer Plus, 1*(73), 1–10. 10.1186/2193-1801-1-73

Ahmad, F., Ahmad, I., & Khan, M.S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research, 163*, 173–181. https://doi.org/10.1016/j.micres.2006.04.001

Andrade, S. G., Arón N., & Flores, F. (2013). Matriz de insumo producto vitivinícola de Baja California México. *Revista de Economía, 30*(81), 57–57. https://doi.org/10.33937/reveco.2013.41

Bailey, B. A., Bae, H., Strem, M. D., Crozier, J., Thomas, S. E., Samuels, G. J., Vinyard, B. T., & Holmes K. A. (2008). Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. *Biological Control, 46*(1), 24–35. https://doi.org/10.1016/j.biocontrol.2008.01.003

Bapiri, A., Asgharzadeh, A., Mujallali, H., Khavazi, K., Pazira, E. & Graduated P.D., (2012). Evaluation of zinc solubilization potential by different strains of fluorescent Pseudomonads. *Journal of Applied Sciences and Environmental Management, 16*(3), 295–298.

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

Billar de Almeida, A. B., de Concas, J., Campos, M. D., Materatski, P., Varanda, C., Patanita, M., Murolo, S., Romanazzi, G., & Félix, M. D. R. (2020). Endophytic fungi as potential biological control agents against grapevine trunk diseases in Alentejo region. *Biology, 9*(420), 1–23. https://doi.org/10.3390/biology9120420

Billones-Baaijens, R., & Savocchia, S. (2019). A review of Botryosphaeriaceae species associated with grapevine trunk diseases in Australia and New Zealand. *Australasian Plant Pathology*, *48*, 3–18. https://doi.org/10.1007/s13313-018-0585-5

Blundell, R., Arreguin, M., & Eskalen, A. (2021). *In vitro* evaluation of grapevine endophytes, epiphytes and sap microorganisms for potential use to control grapevine trunk disease pathogens. *Phytopathologia Mediterranea, 60*(3), 535–548. https://doi.org/10.36253/phyto-12500

Bustamante, M. I., Elfar, K., & Eskalen, A. (2022). Evaluation of the antifungal activity of endophytic and rhizospheric bacteria against grapevine trunk pathogens. *Microorganisms, 10*(10), 2035. 10.3390/microorganisms10102035

Crowley, W.K. (1989). The Growth of the Mexican wine industry. *Yearbook of the Association of Pacific Coast Geographers, 51*, 25–47.

Datta, C., & Basu, P.S. (2000). Indole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub, *Cajanus cajan*. *Microbiological Research*, *155*, 123–127. 10.1016/S0944-5013(00)80047-6

Delgado-Ramírez, C. S., Hernández-Martínez, R., & Sepúlveda, E. (2021). Rhizobacteria Associated with a Native Solanaceae Promote Plant Growth and Decrease the Effects of *Fusarium oxysporum* in Tomato. *Agronomy, 11*(3), 579. https://doi.org/10.3390/agronomy11030579

Díaz-Gutiérrez, C., Arroyave, C., Llugany, M., Poschenrieder, C., Martos, S., & Peláez, C. (2021). *Trichoderma asperellum* as a preventive and curative agent to control Fusarium wilt in *Stevia rebaudiana*. *Biological Control 155*, 104537. https://doi.org/10.1016/j.biocontrol.2021.104537

Dini-Andreote, F. (2020). Endophytes: The second layer of plant defense. *Trends in Plant Science, 25*(4): 319–322. https://doi.org/10.1016/j.tplants.2020.01.007

Druzhinina, I., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B.A., Kenerley, Monte, E., Mukherjee, P. K., Zeilinger, S., Grigoriev, I. V., & Kubicek, C.P. (2011). *Trichoderma*: the genomics of opportunistic success. *Nature Reviews Microbiology, 9*, 749–759. 10.1038/nrmicro2637

Finkel, O. M., Castrillo, G., Herrera-Paredes, S., Salas González, I. & Dangl, J. L. (2017). Understanding and exploiting plant beneficial microbes. *Current Opinion in Plant Biology, 38*, 155–163. https://doi.org/10.1016/j.pbi.2017.04.018

Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Wilson, B. A., & Olsen G. J. (2008). Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Applied and Environmental Microbiology*, *74*(8), 2461–2470. https://doi.org/10.1128/AEM.02272-07

Frascella, A., Sarrocco, S., Mello, A., Venice, F., Salvatici, C., Danti, R., Emiliani, G., Barberini, S., & Della Rocca G. (2022). Biocontrol of *Phytophthora xcambivora* on *Castanea sativa*: Selection of local *Trichoderma* spp. isolates for the management of Ink disease. *Forests, 13*, 1065. https://doi.org/10.3390/f13071065

Gotor-Vila, A., Teixidó, N., Di Francesco, A., Usall, J., Ugolini L., Torres, L., & Mari, M. (2017). Antifungal effect of volatile organic compounds produced by *Bacillus amyloliquefaciens* CPA-8 against fruit pathogen decays of cherry. *Food Microbiology, 64*, 219–225. 10.1016/j.fm.2017.01.006

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

Guevara-Avendaño, E., Carrillo, J. D., Ndinga-Muniania, C., Moreno, K., Méndez-Bravo, A., Guerrero-Analco, J.A., Eskalen, A., & Reverchon, F. (2018). Antifungal activity of avocado rhizobacteria against *Fusarium euwallaceae* and *Graphium* spp., associated with *Euwallacea* spp. nr. *fornicatus*, and *Phytophthora cinnamomi*. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology, 111*(4), 563–572. 10.1007/s10482-017-0977-5

Guevara-Avendaño, E., Bravo-Castillo, K. R., Monribot-Villanueva, J. L., Kiel-Martínez, A. L., Ramírez-Vázquez, M., Guerrero-Analco, J. A., & Reverchon F. (2020). Diffusible and volatile organic compounds produced by avocado rhizobacteria exhibit antifungal effects against *Fusarium kuroshium*. *Brazilian Journal of Microbiology*, *51*(3), 861–873. 10.1007/s42770-020-00249-6

Gupta, R., Singal, R., Shankar, A., Kuhad, R. C., & Saxena, R. K. (1994). A modified plate assay for screening phosphate solubilizing microorganisms. *Journal of General and Applied Microbiology, 40*(3), 255–260. https://doi.org/10.2323/jgam.40.255

Haidar, R., Roudet, J., Bonnard, O., Dufour, M.C., Corio-Costet, M. F., Fert, M., Gautier, T., Deschamps A., & Fermaud, M. (2016). Screening and modes of action of antagonistic bacteria to control the fungal pathogen *Phaeomoniella chlamydospora* involved in grapevine trunk diseases. *Microbiological Research,* 192, 172–184. https://doi.org/10.1016/j.micres.2016.07.003

Haidar, R., Amira, Y., Roudet, J., Marc, F., & Patrice R. (2021). Application methods and modes of action of *Pantoea agglomerans* and *Paenibacillus* sp. to control the grapevine trunk disease-pathogen, *Neofusioccum parvum*. *OENO One, 55*(3), 1–16. https://doi.org/10.20870/oeno-one.2021.55.3.4530

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I.M & Lorito, M. (2004). *Trichoderma* specie opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology, 2*, 43–56. 10.1038/nrmicro797

Instituto Nacional de Estadística y Geografía (INEGI) (2017). Anuario Estadístico y Geográfico de Baja California 2017. https://www.inegi.org.mx/contenido/productos/prod_serv/contenidos/espanol/bvinegi/productos/nueva_estruc/anuarios_2017/702825094874.pdf

Jacob, J., Krishnan, G. V., Thankappan, D., & Amma, D. K. (2020). Endophytic bacterial strains induced systemic resistance in agriculturally important crop plants. In: *Microbial Endophytes* (Ajay K., Radhakrishnan E.K. ed.) Elsevier, Duxford, Cambridge, United Kingdom, 75–105. 10.1016/B978-0-12-819654-0.00004-1

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution, 35*(6), 1547–1549. 10.1093/molbev/msy096

Langa-Lomba, N., Martín-Ramos, P., Casanova-Gascón, J., Julián-Lagunas, C., & González-García V. (2022). Potential of native *Trichoderma* strains as antagonists for the control of fungal wood pathologies in young grapevine plants. *Agronomy, 12*(2), 336. https://doi.org/10.3390/agronomy12020336

Leal, C., Richet, N., Guise, J. F., Gramaje, D., Armengol, J., Fontaine, F., & Trotel-Aziz, P. (2021). Cultivar contributes to the beneficial effects of *Bacillus subtilis* PTA-271 and *Trichoderma atroviride* SC1 to Protect Grapevine Against *Neofusicoccum parvum*. *Frontiers in Microbiology, 12*, 726132. 10.3389/fmicb.2021.726132

Leiva, S., Oliva, M., Hernández, E., Chuquibala, B., Rubio, K., Garcia, F., & Torres de la Cruz M. (2020). Assessment of the potential of *Trichoderma* spp. strains native to Bagua (Amazonas, Peru) in the biocontrol of frosty pod rot (*Moniliophthora roreri*). *Agronomy, 10*(9), 1376. https://doi.org/10.3390/agronomy10091376

Lipový, B., Raška, F., Kocmanová, I., Hanslianová, M., Hladík, M., Holoubek, J., Bezdícek, M., & Macháček, C. (2021). *Trichoderma longibrachiatum* and *Aspergillus fischeri* Infection as a cause of skin graft failure in a patient with critical burns after liver transplantation. *Journal of Fungi, 7*(6), 487. 10.3390/jof7060487

Lynne, A. M., Haarmann, D., & Louden, B. C. (2011). Use of blue agar CAS assay for siderophore detection. *Journal of Microbiology & Biology Education, 12*, 51–53. https://doi.org/10.1128/jmbe.v12i1.249

Magoni, C. (2009). *Historia de la vid y el vino en la Península de la Baja California*. 1ra ed.Universidad Iberoamericana, Tijuana (pp: 283)

Martínez-Diz, M. del P., Díaz-Losada, E., Díaz-Fernández, Á., Bouzas-Cid, Y., & Gramaje, D. (2020). Protection of grapevine pruning wounds against *Phaeomoniella chlamydospora* and *Diplodia seriata* by commercial biological and chemical methods. *Crop Protection, 143*, 105465. https://doi.org/10.1016/j.cropro.2020.105465

Mayo-Prieto, S., Campelo, M. P., Lorenzana, A., Rodríguez-González, A., Reinoso, B., & Gutiérez-Casquero, P. A. (2020). Antifungal activity and bean growth promotion of *Trichoderma* strains isolated from seed vs soil. *European Journal of Plant Pathology, 158,* 817–828. https://doi.org/10.1007/s10658-020-02069-8

Méndez-Bravo, A., Cortazar-Murillo, E. M., Guevara-Avendaño, E., Ceballos-Luna, O., Rodríguez-Haas, B., Kiel-Martínez, A., Hernández-Cristóbal, O., Guerrero-Analco, J. A., & Reverchon F. (2018). Plant growth-promoting rhizobacteria associated with avocado display antagonistic activity against *Phytophthora cinnamomi* through volatile emissions. *PLOS ONE, 13*(3), e0194665. 10.1371/journal.pone.0194665

Myoken, Y., Sugata, T., Fujita, Y., Asaoku, H., Fujihara, M., & Mikami, Y. (2002). Fatal necrotizing stomatitis due to *Trichoderma longibrachiatum* in a neutropenic patient with malignant lymphoma: a case report. *International Journal of Oral and Maxillofacial Surgery, 31*(6), 688-691. 10.1054/ijom.2001.0211.

Mondello, V., Larignon, P., Armengol, J., Kortekamp, A., Vaczy K., Serrano, E., Rego, C., Mugnai, L., & Fontaine F. (2018). Management of grapevine trunk diseases: Knowledge transfer, current strategies and innovative strategies adopted in Europe. *Phytopathologia Mediterranea, 57*(3), 369–383. 10.14601/Phytopathol_Mediterr-23942

Mukherjee, P. K., Mendoza-Mendoza, A., Zeilinger, S., & Horwitz, B. A. (2022). Mycoparasitism as a mechanism of Trichoderma-mediated suppression of plant diseases. *Fungal Biology Reviews*, *39*, 15–33. 10.1016/j.fbr.2021.11.004

Mutawila, C., Halleen, F., & Mostert, L. (2016). Optimization of time of application of *Trichoderma* biocontrol agents for protection of grapevine pruning wounds. *Australian Journal of Grape and Wine Research, 22*(2), 279–287. 10.1111/ajgw.12218

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. 10.3389/fmicb.2020.00477

Nusaibah, A., & Musa, H. (2019). A review report on the mechanism of *Trichoderma* spp. as biological control agent of the basal stem rot (BSR) disease of *Elaeis guineensis.* In *Trichoderma: The most widely used fungicide* (Shah M.M., Sharif U., Buhari R.T., ed), IntechOpen, London, United Kingdom, 1–12. 10.5772/intechopen.84469

O'Donnell, K., Kistlerr, H. C., Cigelnik, E., & Ploetz, R. C. (1998). Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences of the United States of America, 95*(5), 2044–2049. https://doi.org/10.1073/pnas.95.5.2044

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiology 17*, 362–370.

Pimentel, M. F., Arnao, E., Warner, A. J., Subedi, A., Rocha L. F., Srour, A., Bond, J. P., & Fakhoury, A. (2020). *Trichoderma* isolates inhibit *Fusarium virguliforme* growth, reduce root rot, and induce defense-related genes on soybean seedlings*. Plant Disease, 104*(7), 1949–1959. 10.1094/PDIS-08-19-1676-RE

Poudel, M., Mendes, R., Costa, L. A. S., Bueno, C. G., Meng, Y., Folimonova, S. Y., Garrett, K. A., & Martins S.J. (2021). The role of plant-associated bacteria, fungi, and viruses in drought stress mitigation. *Frontiers in Microbiology, 12*, 744512. 10.3389/fmicb.2021.743512

Rangel-Montoya, E. A. (2021). Characterization, evaluation of the colonization process and search for biological control strategies of fungi of the Botryosphaeriaceae family associated with grapevines in Mexico. [Doctoral thesis [Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California]

Rangel-Montoya, E. A., Paolinelli, M., Rolshausen, P. E., Valenzuela-Solano, C., & Hernandez-Martinez, R. (2021). Characterization of *Lasiodiplodia* species associated with grapevines in Mexico. *Phytopathologia Mediterranea*, *60*(2), 237–251. 10.36253/phyto-12576

Rangel-Montoya, E. A., Delgado-Ramírez, C. S. Sepulveda, E., & Hérnandez-Martínez, R. (2022). Biocontrol of *Macrophomina phaseolina* using *Bacillus amyloliquefaciens* strains in cowpea (*Vigna unguiculata* L.). *Agronomy, 12*(3), 676. 10.3390/agronomy12030676

Reyes-Figueroa, O., Ortiz-García, C. F., Torres-de la Cruz, M., Lagunes-Espinoza, L. D. C., & Valdovinos-Ponce, G. (2016). *Trichoderma* species from the cacao agroecosystem with biocontrol potential of *Moniliophthora roreri***.** *Revista Chapingo serie ciencias forestales y del ambiente,* 22(2), 149-163. 10.5154/r.rchscfa.2015.08.036

Rezgui, A., Ben Ghnaya-Chakroun, A., Vallance, J., Bruez, E., Hajlaoui, M. R., Sadfi-Zouaoui, N., & Rey, P. (2016). Endophytic bacteria with antagonistic traits inhabit the wood tissues of grapevines from Tunisian vineyards. *Biological Control, 99,* 28–37. 10.1016/j.biocontrol.2016.04.005

Riseh, R. S., Ebrahimi-Zarandi, M., Gholizadeh-Vazvani, M., & Skorik, Y. A. (2021). Reducing drought stress in plants by encapsulating plant growth-promoting bacteria with polysaccharides. *International Journal of Molecular Sciences, 22*(23), 12979. 10.3390/ijms222312979

Rocha-Ramirez, V., Omero, C., Chet, I., Horwitz, B. A., & Herrera-Estrella, A. (2002). *Trichoderma atroviride* G-protein alpha-subunit gene tga1 is involved in mycoparasitic coiling and conidiation. *Eukaryotic Cell, 1*(14), 594-605. 10.1128/EC.14.594-605.2002

Salvatierra-Martinez, R., Arancibia, W., Araya, M., Aguilera, S., Olalde, V., Bravo, J., & Stoll, A. (2018). Colonization ability as an indicator of enhanced biocontrol capacity an example using two *Bacillus amyloliquefaciens* strains and *Botrytis cinerea* infection of tomatoes. *Journal of Phytopathology, 166*(9), 601–612. 10.1111/JPH.12718

Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, *160*(1), 47–56. 10.1016/003-2697(87)90612-9

Secretria de Fomento Agropecuario del Gobierno del Estado (SEFOA). (2011). Estudio estadístico sobre producción de uva en Baja California. https://www.nacionmulticultural.unam.mx/empresasindigenas/docs/1873.pdf

Silva-Valderrama, I., Toapanta, D., Miccono, M. de los A., Lolas, M., Díaz G. A., Cantu, D., & Castro, A. (2021). Biocontrol potential of grapevine endophytic and rhizospheric fungi against trunk pathogens. *Frontiers in Microbiology, 11*, 614620. 10.3389/fmicb.2020.614620

Spagnolo, A., Magnin-Robert, M., Alayi, T. D., Cilindre, C., Schaeffer-Reiss, C., Van Dorsselaer A., Clément C., Larignon, P., Ramírez, S., Chong, J., Bertsch C., Abou-Mansour, E., & Fontaine, F. (2014). Differential responses of three grapevine cultivars to Botryosphaeria dieback. *Phytopathology, 104*(10), 1021–1035. 10.1094/PHYTO-01-14-0007-R

Sood, M., Kapoor, D., Kumar, V., Sheteiwy, M. S., Ramakrishnan, M., Landi, M., Araniti, F., & Sharma, A., (2020). *Trichoderma*: The "secrets" of a multitalented biocontrol agent. *Plants, 18*(6), 762. 10.3390/plants9060762

Úrbez-Torres, J. R., & Gubler W. D. (2009). Pathogenicity of Botryosphaeriaceae species isolated from grapevine cankers in California. *Plant Disease*, *93*(6), 584–592. 10.1094/PDIS-93-6-0584

Úrbez-Torres, J. R., Peduto, F., Striegler, R. K., Urrea-Romero, K. E., Rupe J. C., Cartwright, R. D., & Gubler, W. D. (2012). Characterization of fungal pathogens associated with grapevine trunk diseases in Arkansas and Missouri. *Fungal Diversity, 52*, 169–189. 10.1007/s13225-11-0110-4

Úrbez-Torres, J. R., Tomaselli, E., Pollard-Flamand, J., Boulé, J., Gerin, D., & Pollastro, M. (2020). Characterization of *Trichoderma* isolates from southern Italy. And their potential biocontrol activity against grapevine trunk disease fungi. *Phytopathologia Mediterranea, 59*(3), 425–439. 10.14601/Phyto-11273

Vasiliki, E.G., Despoina, M., Konstantinos, M., Christos, D., Aikaterini, G., Serafeim, C., Nikolaos, G., Anna, G., Pagona, S., Nikolaos, T., & Xanthi T. (2021). First case of pneumonia-parapneumonic effusion due to *Trichoderma longibrachiatum*. *IDCases, 25*, e01239. 10.1016/j.idcr.2021.e01239.

Wagner, D. B., Furnier, G. R., Saghai-Maroof, M. A., Williams, S. M., Dancik, B. P., & Allard, R. W. (1987). Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. *Proceedings of the*

National Academy of Sciences of the United States of America, 84(7), 2097–2100. 10.1073/pnas.84.7.2097

Walker, M. A., Heinitz, C., Riaz, S., & Uretsky, J. (2019). Grape taxonomy and germplasm. In: *Compendium of plant genomes* (D. Cantu M. A. Walker ed.), Springer, Cham, California, United States of American, 25–38. 10.1007/978-3-030-19601-2

Zehra, A., Raytekar, N. A., Meena, M., & Swapnil, P. (2021). Efficiency of microbial bio-agents as elicitors in plant defense mechanism under biotic stress*:* A review*. Current Research in Microbial Sciences, 2*, 100054. 10.1016/j.crmicr.2021.100054

Zhang, W., Groenewald, J. Z., Lombard, L., Schumacher, R. K., Phillips, A. J. L., & Crous, P. W. (2021). Evaluating species in *Botryosphaeriales*. *Persoonia, 46*, 63–115. 10.3767/persoonia.2021.46.03

Zhao, X., Zhou, J., Tian, R., & Liu, Y. (2022). Microbial volatile organic compounds: Antifungal mechanisms, applications, and challenges. *Frontiers in Microbiology, 13*, 922450. 10.3389/fmicb.2022.922450

Zheng, B. X., Ibrahim, M., Zhang, D. P., Bi Q. F., Li, H. Z., Zhou, G. W., Ding, K., Zung, Y. G., & Yang X. R. (2018). Identification and characterization of inorganic-phosphate-solubilizing bacteria from agricultural fields with a rapid isolation method. *AMB Express, 8*(1), 47. 10.1186/s13568-018-0575-6

Chapter 4. General discussion

This work identified 'Mission' grapevines as a reservoir for grapevine trunk diseases fungi, as well as biological control agents. In Baja California, this cultivar has demonstrated adaptation to saline soils, arid climate, low water availability, resistance to certain diseases, and the ability to grow without tutoring, requiring minimal agricultural management and growing under rainfed conditions (González-Andrade and Fuentes-Flores, 2013; SEFOA, 2011; Magoni, 2021; Martain-Amozurrutia, 2021). Recently, consumers have shown increasing interest in purchasing unique, historically significant wines produced in an artisanal manner. In this context, wines from 'Mission' grapes are an excellent option. However, the production of such wines is constrained by the limited area of vineyards planted with this variety and the damage caused by pests and diseases.

Botryosphaeria dieback, regarded as one of the most destructive grapevine trunk diseases, is a major issue in Baja California vineyards. This disease is caused by fungi of the Botryosphaeriaceae family, which colonize the xylem of plants. Once the infection occurs, symptoms such as necrosis in the wood, cankers, dieback, and ultimately the death of the plant are observed (Gramaje et al., 2018; Hrycan et al., 2020; Niem et al., 2020). In commercial grapevine varieties, including 'Cabernet Sauvignon,' 'Carignane,' 'Chenin Blanc,' 'Sauvignon Blanc,' and 'Petit Sirah,' among others, at least 16 species of fungi of the genera *Botryosphaeria*, *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* causing Botryosphaeria dieback have been identified (Candolfi-Arballo et al., 2010; Paolinelli-Alfonso 2015; Rangel-Montoya et al., 2021; Rangel-Montoya et al., 2024). For the 'Mission' cultivar, plants in different vineyards have shown symptoms such as canker formation, dieback, or plant death, all associated with Botryosphaeria dieback. Analysis of the symptomatic tissue from these plants yielded 13 strains, morphologically and molecularly identified as *Diaporthe ampelina* (one isolate) and *Diplodia seriata* (12 isolates).

It has been previously observed that many fungal species causing Botryosphaeria dieback are present in commercial grapevine varieties in the region. In contrast, this study found that only two species were in 'Mission' grapevines. This variation in pathogen diversity may be attributed to the sources of infected material (Gramaje and Armengol, 2011; Carbone et al., 2022); as most vineyards in Mexico obtain propagation material from other countries such as France, Spain, the United States of America, and Italy (CMV, 2020). Meanwhile, the distribution and establishment of 'Mission' plant material has been limited to Baja California.

Differences in the virulence levels of grapevine trunk fungi from commercial varieties and 'Mission' grapevines are evident. Highly virulent fungi, such as *L. brasiliensis*, *L. exigua*, and *N. parvum,* have been isolated from commercial varieties (Rangel-Montoya et al., 2021; Rangel-Montoya et al., 2024). In contrast, fungal strains associated with 'Mission' grapevines have been characterized by their low infective capacity.

Strains of GTDs fungi are known for their high capacity to rapidly degrade cell-wall components of the grapevine wood (Cruz-Lopes et al., 2014). When evaluating the growth of *D. ampelina* and *D. seriata* on different carbon sources related to wood components, we observed that all strains had low growth rates and minimal aerial mycelium formation. This contrast with finding by Rangel Montoya et al., (2023), who reported that *L. brasiliensis* MXBCL28 strain had a growth rate of up to 18.7±1.764 mm/day. This discrepancy is primarily related to the capacity of the fungus to degrade and absorb wood components.

There is no effective control method for GTDs, so various management strategies are recommended, including the application of fungicides, cultural practices, and biological control agents (Mondello et al., 2018; Mesguida et al., 2023). Biological control has emerged as a sustainable option for grape cultivation because biological control agents often use different modes of action to inhibit pathogens, promote plant growth, colonize plant tissue, and maintain a biocontrol effect for longer (Köhl et al., 2019; Alsharif et al., 2020). However, biological control agents often show low effectiveness in field applications, which is attributed to the impact of climatic conditions on the ability of microorganisms to colonize and persist in the plant tissues (Sarma et al., 2015; Ben Zineb et al., 2020; Lahlali et al., 2022).

When analyzing and evaluating the microorganisms associated with grapevines, it was found that strains of *Bacillus* and *Trichoderma* demonstrated the ability to solubilize nutrients and produce plant growth hormones in *in vitro*. Additionally, these strains exhibited antifungal activity against highly virulent grapevine trunk disease fungi, such as *L. brasiliensis*, through different mechanisms, including competition for space, production of volatile and non-volatile compounds, and mycoparasitism. The presence of multiple modes of action in biological control agents enhances their antagonistic activity against pathogens (Köhl et al., 2019).

Typically, the antagonistic activity of beneficial microorganisms is assessed only under laboratory conditions, which does not guarantee their effectiveness in field applications. However, the *Bacillus* and *Trichoderma* strains identified in association with 'Mission' grapevines demonstrated high adaptability and biocontrol potential. These strains showed significant inhibition percentages *in vitro* and maintained their

antifungal activity when applied preventively to plants under both greenhouse and field conditions. Strains from the *Bacillus* and *Trichoderma* are increasingly recognized as effective biological control agents due to their antagonistic activity, ability to improve nutrient availability, promotion of plant growth, and reduction in the need for chemical fungicides and environmental pollution (Khan et al., 2021; Yao et al., 2023). Although the microorganisms applied in the greenhouse and field trials showed good efficacy, it is necessary to develop a bioformulation to ensure the functionality and survival of the *Trichoderma* and *Bacillus* strains and to facilitate their application.

The effectiveness of biological control agents is directly related to the characterization and selection process under *in vitro* conditions, their ability to survive in various environmental conditions, the formulation, and the method of application (Lefort et al., 2016; Elnahal et al., 2022). Based on the results obtained in this work, the methodology used to isolate and characterize biocontrol agents with antifungal activity against *L. brasiliensis* effectively identified beneficial microorganisms.

In Baja California, the prevailing arid climate with very low precipitation, significantly impact grapevine yields. With the increasing prevalence of arid conditions in the state, selecting grapevine cultivars that can thrive under such conditions is crucial. 'Mission' grapevines are particularly promising, as they have shown the capacity to sustain production for many years, even without irrigation. This study offers valuable insights into the grapevine trunk disease fungi associated with 'Mission' grapevines, which will be instrumental in developing strategies for managing and preserving this cultivar.

Chapter 5. General conclusions

'Mission' grapevines serve as a reservoir for grapevine trunk diseases fungi, specifically from the genera *Diaporthe* and *Diplodia*. Among these, *Diplodia seriata* was the most prevalent, with strains isolated from all sampled locations. The virulence of grapevine trunk disease fungi associated with 'Mission' grapevines is relatively low compared to the highly virulent *L. brasiliensis* MXBCL28.

'Mission' grapevines are associated with beneficial microorganisms of the genera *Bacillus* and *Trichoderma*. The *Bacillus* and *Trichoderma* strains identified in this study have potential as biological control agents against *L. brasiliensis* MXBCL28 when applied preventively to soil and pruning wound.

The findings from this study will enable the development of effective management strategies for trunk diseases fungi associated with 'Mission' grapevines.

Bibliography

Aliquó, G., Torres, R., Lacombe, T., Boursiquot, J. M., Laucou, V., Gualpa, J., Fanzone, M., Sari, S., Perez Peña, J., & Prieto, J. A. (2017). Identity and parentage of some South American grapevine cultivars present in Argentina. *Australian Journal of Grape and Wine Research*, *23*, 452-460. https://doi.org/10.1111/ajgw.12282

Álvarez-Pérez, J.M., González-García, S., Cobos, R., Olego, M.Á., Ibañez, A., Díez-Galán, A., Garzón-Jimeno E., & Coque, J. J. R. (2017). Use of Endophytic and Rhizosphere Actinobacteria from Grapevine Plants To Reduce Nursery Fungal Graft Infections That Lead to Young Grapevine Decline. *Applied and Environmental Microbiology, 83*, e01564-17. https://doi.org/10.1128/AEM.01564-17

Andreolli, M., Lampis, S., Zapparoli, G., Angelini, E., & Vallini, G. (2016). Diversity of bacterial endophytes in 3 and 15 year-old grapevines of *Vitis vinifera* cv. Corvina and their potential for plant growth promotion and phytopathogen control. *Microbiological Researc*h, *183*, 42-52. 10.1016/j.micres.2015.11.009

Alsharif, W., Saad, M. M., & Hirt, H. (2020). Desert Microbes for Boosting Sustainable Agriculture in Extreme Environments. *Frontiers in Microbiology*, *22*(11), 1666. https://doi.org/10.3389/fmicb.2020.01666

Ben Zineb, A., Lamine, M., Khallef, A., Hamdi, H., Ahmed, T., Al-Jabri., H., Alsafran, M., Mliki, A., Sayadi, S., & Gargouri, M. (2024). Harnessing rhizospheric core microbiomes from arid regions for enhancing date palm resilience to climate change effects. *Frontiers in Microbiology*, *15*, 1362722. https://doi.org/10.3389/fmicb.2024.1362722

Bettenfeld, P., Cadena, J. C., Jacquens, L., Fernandez, O., Fontaine, F., van Schaik, E., Courty, P. E., & Trouvelot, S. (2021). The microbiota of the grapevine holobiont: A key component of plant health. Journal of Advanced *Research*, *40*, 1-15. https://doi.org/10.1016/j.jare.2021.12.008

Billones-Baaijens, R., & Savocchia, S. (2019). A review of Botryosphaeriaceae species associated with grapevine trunk diseases in Australia and New Zealand. *Australasian Plant Patho*logy, *48*, 3–18. https://doi.org/10.1007/s13313-018-0585-5

Bonatelli, M. L., Lacerda-Júnior, G. V., Dos Reis Junior, F. B., Fernandes-Júnior, P. I., Melo, I. S., & Quecine, M. C. (2021). Beneficial Plant-Associated Microorganisms From Semiarid Regions and Seasonally Dry Environments: A Review. *Frontiers in Microbiology 15*, 11:553223. 10https://doi.org/10.3389/fmicb.2020.553223

Blundell, R., & Eskalen, A. (2022). Biological and chemical pruning wound protectants reduce infection of grapevine trunk disease pathogens. *Califronia Agriculture*, *75*(3), 128-134. https://doi.org/10.3733/ca.2021a0018

Candolfi-Arballo, O. (2009). Caracterización de anamorfos de Botryosphaeriaceae asociados a deterioro progresivo en vid (*Vitis vinifera* L.) aislados de la región vitivinícola de Baja California. [Tesis de Maestría en Ciencias de la Vida, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California]

Carbone, M. J., Gelabert, M., Moreira, V., Mondino, P., & Alaniz, S. (2022). Grapevine nursery propagation material as source of fungal trunk disease pathogens in Uruguay. *Frontiers Fungal Biology*, *3*, 958466. https://doi.org/10.3389/ffunb.2022.958466

Consejo Mexicano Vitivinícola (CMV). (2020). Importaciones de Planta de Vid de Viveros De USA, Francia, España e Italia: Problemas y Propuestas. https://uvayvino.org.mx/wp-content/uploads/2020/07/Importaciones-de-Planta-de-Vid.pdf

Comisión Nacional del Agua (CONAGUA). (2023). Reporte anual del clima en México 2023. Coordinación General del Servicio Meteorologico Nacional, 13 (13). https://smn.conagua.gob.mx/tools/DATA/Climatolog%C3%ADa/Diagn%C3%B3stico%20Atmosf%C3%A9rico/Reporte%20del%20Clima%20en%20M%C3%A9xico/Anual2023.pd

Cruz-Lopes, L., Prozil, S., Evtuguin, D. V., Arshanitsa, A., Solodovnik, V., & Telysheva, G. M. (2014). Evaluation of grape stalks as a feedstock for pellets production. In *Proceedings of the 13th European Workshop on Lignocellulosics and Pulp*, Seville, Spain

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

Dini-Andreote, F. 2020. Endophytes: The second layer of plant defense. *Trends in Plant Science, 25*(4), 319–322. https://doi.org/10.1016/j.tplants.2020.01.007

Elnahal, A. S. M., El-Saadony, M. T., Saad, A. M., Desoky, E. S. M., El-Tahan, A. M., Rady, M. M., AbuQamar, S., & El-Tarabily, K. A. (2022). The use of microbial inoculants for biological control, plant growth promotion, and sustainable agriculture: A review. *European Journal of Plant Pathology*, *162*, 759–792. https://doi.org/10.1007/s10658-021-02393-7

Góngora-Rosado, M. A. (2016). Propuestas de prácticas sustentables en la industria vitivinícola de Baja California, México. [Tesis de Maestría en Administración Integral del Ambiente. Colegio de la Frontera Norte, A. C. y el Centro de Estudios de Investigación Científica y de Educación Superior de Ensenada. B.C.]

González-Andrade, S., & Fuentes-Flores, N. (2013). Matriz de insumo productivo vinícola de Baja California México. *Revista de Economía, 30*(81), 1-32. https://doi.org/10.33937/reveco.2013.41

Gramaje, D., & Armengol, J. (2011). Fungal trunk pathogens in the grapevine propagation process: potential inoculum sources, detection, identification, and management strategies. *Plant Diseases*, *95*, 1040–1055. https://doi.org/10.1094/PDIS-01-11-0025

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

Gutiérrez-Gamboa, G., Liu, S., & Pszczólkowski, P. (2019). Resurgence of minority and autochthonous grapevine varieties in South America: A review about their oenological potential. *Journal of the Science of Food and Agriculture.* 10.1002/jsfa.10003

Haidar, R., Roudet, J., Bonnard, O., Dufour, M. C., Corio-Costet, M. F., Fert, M., Gautier, T., Deschamps, A., & Fermaud, M. (2016). Screening and modes of action of antagonistic bacteria to control the fungal pathogen *Phaeomoniella chlamydospora* involved in grapevine trunk diseases. *Microbiological Research, 192*, 172–184. https://doi.org/10.1016/j.micres.2016.07.003

Hrycan, J., Hart, M., Bowen, P., Forge, T., & Úrbez-Torres. (2020). Grapevine trunk disease fungi: their roles as latent pathogens and stress factors that favour disease development and symptom expression. *Phytopathologia Mediterranea, 59*(3), 395-424. https://doi.org/10.14601/Phyto-11275

International Organization of Vine and Wine (OIV). (2018). *Distribution of the world's grapevine varieties. Focus OIV 2017*. https://www.oiv.int/public/medias/5888/en-distribution-of-the-worlds-grapevine-varieties.pdf

International Organization of Vine and Wine (OIV). *Annual assessment of the world vine and wine sector in 2021*. Intergobemental organization. https://www.oiv.int/sites/default/files/documents/OIV_Annual_Assessment_of_the_World_Vine_and_Wine_Sector_in_2021.pdf

International Organization of Vine and Wine (OIV). (2022). *State of the world vine and wine sector in 2022.* https://www.oiv.int/sites/default/files/documents/OIV_State_of_the_world_Vine_and_Wine_sector_in_2022_2.pdf

Jacob, J., Krishnan, G. V., Thankappan, D., & Amma, D. K. (2020). Endophytic bacterial strains induced systemic resistance in agriculturally important crop plants. In: *Microbial Endophytes* (Ajay K., Radhakrishnan E.K. ed.), Elsevier, Duxford, Cambridge, United Kingdom, 75–105. https://doi.org/10.1016/B978-0-12-819654-0.00004-1

Köhl, J., Kolnaar, R., & Ravensberg, W. J. (2019). Mode of Action of Microbial Biological Control Agents Against Plant Diseases: Relevance Beyond Efficacy. *Frontiers in Plant Science*, *10*(2019). https://doi.org/10.3389/fpls.2019.00845

Khan, M., Salman, M., Jan, S.A., & Shinwari, Z. K. (2021) Biological control of fungal phytopathogens: A comprehensive review based on Bacillus species. *MOJ Biology and Medicine*, *6*(2), 90–92. 10.15406/mojbm.2021.06.00137

Lahlali, R., Ezrari, S., Radouane, N., Kenfaoui, J., Esmaeel, Q., El Hamss, H., Belabess, Z., & Barka, E. A. (2022). Biological Control of Plant Pathogens: A Global Perspective. *Microorganisms*, *10*, 596. https://doi.org/10.3390/microorganisms10030596

Lefort, M. C., McKinnon, A., Nelson, T. L., & Glare, T. (2016). Natural occurrence of the entomopathogenic fungi *Beauveria bassiana* as a vertically transmitted endophyte of *Pinus radiata* and its effect on above-and below-ground insect pests. *New Zealand Plant Protection, 69*, 68–77. https://doi.org/10.30843/nzpp.2016.69.5917

Liu, D., & Howell, K. (2021). Community succession of the grapevine fungal microbiome in the annual growth cycle. *Enviromental Microbiology*, *23*, 1842-1857. https://doi.org/10.1111/1462-2920.15172

López-Franco, F. (2012). Los vitivinicultores de la región de Baja California: un acercamiento a su comportamiento informativo . *Biblioteca Universitaria*, *15*(1), 49-61. Doi: https://doi.org/10.22201/dgb.0187750xp.2012.1.41

Magoni, C. (2009). *Historia de la vid y el vino en la Península de la Baja California*. Universidad Iberoamericana.

Magoni, C. (2021). Recuento histórico de la vitivinicultura en México, con énfasis en Baja California. *En Vinos y variedades patrimoniales. Resumen de las primeras jornadas Latinoamericanas*. Prieto, J.A (Ed) INTA Ediciones.

Martain-Amozurrutia., F. (2021). Experiencias comerciales: México. En Vinos y variedades patrimoniales. *Resumen de las primeras jornadas Latinoamericanas*. Prieto, J.A (Ed) INTA Ediciones.

Martínez-Diz, M. D. P., Díaz-Losada, E., Andrés-Sodupe, M., Bujanda, R., Maldonado-González, M. M., Ojeda, S., Yacoub, A., Rey, P., & Gramaje, D. (2021). Field evaluation of biocontrol agents against black-foot and Petri-diseases of grapevine. *Pest Management Science*, 77, 697–708. https://doi.org/10.1002/ps.6064

Mesguida, O., Haidar, R., Yacoub, A., Dreux-Zigha, A., Berthon, J.-Y., Guyoneaud, R., Attard, E., & Rey, P. (2023). Microbial Biological Control of Fungi Associated with Grapevine Trunk Diseases: A Review of Strain Diversity, Modes of Action, and Advantages and Limits of Current Strategies. *Journal of Fungi*, *9*, 638. https://doi.org/10.3390/jof9060638

Mondello, V., Larignon, P., Armengol, J., Kortekamp, A., Vaczy K., Serrano, E., Rego, C., Mugnai, L., & Fontaine, F. (2018). Management of grapevine trunk diseases: Knowledge transfer, current strategies and innovative strategies adopted in Europe. *Phytopathologia Mediterranea, 57*(3), 369–383. https://doi.org/10.14601/Phytopathol_Mediterr-23942

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

Niem, J. M., Billones-Baaijens, R., Stodart, B. J., Reveglia, P., & Savocchia, S. (2023). Biocontrol Potential of an Endophytic *Pseudomonas poae* strain against the Grapevine Trunk Disease Pathogen *Neofusicoccum luteum* and Its Mechanism of Action. *Plants*, *12*, 2132. https://doi.org/10.3390/plants12112132

Paolinelli-Alfonso, M., Serrano-Gómez, C., & Hernández-Martínez, R. 2015. Occurrence of *Eutypella microtheca* in grapevine cankers in Mexico. *Phytopathologia Mediterranea*, *54*(1), 86–93, 2015. https://doi.org/10.14601/Phytopathol_Mediterr-14998

Papadopoulou, E., Bekris, F., Vasileiadis, S., Papadopoulou, K. K., & Karpouzas, D. G. (2022). Different factors are operative in shaping the epiphytic grapevine microbiome across different geographical scales: biogeography, cultivar, or vintage. *Journal of Sustainable Agriculture and Enviroment*, *1*, 287-301. https://doi.org/10.1002/sae2.12030

Pollard-Flamand, J., Boulé, J., Hart, M., & Úrbez-Torres, J.R. (2022). Biocontrol Activity of *Trichoderma* Species Isolated from Grapevines in British Columbia against Botryosphaeria Dieback Fungal Pathogens. *Journal of Fungi*, *8*, 409. https://doi.org/10.3390/jof8040409

Poudel, M., Mendes, R., Costa, L. A. S., Bueno, C. G., Meng, Y., Folimonova, S. Y., Garrett, K. A., & Martins S.J. (2021). The role of plant-associated bacteria, fungi, and viruses in drought stress mitigation. *Frontiers in Microbiology, 12*, 744512. https://doi.org/10.3389/fmicb.2021.743512

Rabiey, M., Hailey, L. E., Roy, S. R., Al-Zadjali, M. A. S., & Barrett, G. A. (2019) Endophytes vs tree pathogens and pests: can they be used as biological control agents to improve tree health? *European Journal of Plant Pathology*, *155*, 711–729. https://doi.org/10.1007/s10658-019-01814-y

Rangel-Montoya, E. A., Paolinelli, M., Rolshausen, P. E., Valenzuela-Solano, C., & Hernandez-Martinez, R., (2021). Characterization of *Lasiodiplodia* species associated with grapevines in Mexico. *Phytopathologia Mediterranea, 60*(2), 237–251. https://doi.org/10.36253/phyto-12576

Rangel-Montoya, E.A., Delgado-Ramírez, C.S. Sepulveda, E., & Hérnandez-Martínez, R. (2022). Biocontrol of *Macrophomina phaseolina* using *Bacillus amyloliquefaciens* strains in cowpea (*Vigna unguiculata* L.). *Agronomy, 12*(3), 676. https://doi.org/10.3390/agronomy12030676

Rangel-Montoya, E.A., Rolshausen, P.E., & Hernández-MartÍnez. R. (2023). Unravelling the colonization mechanism of *Lasiodiplodia brasiliensis* in grapevine plants. *Phytopathologia Mediterranea, 62*(2). 135-149. https://doi.org/10.36253/phyto-14198

Rangel-Montoya, E. A., Candolfi-Arballo, O., Obrador-Sánchez, J. A., Valenzuela-Solano, C., & Hernández-Martínez, R. (2024). Enhancing epidemiological knowledge of Botryosphaeriaceae in Mexican vineyards. *Phytopathologia Mediterranea*, 191–206. https://doi.org/10.36253/phyto-15292

Riseh, R. S., Ebrahimi-Zarandi, M., Gholizadeh-Vazvani, M., & Skorik, Y.A. (2021). Reducing drought stress in plants by encapsulating plant growth-promoting bacteria with polysaccharides. *International Journal of Molecular Sciences 22*(23), 12979. https://doi.org/10.3390/ijms222312979

Rodríguez-Torres, I. (2017). Variedades de vid cultivadas en Canarias: Descriptores morfólogicos, caracterización morfológica molecular, agrónomica y enológica. 2da ed. ICIA

Sarma, B. K., Yadav, S. K., Singh, S., & Singh, H.B. (2015). Microbial consortium-mediated plant defense against phytopathogens: readdressing for enhancing efficacy. *Soil Biology Biochemistry, 87*, 25–33. https://doi.org/10.1016/j.soilbio.2015.04.001

Secretaria de Fomento Agropecuario del Gobierno del Estado (SEFOA). (2011). Estudio estadístico sobre producción de uva en Baja California, GobBC, OEIDRUS, SAGARPA. https://www.nacionmulticultural.unam.mx/empresasindigenas/docs/1873.pdf

Servicio de Información Agroalimentaria y Pesquera (SIAP). (2022a). Producción de uva en México. https://www.gob.mx/cms/uploads/attachment/file/771603/Producci_n_Uva_en_M_xico.pdf

Servicio de Información Agroalimentaria y Pesquera (SIAP). (2022b). Baja California Infografía agroalimentaria. https://nube.siap.gob.mx/avance_agricola/

Servicio de Información Agroalimentaria y Pesquera (SIAP). (2022c). Avance de siembras y cosechas. https://nube.siap.gob.mx/infografias_siap/pag/2022/Baja-California-Infografia-Agroalimentaria-2022

Servicio de Información Agroalimentaria y Pesquera (SIAP). (2023). Avance de siembras y cosechas. https://nube.siap.gob.mx/avance_agricola/

Silva-Valderrama, I., Toapanta, D., Miccono, M. de los A., Lolas, M., Díaz G.A., Cantu, D., & Castro, A. (2021). Biocontrol potential of grapevine endophytic and rhizospheric fungi against trunk pathogens. *Frontiers in Microbiology*, *11*, 614620. https://doi.org/10.3389/fmicb.2020.614620

Sood, M., Kapoor, D., Kumar, V., Sheteiwy, M.S., Ramakrishnan, M., Landi, M., Araniti, F., & Sharma, A. (2020). *Trichoderma*: The "Secrets" of a Multitalented Biocontrol Agent. *Plants*, *9*, 762. https://doi.org/10.3390/plants9060762

Swift, J. F., Hall, M. E., Harris, Z. N., Kwasniewski, M. T., & Miller, A. J. (2021). Grapevine Microbiota Reflect Diversity among Compartments and Complex Interactions within and among Root and Shoot Systems. *Microorganisms, 9*,92. https://doi.org/10.3390/microorganisms9010092

This, P., Lacombe, T., & Thomas, M. (2006). Historical origins and genetic diversity of wine grapes. *Trends in Genetics, 22*(9), 511–519. https://doi.org/10.1016/j.tig.2006.07.008

Úrbez-Torres, J. R., & Gubler, W. D. (2009). Pathogenicity of *Botryosphaeriaceae* species isolated from grapevine cankers in California. *Plant Disease, 93*(6), 584–592. https://doi.org/10.1094/PDIS-93-6-0584

Úrbez-Torres, J. R., Peduto, F., Striegler, R. K., Urrea-Romero, K. E., Rupe, J.C., Cartwright, R. D., & Gubler, W.D., (2012). Characterization of fungal pathogens associated with grapevine trunk diseases in Arkansas and Missouri. *Fungal Diversity, 52*, 169–189. https://doi.org/10.1007/s13225-011-0110-4

Yao, X., Guo, H., Zhang, K., Zhao, M., Ruan, J., & Chen, J. (2023). *Trichoderma* and its role in biological control of plant fungal and nematode disease. *Frontiers in Microbiology*, *3*(14), 1160551. https://doi.org/10.3389/fmicb.2023.1160551

Annex

Figure 18. Vineyard biocontrol assay establishment. 1) A pruning cut was made in one of the grapevine branches. 2) First inoculation of beneficial microorganisms. 3) Second inoculation of beneficial microorganisms and inoculation of L. brasiliensis. 4) A moistened cotton swab was applied at the pruning site. 5) The inoculated branches were sealed with parafilm. 6) Inoculated branches were covered with a paper bag to avoid fungus spread.

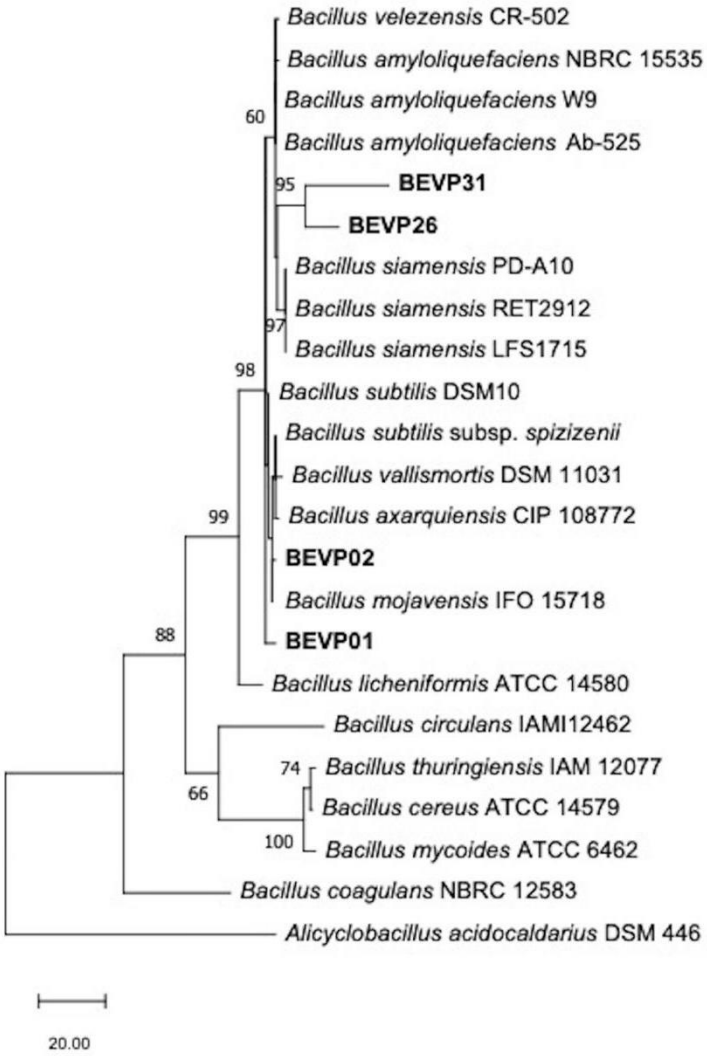

Figure 19. Maximum Parsimony tree of *Bacillus* isolates, using the analyses of partially sequenced *tef-1α*. Bold font indicates isolates described in the present study. Values above nodes are bootstrap values obtained from 1000 replicates.

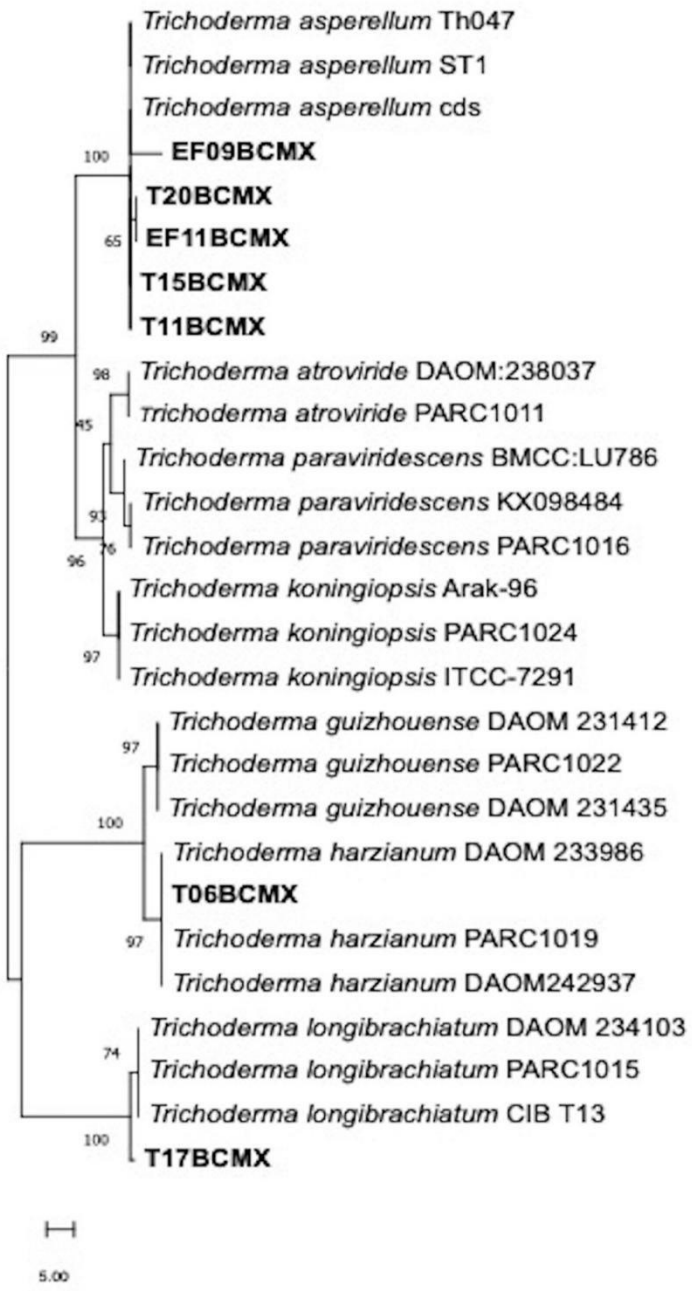

Figure 20. Maximum Parsimony tree of *Trichoderma* isolates, using analyses of partially sequenced *tef-1α*. Bold font indicates isolates described in the present study. Values above nodes are bootstrap values obtained from 1000 replicates.

Table 11. Mean percent inhibition *of Lasiodiplodia brasiliensis* by different potential biocontrol microorganisms and their respective production of plant growth promotion compounds for *Bacillus* and *Trichoderma* isolates.

Microorganisms	Isolates	Inhibition (AA)	Production				Solubilization		
			SID	CHI	HCN	IAA	P	K	Zn
Bacteria	BEVP13BCMX	51.6	-	-	-	-	-	-	-
	BEVP15BCMX	52.0	+	+	-	-	-	-	-
	BEVP01BCMX	51.28	+	+	-	+	-	-	-
	BEVP02BCMX	17.09	-	+	-	+	+	+	-
	BEVP03BCMX	14.87	-	-	-	-	+	-	-
	BEVP05BCMX	7.69	+	-	-	-	-	-	-
	BEVP08BCMX	33.33	-	-	-	-	+	-	-
	BEVP10BCMX	13.68	-	-	-	-	-	-	-
	BEVP11BCMX	40.17	+	-	-	-	-	-	-
	BEVP19BCMX	18.12	-	-	-	-	-	-	-
	BEVP26BCMX	37.95	+	+	-	+	+	-	-
	BEVP29BCMX	50.43	-	-	-	-	-	-	-
	BEVP30BCMX	23.93	-	-	-	-	-	-	-
	BEVP31BCMX	50.60	-	-	-	+	-	-	-
	BEV15BCMX	51.62	+	+	-	+	-	-	-
	BEVPRP11BCMX	21.34	+	-	-	+	-	+	-
	BEVPRP12BCMX	12.72	-	-	-	-	-	+	-
	BEVPRP22BCMX	16.85	+	-	-	+	-	-	-
	BEVPRP25BCMX	33.43	+	-	-	-	-	+	-
	BEVP2-39BCMX	12.1	-	-	-	-	-	-	-
	BEVPE-60BCMX	19.4	+	+	-	-	-	+	-
***Trichoderma* isolates**	EF09BCMX	51.83	+	+	-	+	+	-	-
	EF11BCMX	51.67	+	+	-	+	+	-	-
	T01BCMX	36.29	+	+	-	-	-	-	-
	T02BCMX	23.93	+	+	-	-	-	-	-
	T03BCMX	42.73	+	+	-	-	-	-	-
	T04BCMX	41.02	+	+	-	-	-	-	-
	T05BCMX	37.60	+	+	-	-	-	-	-
	T06BCMX	41.02	+	+	-	+	-	-	-
	T07BCMX	48.71	+	+	-	-	-	-	-
	T08BCMX	45.29	+	+	-	-	-	-	-
	T09BCMX	36.92	+	+	-	-	-	-	-
	T10BCMX	29.91	+	+	-	-	-	-	-
	T11BCMX	29.05	+	+	-	+	-	-	-
	T12BCMX	39.31	+	+	-	-	-	-	-
	T13BCMX	42.72	+	+	-	-	-	-	-
	T14BCMX	33.33	+	+	-	-	-	-	-
	T15BCMX	25.05	+	+	-	+	-	-	-
	T16BCMX	39.31	+	+	-	-	-	-	-
	T17BCMX	52.82	+	+	-	+	-	-	-
	T18BCMX	35.89	+	+	-	-	-	-	-
	T19BCMX	35.04	+	+	-	-	-	-	-
	T20BCMX	39.31	+	+	-	-	-	-	-
	T21BCMX	38.46	+	+	-	-	-	-	-
	T22BCMX	21.14	+	+	-	-	-	-	-

	T23BCMX	16.26	+	+	-	-	-	-	-
	T24BCMX	17.89	+	+	-	-	-	-	-
	T25BCMX	9.75	+	+	-	-	-	-	-
	T26BCMX	35.77	+	+	-	-	-	-	-
	T26BCMX	15.45	+	+	-	-	-	-	-
	T28BCMX	39.35	+	+	-	-	-	-	-
	T29BCMX	37.40	+	+	-	-	-	-	-
	T30BCMX	41.79	+	+	-	-	-	-	-
	T31BCMX	48.71	+	+	-	-	-	-	-
	T32BCMX	34.18	+	+	-	-	-	-	-
	T33BCMX	3.41	+	+	-	-	-	-	-
	T34BCMX	16.67	+	+	-	-	-	-	-
	T35BCMX	36.75	+	+	-	-	-	-	-