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Abstracts

Abstracts of oral and poster papers presented at the 13th International Workshop on Grapevine Trunk Diseases, held in Ensenada, Baja California, Mexico (20–24 July 2025)

The 13th International Workshop on Grapevine Trunk Diseases (IWGTD) was chaired by Dr Rufina Hernández-Martínez, and was organized by the Center for Scientific Research and Higher Education of Ensenada (CICESE). The Workshop was supported by the Ministry of Science, Humanities, Technology and Innovation of Mexico (SECIHTI), and the International Organization of Vine and Wine (OIV). The ICGTD Council meeting took place on July 20, prior to the Workshop welcome reception.

The Workshop scientific programme was opened by Dr Philippe Rolshausen (University of California, Riverside, USA), who outlined recent advances in pathogen genomics that have deepened understanding of grapevine trunk diseases, enabling improvements in vineyard practices, nursery standards, and development of biocontrol strategies. The other Keynote speakers were Dr Catarina da Cunha Maia Leal (Instituto de Ciencias de la Vid y el Vino, Spain), who discussed how grapevine-associated microbial communities shape disease, host defense, and biocontrol, highlighting sequencing-based tools for sustainable vineyard management, and Dr Akif Eskalen (University of California, Davis, USA), who outlined integrated nursery and vineyard disease management strategies, combining prevention, sanitation, and biocontrol, to reduce grapevine trunk diseases and extend vineyard longevity.

The Workshop was attended by 105 participants from 14 countries. A total of 35 oral and 29 poster papers were presented, across the ten Workshop sessions. The papers covered pathogen characterization, detection and disease epidemiology, host plant interactions, and disease management.

During the Workshop field trip to the Guadalupe Valley, delegates visited vineyards affected by GTD pathogens, where local growers shared their perspectives and key challenges in managing vineyards under semiarid conditions, as well as the strategies they have adopted. The field trip concluded with a wine tasting at Mexico's largest vineyard, L.A. Cetto.

Student competitions for best oral and poster paper presentations included contributions from 16 students. The best student poster paper award was presented to MSc student Grecia Paniagua Pérez (CICESE, Mexico) for her paper "Comparative cellular morphology and stress tolerance of *Botryosphaeriaceae* fungi affecting grapevines in Mexico. Second place went to PhD student Angelos Floudas (Aristotle University of Thessaloniki, Greece) for paper "Evaluation of selected fungal biological control agents for the protection of grapevine pruning wounds against *Diplodia seriata*". Third place was awarded to MSc student Yessica Osorio Sánchez (CICESE, Baja California, Mexico) for her paper "Evaluation and characterization of the biological activity of secondary metabolites from actinobacteria of the genus *Streptomyces*".

In the oral student competition, first place went to PhD student Isidora Silva Valderrama (University of British Columbia, Vancouver, Canada) for her presentation "Host phylogenetic diversity and virulence in *Botryosphaeriaceae*". Second place was awarded to PhD student Karen Andrea Corrales Adame (Institut des Sciences Analytiques et de Physico-Chimie pour l'Environnement et les Matériaux, France) for her paper, "Limited influence of pruning practice on the fungal community of asymptomatic Esca-infected grapevines", and third place went to PhD student Martín Puebla (University of California, Riverside, USA) for his paper "Evaluation of commercial bioinoculant products on the health of potted nursery vines".

The 14th IWGTD will be held in Greece in 2026.

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Delegates attending the 13th International Workshop on Grapevine Trunk Diseases, in Ensenada, Baja California, Mexico.

Publication disclaimer

This Supplement to *Phytopathologia Mediterranea* publishes abstracts of the papers presented at the 13th International Workshop on Grapevine Trunk Diseases. The abstracts are here presented as submitted by the respective authors. The texts have been checked for consistency of presentation, but have not been through the peer review processes used for research papers published in *Phytopathologia Mediterranea*.

ORAL PAPERS

Closing the Gaps on Grapevine Trunk Diseases' Research. P. ROLSHAUSEN. *University of California, Riverside. Department of Botany and Plant Sciences, USA. E-mail: philrols@ucr.edu*

Knowledge on grapevine trunk diseases etiology has improved significantly with the emergence of omics technologies. The increasing availability of reference genomes of trunk disease pathogens has enabled studies spanning from gene function to evolutionary relationships within and across species. Our scientific community has gained clarity on the GTD disease complex and the dynamics of microbial communities including the interaction of those community members with each other, but also with the plant host and its environment, and how it affects disease outcome. That knowledge has enabled the improvement of plant quality standards in nursery and the adoption of best cultural practices in vineyards. This work has also facilitated understanding of the beneficial organisms associated with grapevine and how these could be developed and marketed into biological control agents or plant biostimulants to better manage infection with trunk pathogens. My talk will highlight the progress that has been made in the past decade and where our research efforts should be invested in the future to improve vineyard health, longevity and productivity.

Microbial balance in grapevine: from pathogen pressure to biocontrol protection. C. LEAL[1]. [1]*Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain. E-mail:* catarina.leal@icvv.es

Grapevines exist within a highly dynamic microbial ecosystem where plant health is determined by the complex interplay between pathogenic and beneficial microorganisms. The microbial communities inhabiting the rhizosphere and xylem play pivotal roles in both the expression of grapevine trunk diseases and the activation of plant defense mechanisms. This presentation scanned recent advances in microbial ecology applied to viticulture, highlighting the functional roles of key fungal pathogens and the potential of bacterial and fungal biological control agents. By integrating data from transcriptomic analyses and high throughput metabarcoding, this talk will explore how these microbes shape the grapevine's immune landscape and how their establishment and efficacy are influenced by environmental factors, including soil conditions and drought stress. Studies of both disease development and biocontrol implementation reveal a context-dependent response of the plant and its microbiome, underscoring the need for ecologically tailored management strategies. Emphasis will be placed on the potential of sequencing-based approaches to serve as predictive tools in sustainable viticulture, aiding in the design of biologically informed, site-specific disease control programs.

From propagation to production: integrated strategies for Grapevine Trunk Disease Management in nurseries and vineyards. A. ESKALEN. *Department of Plant Pathology, University of California, Davis, CA 95616, U.S.A. E-mail: aeskalen@ucdavis.*edu

Grapevine trunk diseases (GTDs) are among the most economically damaging challenges in viticulture, often establishing early during propagation and progressing silently until vine productivity declines. This presentation will provide an overview of integrated disease management strategies that bridge the nursery and vineyard systems. Emphasis will be placed on preventative practices, including sanitation, grafting hygiene, pruning wound protection, and the strategic use of biocontrol agents. Drawing from multi-year research trials across diverse California regions, I will highlight how combining biological and cultural tools can reduce GTD incidence and severity in both young and mature vines. The lecture will also address the practical challenges of implementation in commercial settings and offer regionally adaptable, sustainable solutions for growers and nurseries. Attendees will gain a comprehensive understanding of how early intervention and coordinated management can improve vine health and vineyard longevity.

Elucidating the distribution and pathogenicity of *Cryptovalsa ampelina* in Australian **vineyards.** M. ANDRES-SODUPE[1,2], M.R. SOSNOWSKI[3,4], J. HRYCAN[2], C.C. STEEL[1,2], S. SAVOCCHIA[1,2]. *[1] Faculty of Science and Health, School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, New South Wales, 2678, Australia.[2] Gulbali Institute, Charles Sturt University, Wagga Wagga, New South Wales, 2678, Australia. [3]South Australian Research and Development Institute, Adelaide, SA, 5001, Australia.[4] School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, Adelaide, SA 5005, Australia. E-mail: mandressodupe@csu.edu.au*

Cryptovalsa ampelina is a fungal species associated with the Eutypa dieback (ED) complex. Limited information is available regarding its biology and pathogenicity, despite its presence worldwide. Previous studies have reported low to moderate levels of virulence, with some suggesting pathogenicity comparable to *Eutypa lata*, the primary cause of ED. To investigate the distribution of *C. ampelina* in Australian vineyards, dead grapevine wood tissues were collected from 43 vineyards across 12 wine regions in South Australia, New South Wales, Western Australia and Tasmania. Two methods were used to detect the pathogen: internal wood tissues were cultured on potato dextrose agar amended with chloramphenicol (PDA-C), and perithecia on the wood surface were dissected and asci inspected under a microscope. When asci were observed with 32 spores, characteristic of *C. ampelina*, they were cultured on PDA-C. Cultures were identified morphologically and confirmed using Loop-mediated isothermal amplification (LAMP) with species-specific primers and DNA sequencing of the internal transcribed spacer (ITS) region. A total of 36 *C. ampelina* isolates were identified. Pathogen prevalence varied between regions, providing insight into the distribution of this pathogen in Australian vineyards. To assess pathogenicity, a detached cane assay was performed using 26 isolates of *C. ampelina* and six of *E. lata* for comparison. After 3 months, the bark was removed, and the lesion length was measured. Koch's postulates were confirmed by re-isolating the pathogens from necrotic tissue plated on PDA-C. Additionally, the extent of colonisation was assessed by sampling tissue at 5 mm intervals above and below the lesion margins. *C. ampelina* was found to colonise wood up to 25 mm beyond the visible lesion boundaries. *E. lata* showed longer lesions and colonisation than *C. ampelina*. These results suggest the ability of these fungi to spread extensively within grapevine tissues and far beyond the staining lesion.

This research was financially supported by Wine Australia, with levies from Australia's grape growers and winemakers and matching funds from the Australian Government.

Phytosanitary analysis of Misión grapevines from Baja California, Mexico, focusing on grapevine trunk disease fungi.

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In Baja California, Misión grapevines were introduced in 1869 and are currently cultivated under rainfed conditions with minimal agricultural management. There is growing interest in using this variety for wine production, which requires the evaluation of its phytosanitary status to support large-scale propagation. Like other varieties, Misión grapevines show symptoms associated with grapevine trunk diseases, although the responsible pathogens have yet to be identified. In this study, endophytic fungi were isolated from Misión grapevines. Tissue samples from eight vineyards were analyzed, from which 78 fungal strains were obtained. Thirteen fungi were morphologically and molecularly identified as grapevine trunk pathogens, specifically *Diaporthe ampelina* and *Diplodia seriata*. For these isolates, optimal growth temperatures and pathogenicity on three grapevine varieties were determined. All evaluated strains showed optimal growth temperatures between 25 and 28°C. In the pathogenicity assay, all thirteen strains induced necrotic lesions in the wood of the Tempranillo, Malvasía Blanca, and Misión cultivars, confirming their infective capacity. A histological analysis was performed to observe changes in starch, cellulose, and lignin content in tissue from two grapevine varieties inoculated with *Lasiodiplodia brasiliensis* MXBCL28 and *D. seriata* HP12BCMX. Plants inoculated with *L. brasiliensis* MXBCL28 showed lower levels of the analyzed components compared to those inoculated with *D. seriata*, which might be related to its higher virulence. In conclusion, Misión grapevines serve as a reservoir for grapevine trunk fungi with intermediate virulence. This finding is crucial for supporting future decisions regarding the propagation and management of these grapevines in the region.

This research was financially supported by the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI), Mexico.

Environmental variables associated with *Botryosphaeriaceae* spore release in Oregon vineyards.

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Botryosphaeria dieback is one of the most common Grapevine Trunk Disease (GTD) in Oregon vineyards. Due to winter rain and possible availability of spores, the pruning wounds become most susceptible to infection during these months (November to March). To understand the temporal dispersal pattern of *Botryosphaeriaceae* spores and the effect of environmental variables on their availability, four Burkard seven-day recording volumetric spore traps were placed in vineyard blocks in northern and southern Oregon. The traps were installed from December 2019 to March 2021 to collect 477 days of samples from each spore trap. Total DNA was extracted from the spore tapes and qPCR was performed to quantify the number of spores. Weather data including daily average and hourly temperature, relative humidity (RH), wind speed, total daily precipitation, and total hours of precipitation per day, were obtained from nearby weather stations. In addition to individual weather variables, ten days cumulative precipitation, hours of rain, and growing degree days (GDD) were calculated. Pearson Correlation analysis was performed between these variables and the total number of spores detected at both locations. In northern Oregon, the detection occurred between December and February; and the first spore detection occurred when cumulative growing degree day (GDD) totaled to 4357 and 4351units during the first and second seasons respectively. Similarly, in southern Oregon, the detection occurred between November and January; and the first spore detection occurred when cumulative GDD was 4405 units during the second season. Hours of continuous RH >86% was significantly associated with numbers of spores released ($P = 0.026$; $r = 0.42$). During the spore detected dates, the RH was >86% for at least 19 consecutive hours. This study suggests environmental variables such as GDD and RH are the critical components of *Botryosphaeriaceae* spores availability during the main pruning season in Oregon.

This research was financially supported by the Oregon Wine Board.

Monitoring trunk disease pathogen spore deposits on grapevine pruning wounds.

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Eutypa (ED) and Botryosphaeria dieback (BD) are caused by fungal species of the Diatrypaceae and *Botryosphaeriaceae*, respectively. Perithecia on infected wood release spores during rain events, which are dispersed by wind and rain splash to land on fresh pruning wounds and infect the exposed vascular tissue to cause dieback and eventually vine death. Research conducted in the 1960's on apricots reported that as little as 10 *Eutypa lata* spores could land on a wound in an orchard. Since then, researchers have used a large range of artificial inoculum spore concentrations for experiments, but there are no reports quantifying natural spore deposits on pruning wounds in grapevines. This research aims to determine the likelihood and quantity of ED and BD pathogen spores naturally landing on pruning wounds in vineyards. Glass microscope slides containing Melinex tape covered with Vaseline, to simulate small (1 cm^2), medium (2 cm^2) and large (4 cm^2) wounds, were placed in Shiraz blocks during winter in 2023 and 2024. Different scenarios regarding vine age, timing, rainfall and location were tested. DNA was extracted from spores on the tapes and analysed using qPCR to quantify the number of ED and BD pathogen spores deposited. Both incidence of tapes with spores and spore numbers varied greatly between years, most likely due to rainfall differences. Although ED pathogen spores were deposited in greater numbers than those of BD, the incidence of tapes detected with spores was greater for BD pathogens, possibly due to different spore dispersion dynamics. This is the first known report quantifying spore numbers deposited on grapevine pruning wounds naturally in a vineyard. Monitoring of spore deposits will continue in 2025, and results will provide valuable insights on the epidemiology of ED and BD to provide better guidance for managing infections in the vineyard.

This research was supported by Wine Australia, with levies from Australia's grape growers and winemakers and matching funds from the Australian Government.

Status of young vine decline fungi in source blocks, commercial nursery grapevines, and young vineyards in Australia. J. HRYCAN[1], M. LIU[1], M.R. SOSNOWSKI[2,3], R. BILLONES-BAAIJENS[1,4], T. FURLAN[2,3], S. SAVOCCHIA[1]. *[1]Gulbali Institute, School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, NSW. [2]South Australian Research and Development Institute, Adelaide SA 5001, Australia, [3]School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, SA 5005, [4]Affinity Labs, Australian Wine Research Institute, PO Box 46, Glenside, SA 5064, Australia. E-mail: jhrycan@csu.edu.au*

Young vine decline (YVD) is caused by several fungal species that colonize the grapevine xylem, causing internal necrosis and leading to grapevine decline and death within the first few years after planting. Nursery studies worldwide have identified *Botryosphaeriaceae* spp., *Cadophora luteo-olivacea, Dactylonectria* spp., *Ilyonectria* spp., *Phaeoacremonium minimum,* and *Phaeomoniella chlamydospora* as the most prevalent YVD fungi, but the status in Australia is not known. Dormant canes from nursery source blocks in Australia were collected in the winters of 2023 and 2024. Canes were screened for mechanical damage and internal necrosis and DNA was extracted from wood of composite samples of five canes per block. Presence and abundance of the most prevalent YVD pathogens was analysed with qPCR. Pathogen presence and abundance varied widely between source blocks in 2023 and 2024, and YVD incidence in composite samples ranged from 0 to 100% infection. *Botryosphaeriaceae* spp. were the most prevalent fungi. In 2024, commercial nursery grapevines propagated from 2023 source block material were also analysed with qPCR, and 100% of composite samples were positive for YVD fungi, with *C. luteo-olivaceae, Dactylonectria* spp. and *P. minimum* the most prevalent. To understand the potential impact infected nursery material has on young grapevine health, vineyard surveys were conducted on 0 to 5-year-old grapevines in New South Wales (NSW), South Australia (SA), and Victoria (VIC). Symptoms of YVD included reduced vigour, stunted growth, leaf chlorosis or reddening, wilting and grapevine death. Symptomatic grapevines were screened for internal necrosis and fungal isolation onto artificial media. Young vine decline symptoms were recorded in 3.1%, 0% and 0.3% of young grapevines in NSW, SA, and VIC, respectively. The incidence of symptomatic grapevines in individual vineyard assessments varied between 0% to 40.7%. These results provide insight into the health status of source blocks and young vineyards regarding YVD in Australia.

This research was supported by Wine Australia, with levies from Australia's grape growers and winemakers and matching funds from the Australian Government.

Epidemiology, aggressiveness and fungicide sensitivity of *Aspergillus* species causing vine canker and sour rot in California vineyards. M.I. BUSTAMANTE, A. ADASKAVEG, J. CHAN, K. ELFAR, A. ESKALEN.

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Black aspergilli, primarily *Aspergillus tubingensis*, followed by *A. niger* and *A. carbonarius*, are the main causal agents of Aspergillus vine canker and summer bunch rot (sour rot) of table grapes in California vineyards. Current management relies largely on cultural practices. However, despite routine fungicide applications targeting *Erysiphe necator* (powdery mildew) and *Botrytis cinerea* (Botrytis bunch rot), high incidences of *Aspergillus* spp. are still frequently observed at harvest, leading to significant economic losses. In this study, we analyzed populations of black aspergilli obtained from vine canker and bunch rot samples, focusing on temperature response, pathogenicity, and sensitivity to six fungicides commonly used in California vineyards. *Aspergillus tubingensis* and *A. niger* exhibited similar mycelial growth rates between 15°C and 40°C, with optimal growth observed between 30°C and 35°C. *Aspergillus carbonarius* isolates showed significantly higher growth rates between 25°C and 35°C, with an optimum at 35°C. Pathogenicity assays on one-year-old grape canes revealed that *A. tubingensis* and *A. niger* isolates induced more aggressive cankers—larger in length, width and vascular discoloration—than *A. carbonarius*. However, on inoculated berries, *A. carbonarius* caused significantly larger lesions compared to the other two species. Fungicide sensitivity assays revealed that all isolates were sensitive to fludioxonil and iprodione. Low levels of resistance were observed for tebuconazole and cyprodinil, while high levels of resistance were detected for boscalid and pyraclostrobin. These findings provide critical insights into the epidemiology and aggressiveness of black aspergilli in both wood and fruit tissues and highlight the need for targeted fungicide programs to effectively manage *Aspergillus*-related diseases in California table grape vineyards.

Exploring the role of soil in Esca Complex: a first integrated assessment of physical, chemical, and biological properties in a Tuscan vineyard. F. BIGAZZI[1], G. CARELLA[1], S. DEL DUCA[2], S. MOCALI[2], S. PRIORI[3], F. VITALI[2], L. MUGNAI[1] *[1]Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant pathology and Entomology section, P.le delle Cascine 28, 50142 Firenze, Italy. [2]Council for Agricultural Research and Economics - Research Centre for Agriculture and Environment (CREA-AA), Via di Lanciola 12/A, 50125 Firenze, Italy. [3] University of Tuscia, Department of Agriculture and Forest Sciences (DAFNE), via San Camillo de Lellis, 01100 Viterbo, Italy. E-mail: francesco.bigazzi1@unifi.it*

Although multiple fungal pathogens and heavy May–June rainfall are recognized drivers of the Esca complex, the role of soil properties in symptom expression remains largely unexplored. In this study, we present the first integrated analysis of soil physicochemical and microbiological characteristics in relation to Esca incidence. We focused on a 1.5-ha Cabernet Sauvignon vineyard in Tuscany (Italy), where five years of monitoring data allowed us to delineate zones of high and low disease prevalence. To assess soil spatial heterogeneity, we employed an electromagnetic induction (EMI) sensor to map apparent soil electrical conductivity (ECa). By overlaying disease incidence maps with ECa data, we identified four locations for detailed pedological profiling and sampling. From each soil horizon within these profiles, three rhizosphere samples were collected and analyzed to characterize microbial community composition and functional traits using Biolog EcoPlate assays. Low-disease-incidence soils were characterized by a clay-loam texture, high lime content, alkaline pH (8.3–8.5), and low levels of bioavailable manganese (6.8–12.3 mg/kg). In contrast, high-incidence soils exhibited deeper profiles with a sandy clay-loam texture, minimal lime (<3%), sub-alkaline pH (7.8–8.1), higher nitrogen content, and increased organic matter. Bioavailable manganese was also higher (13–18 mg/kg). Preliminary microbiome analyses will be presented. Biolog EcoPlate assays showed a non-significant trend toward higher AWCD (Average Well Color Development) values in low-incidence zones, suggesting increased microbial metabolic activity in soils where vines were consistently less affected by the disease. Together, these findings indicate that soil texture, pH, and organic matter buffering capacity can substantially influence micronutrient dynamics—particularly manganese availability—potentially enhancing the wood-degrading enzymatic activity of *Fomitiporia mediterranea*. This, in turn, may lead to greater production of toxic byproducts that contribute to foliar symptom development. This study highlights the critical role of soil properties in Esca disease dynamics.

Epidemiology and impact of Grapevine Trunk Diseases on Chilean patrimonial vineyards. D. GRINBERGS[1], J. CHILIAN[1], R. ORREGO[2], M. ISLA[1], C. FERNÁNDEZ[1]. *[1]Instituto de Investigaciones Agropecuarias INIA-Quilamapu. Av. Vicente Méndez 515, Chillán. Chile. [1]Fruit Pathology Lab, [2]Climate change risk unit. E-mail: dgrinbergs@inia.cl*

Grapevine Trunk Diseases (GTDs) are a major threat to Chilean patrimonial vineyards, primarily composed of País, Moscatel, Cinsault, and Carignan cultivars. While GTDs have been extensively studied in commercial cultivars such as Cabernet Sauvignon, Sauvignon Blanc, Merlot, and Chardonnay, information on their impact in patrimonial vineyards remains scarce. Previous studies identified *Neofusicoccum* spp., *Diplodia* spp., *Seimatosporium vitifusiforme*, and *Arambarria destruens* as the most prevalent and virulent pathogens. This study aimed to evaluate the effects of GTDs on vine physiology, fruit yield, and the quality of both grapes and wine. Symptomatic and apparently healthy País vines (n = 30) were selected. Measurements included water potential, chlorophyll fluorescence, chlorophyll content, and gas exchange. Grapes were analyzed and subjected to microvinification. Results showed that the most affected parameters in symptomatic vines were water potential and fluorescence. Fruits from symptomatic plants were smaller and had lower soluble solids content. In addition, wines exhibited higher acidity and lower color intensity compared to those from healthy vines. A second objective was to investigate the epidemiology of *Neofusicoccum* spp. and *Diplodia* spp., identifying periods of infection risk in the Ñuble region of Chile. A País vineyard was monitored weekly for 24 months using glass spore traps to collect airborne inoculum. DNA was extracted, and standard qPCR curves were developed for each pathogen, relating Ct values to log DNA concentrations. Inoculum data were correlated with climate variables from nearby meteorological stations, after removing the seasonal component. Precipitation and humidity were the most influential factors, with a stronger correlation for *Diplodia* spp. (87%) compared to *Neofusicoccum* spp. (45%). Temperature also played a role, showing a stronger correlation with *Neofusicoccum* spp.

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Influence of rootstock-scion interaction on vine decline in different grapevine cultivars in Hungary. A. KUN[1], A. CSIKÁSZ-KRISZICS[1], B. SZABÓ[1], P. TESZLÁK[1]. [1]*University of Pécs, Research Institute for Viticulture and Oenology, Hungary. E-mail: kun.agnes@pte.hu*

The productivity of the vineyards declines with the appearance of vine decline symptoms, which in turn reduces both competitivity and sustainability, key aspects in modern viticulture. While the susceptibility of cultivated grapevine varieties to vine decline has been studied, the influence of rootstock-scion interactions remains less understood. This study aimed to evaluate the effect of rootstock-scion combinations on vine decline expression in a long-term field experiment involving 'Cabernet Sauvignon', 'Chardonnay', and 'Merlot' grafted onto six different rootstocks: 'T5C', 'TK5BB', '125AA', (V. *berlandieri* x V. *riparia*), '110R', '140Ru' (V. *berlandieri* x V. *rupestris*), and 'Fercal' (V. *vinifera* x V. *berlandieri*). The trial is located on non-irrigated, south-facing steep slopes in the Mecsek Hills, Hungary (46°07' N, 18°17' E, 210-230 m a.s.l.). Twenty-four-year-old vines, cultivated under uniform agronomic conditions, were visually assessed for grapevine trunk disease (GTD)-related symptoms before ripening in 2023-2024. In addition to symptoms scoring, meteorological data, nutrient content of leaf samples, and yield-related parameters (pH, sugar content, and acidity) were evaluated. The results were compared with previous years' data to assess the evolution of vine health and trunk viability. Among the cultivars, 'Cabernet Sauvignon' exhibited the highest incidence of GTD symptoms, while 'Merlot' showed the lowest, based on the average across all rootstocks. Although cultivar susceptibility varied significantly, no consistent pattern of susceptibility emerged among rootstocks. Nevertheless, the rootstock-scion combination clearly influenced vine resilience, longevity, and overall vineyard productivity.

Anatomical characterization of table grape cultivars in relation to susceptibility to *Lasiodiplodia brasiliensis.* E.A. RANGEL-MONTOYA[1,2], I. CÓRDOVA-GUERRERO[1], R. HERNANDEZ-MARTINEZ[2]. [1]*Facultad de Ciencias Químicas e Ingeniería, UABC.* [2]*Centro de Investigación Científica y de Educación Superior de Ensenada. E-mail: erangel@cicese.mx*

In Mexico, the state of Sonora is the leading producer of table grapes. This crop is significantly affected by trunk diseases fungi, resulting in considerable economic losses. *Lasiodiplodia brasiliensis* is among the most virulent among the trunk pathogens recorded, causing xylem necrosis and vessel occlusion, which disrupt water transport and ultimately lead to plant death. This study aimed to evaluate the anatomical characteristics of eleven table grape cultivars and their potential relationship with susceptibility to *L. brasiliensis* strain MXBCL28. Detached shoots from 'Cotton Candy', 'Flame Seedless', 'Prime Seedless', 'Summer Royal', 'Sweet Celebration', 'Superior', 'Timpson', 'Candy Snaps', 'Sweet Bond', 'Candy Hearts', and 'Sweet Globe' were mechanically wounded, inoculated, and incubated for 15 days in a growth chamber. Necrotic lesion length was measured to assess susceptibility. For anatomical analysis, non-inoculated shoots were fixed in FAA solution, and 70 µm transverse sections were obtained. Five samples per cultivar were analyzed using a light microscope (Zeiss AxioVert2000), and images captured using a Zeiss AxioCam HRc. Xylem vascular bundle areas were measured using IMAGE J, and the equivalent diameter (D) was calculated using the formula: $D = \sqrt{(4A/\pi)}$. Results showed that 'Cotton Candy' had the largest average vascular bundle diameter (127.1±26.1 µm), followed by 'Summer Royal' (108.5±29.7 µm) and 'Sweet Globe' (108.1±25.9 µm). The smallest diameters were observed in 'Candy Hearts' (53.9±9.8 µm) and 'Candy Snaps' (54.2±9.7 µm). Regarding susceptibility, Prime Seedless' was the most affected cultivar, followed by 'Summer Royal'. In contrast, Candy Hearts', 'Sweet Bond' and 'Flame Seedless' showed lower susceptibility. Further analysis of wood composition is ongoing to allow a better understanding of the relationship between xylem anatomy and susceptibility to *L. brasiliensis*, which may support the development of more resistant grapevine cultivars.

This research was financially supported by the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI), Mexico and Molina group of Hermosillo, Sonora.

The broad host range in *Botryosphaeriaceae*: a result of anthropogenic movement of plant species. I. SILVA-VALDERRAMA[1,2], J.R. ÚRBEZ-TORRES[2], T.J. DAVIES[1,3]. *[1]Department of Botany, University of British Columbia, Vancouver, BC, Canada. [2]Agriculture and Agri-Food Canada, Summerland Research and Development Centre, Summerland, BC, Canada. [3]Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, BC, Canada. E-mail: ibsilva26@gmail.com*

The *Botryosphaeriaceae* (*Ascomycota*, *Dothideomycetes*) is a diverse fungal family that includes many emerging pathogens of global concern. Some species are considered among the most aggressive pathogens in endophyte communities, causing diseases in a wide array of economically important plants, including Botryosphaeria dieback in grapevines. *Vitis vinifera* (grapevine) hosts the highest diversity of *Botryosphaeriaceae* species; however, little is known about their ecology, host breadth, and the factors that shape their association with plants. Over 27,000 DNA sequences from different loci were gathered to construct a comprehensive phylogenetic tree of the *Botryosphaeriaceae*, revealing a significant influence of both pathogen and plant-host evolutionary histories on fungal-plant associations. The majority of *Botryosphaeriaceae* species were found to be moderate generalists, with some notable exceptions of host hyper-generalists, including *Neofusicoccum parvum*, *Macrophomina phaseolina*, *Botryosphaeria dothidea* and *Diplodia seriata*, all commonly associated with *V. vinifera*. Contrary to the theory that suggests that pathogens should evolve towards host specialization, discrete states models of *Botryosphaeriaceae* indicate an increasing trend towards generalism. Analysis of the external factors influencing the host range of this family demonstrate the importance of anthropogenic movement of plant species, such as grapevines, and agricultural practices in determining the current distribution of *Botryosphaeriaceae* fungi across plant hosts. Understanding the factors that model pathogen-host associations can help predict the likelihood of emerging *Botryosphaeriaceae* fungi in new plant hosts and environments, helping inform efficient regulatory policies in plant and plant-derived products trade and influence the design of agricultural systems to reduce their host breadth expansion and the risk of epidemics.

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Host phylogenetic diversity and virulence in *Botryosphaeriaceae*. I. SILVA-VALDERRAMA[1,2], J.R. ÚRBEZ-TORRES[2], T.J. DAVIES[1,3]. *[1]Department of Botany, University of British Columbia, Vancouver, BC, Canada. [2]Agriculture and Agri-Food Canada, Summerland Research and Development Centre, Summerland, BC, Canada. [3]Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, BC, Canada. E-mail: ibsilva26@gmail.com*

Botryosphaeriaceae are one of the most devastating pathogens of grapevines (*Vitis vinifera*) and several other economically important plants. Globalization, international plant trade, and the lowering of geographical dispersal barriers have increased ecological opportunities for associations between *Botryosphaeriaceae* and novel plant hosts, increasing the risk of emergence and disease epidemics. However, the damage caused by new fungal-plant associations is challenging to predict, with few studies exploring the factors that determine pathogen virulence on a new host. A total of 4000 grapevine cv. Chardonnay dormant canes were collected between 2021 and 2023 and inoculated with 155 *Botryosphaeriaceae* isolates belonging to 32 species obtained from *V. vinifera*. After incubation under controlled conditions in a growing chamber for 10 weeks, canes were dissected, and the area of necrotic tissue was assessed as a measure of pathogen virulence. Canes inoculated with *Neoscyta-*

lidium and *Neofusicoccum* species produced the most extensive necrotic lesions, while *Dothiorella* appeared to be the least virulent genus. Most *Botryosphaeriaceae* isolates showed low virulence in grapevines, with an area of necrosis < 1 cm^2 in 90% of the cases. However, quantile regression models revealed a significant positive relationship between virulence and the phylogenetic diversity of host plants a fungal species was associated with. Accurate data on host breadth of fungal species could help predict the virulence posed in new plant hosts, preventing emerging pathogens becoming the cause of a new outbreak.

This work was supported by NSERC Discovery Grant awarded to T. Jonathan Davies (2020).

Susceptibility of spring shoot thinning wounds to *Eutypa lata* and *Diplodia seriata*. M.R. SOSNOWSKI[1,2], T. FURLAN[1,2], M.R. AYRES[1].[1]*South Australian Research and Development Institute, Adelaide SA 5001, Australia.* [2]*School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, SA 5005, Australia. E-mail: mark.sosnowski@sa.gov.au*

The grapevine trunk diseases (GTDs) Eutypa (ED) and Botryosphaeria dieback (BD) are caused primarily by infection of winter pruning wounds with spores of the causal fungi, species of the *Diatrypaceae* and *Botryosphaeriaceae*, respectively. Shoot thinning is a canopy management strategy applied in vineyards during spring to alleviate excessive shading of bunches, improve air flow within the canopy and reduce the number of cuts needed during winter pruning. Spore trapping in Australian vineyards has detected GTD pathogens throughout spring and summer in association with rainfall. It is well documented that BD pathogens can infect green grapevine tissue but there is no evidence of green shoot infection by ED pathogen spores. Preliminary shadehouse experiments showed that wounds left following spring shoot removal, by either tearing off or cutting with secateurs, were highly susceptible to artificial inoculation with *Eutypa lata* (ED) and *Diplodia seriata* (BD) spores. A vineyard trial was established to determine whether shoot thinning wounds are susceptible to infection by GTDs in a natural setting. In late September 2023, during dry weather conditions, Shiraz vines in the Barossa Valley at growth stage EL 12 were shoot thinned by manually tearing or cutting the shoot with secateurs. The wounds were inoculated with *E. lata* and *D. seriata* or left uninoculated to monitor natural infection. Isolations onto potato dextrose agar revealed that pathogen recovery was significantly greater from the rough tear wounds (33 and 83%, respectively) than from the smooth cut wounds (13 and 43%, respectively), but there was negligible pathogen recovery from naturally infected wounds (0-11% for both pathogens). The vineyard trial was repeated in 2024, and in 2025 a new trial will compare shoot thinning in dry conditions with during rainfall, when natural spore infection is most likely.

This research was supported by Wine Australia, with levies from Australia's grape growers and winemakers and matching funds from the Australian Government.

Biocontrol comparison of the pathogens *Diplodia seriata* and *Neofusicoccum parvum* in the context of Botryosphaeria dieback on *Vitis vinifera*. X. BESOAIN[1,3], D. CASTILLO-NOVALES[1,2,3], P. VEGA-CELEDÓN[1,2] A. LARACH[1], M. SEEGER[2,3]. [1]*Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, San Francisco s/n La Palma, Quillota 2260000, Chile.* [2]*Molecular Microbiology and Environmental Biotechnology Laboratory, Department of Chemistry, Center of Biotechnology Daniel Alkalay Lowitt, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso 2390123, Chile.* [3]*Millennium Nucleus Bioproducts, Genomics and Environmental Microbiology (BioGEM), Avenida España1680, Valparaíso 2390123, Chile. E- mail: Ximena.besoain@pucv.cl*

Botryosphaeria dieback is one of the most significant trunk diseases affecting vineyards worldwide, and it is associated with various fungi of the *Botryosphaeriaceae* family. This presentation compares the pathogenic profiles of *Diplodia seriata* and *Neofusicoccum parvum* based on pathogenicity and biocontrol trials conducted under field conditions on shoots and branches of *Vitis vinifera*, using Cabernet Sauvignon and Sauvignon Blanc varieties. These trials were conducted at the La Palma Experimental Station, Quillota, Chile. The pathogens were inoculated with 50 µl of a spore concentration of $1*10^4$ conidia mL^{-1}, and the bacteria were inoculated (1 day before pathogen inoculation) by applying 50 µL of $1*10^8$ cfu mL^{-1} to each cut. The results showed apparent pathogenic differences between the two pathogens according to the type of affected tissue. *D. seriata* was more aggressive in wood older than 7 years, while *N. parvum* showed greater severity in young shoots. At the variety level, Sauvignon Blanc was consistently more susceptible than Cabernet Sauvignon to both pathogens on young shoots. Regarding average severity, *N. parvum* had higher values for lesion length on Cabernet Sauvignon and Sauvignon Blanc compared to *D. seriata* on shoots.

Diplodia seriata had higher values of lesion length on Cabernet Sauvignon and Sauvignon Blanc than *N. parvum* on the trunk (10-year-old tissue. On the other hand, there were interesting biocontrol effects at different temperatures of *N. parvum* and *D. seriata* and their confrontation with biocontrollers at low, medium, and high temperatures. These findings indicate a differentiation in the mechanisms of infection and damage according to the pathogen, tissue, and variety, which is key for designing integrated management strategies and targeted application of biocontrol agents.

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The endophytic bacterium *Erwinia billingiae* may promotes the virulence *Phaeomoniella chlamydospora*. D. SZABÓ[1], Á. NOVÁK[1], N. MOLNÁR[1], A. GOMBA-TÓTH[1], X. PÁLFI[1], K.Z. VÁCZY[1], Z. KARÁCSONY[1]. [1]*Eszterházy Károly Catholic University, Food and Wine Research Institute, Leányka str. 8/G, Eger, H3300, Hungary, E-mail: karacsony.zoltan@uni-eszterhazy.hu*

Phaeomoniella chlamydospora (Pch) among the fungal causal agents of Esca is considered a “pioneer” pathogen. The pathogenesis of Esca has an exceptionally long latent period, suggesting that the pathogens grow as harmless endophytes in the host for a long period and their pathogenic behavior is triggered by rare events like excessive stress, or the establishment of microbial interactions. An increasing number of studies suggest that bacterial endophytes may contribute to the development of Esca. A total of 54 grapevine endophytic bacterial strains were screened in confrontation assays against a Pch isolate. Strain TKP9/2 – belonging to *Erwinia billingiae* species according to 16S rDNA sequence – showed increased growth on the Pch mycelial mat compared to the not pre-inoculated medium. The interaction of TKP9/2 with Pch was tested further using three additional Pch strains. Growth assays carried out on a solid medium showed that both fungi and the bacterium can establish mixed cultures, and their interaction depends on the available carbon source. On a sugar mixture mimicking the grapevine xylem sap, Pch and TKP9/2 mutually promoted each other’s growth. The microbes did not affect the growth of the confrontation partner on cellulose and starch carbon sources, while TKP9/2 inhibited the growth of Pch strains on pectin. Further experiments on media with sucrose carbon source showed that TKP9/2 increases the production of melanin and pullulan by Pch and it also promoted the ability of the fungus to degrade phenolics. This latter process believed to take part in the virulence of Pch. In artificially infected shoots, the co-inoculation of TKP9/2 with the Pch strains led to a significant decrease in photosynthetic pigment content of leaves. The above results suggest that *E. billingiae* may play a role in the pathogenesis of Esca, by increasing the growth as well as the production of some virulence factors in Pch.

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Transcriptional changes in grapevine patrimonial varieties following *Botryosphaeriaceae* infection. J. CHILIAN, D. GRINBERGS, M. ISLA, C. FERNÁNDEZ. *Instituto de Investigaciones Agropecuarias INIA-Quilamapu. Av. Vicente Méndez 515, Chillán. Chile. Fruit Pathology Laboratory. E-mail: jchilian@inia.cl*

Grapevine trunk diseases (GTDs) pose a significant threat to the productivity and longevity of vineyards in Chile. While these diseases have been extensively studied in commercial cultivars such as Cabernet Sauvignon, Sauvignon Blanc, Merlot, and Chardonnay, there is limited information available for patrimonial vineyards, which are primarily composed of cultivars like País, Moscatel, Cinsault, and Carignan. In southern Chile, the most prevalent and virulent GTD pathogens in these vineyards belong to the *Botryosphaeriaceae* family, particularly *Neofusicoccum parvum* and *Diplodia* spp. This study aimed to characterize and compare transcriptional changes in defense-related genes during colonization by *N. parvum* and *Diplodia* spp. in the Cinsault and País cultivars. Healthy plants were inoculated with mycelial discs from virulent strains of both pathogens. Three treatments were applied: (i) plants inoculated with the pathogen, (ii) plants inoculated with sterile agar discs (control), and (iii) uninoculated plants. Wood samples were collected at 0-, 3-, and 24-hours post-inoculation for RNA extraction. Gene expression was assessed using quantitative RT-PCR (qPCR), focusing on key genes associated with fungal defense responses. The results showed that genes involved in reactive oxygen species (ROS) detoxification were expressed at levels up to four times higher in inoculated plants. Additionally, PAL and NPR1, which are associated with downstream defense signaling pathways, were also upregulated. No significant differences in gene expression were observed between the two cultivars. Identifying these differentially expressed genes enhances our understanding of the molecular response to *Botryosphaeriaceae* infection and may inform future grapevine breeding programs, including marker-assisted selection for GTD resistance.

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Mycobiome analysis reveals *Truncatella* and *Seimatosporium* spp. as potential contributors of grapevine trunk disease complex in Oregon vineyards.

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Grapevine trunk diseases (GTDs) are caused by many fungal pathogens that colonize and block xylem vessels and eventually lead to stunted shoot growth and vine decline. To advance our understanding of the grapevine fungal microbiome and to explore how fungal communities vary along gradients of GTD disease incidence, and geography in Oregon, we conducted a metabarcoding study, amplifying the ITS1 region of fungal rDNA extracted from trunk tissues. The samples were collected from 29 vineyards in the Rogue and Willamette Valleys (N = 396). In terms of average relative abundance, it was dominated by *Ascomycota* (85.15%) and *Basidiomycota* (14.29%). Across all OTUs there were 576 genus level identifications, the most abundant of which were *Cladosporium* (30%), *Penicillium* (8%), *Seimatosporium* (7%), *Alternaria* (5%), and *Aureobasidium* (5%). Although many GTD genera had a low relative abundance, vineyard level detection was often high. *Truncatella* and *Seimatosporium* were detected in 100% of vineyards, *Phaeomoniella* and *Phaeoacremonium* in 93%, *Cadophora* and *Botryosphaeria* in 79%, and *Dothiorella* in 57% of the vineyards. On average, GTD associated genera were detected in a higher percentage in the Willamette Valley than in the Rogue Valley (Willamette mean = 12%, Rogue mean = 6%, $P < 0.001$). *Phaeoacremonium*, *Phaeomoniella*, *Botryosphaeria*, and *Neonectria* were detected notably more in the Willamette Valley. Within the two locations, *Seimatosporium* and *Truncatella* were detected in the highest percentage in both valleys. These results suggest that among the major players of GTD in Oregon vineyards, *Seimatosporium* and *Truncatella* spp. also contribute to the GTD complex, and their roles need to be further investigated.

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Limited influence of pruning practice on the fungal community of asymptomatic Esca-infected grapevines.

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Esca complex, one of the main GTDs associated with wood-colonizing fungi, alters the vascular system, reducing water and nutrients transport through the plant. During pruning season, cutting wounds becomes a potential entry point for fungal pathogens. We hypothesized that pruning practices influence the vine microbiome as a proxy for disease progression. For a 2-year survey, we selected ten asymptomatic grapevines (*Vitis vinifera* cv. Ugni Blanc) per sampling time; five pruned with a virtuous method (leaving ~4 cm of the branch to preserve sap flow), and five pruned with a non-virtuous method. We evaluated the diversity of fungal communities (ITS2 region) across 7 different plant tissues: arms, healthy and necrotic trunk and graft union. Here we present the results from the first sampling (February 2024). We showed that *Phaeomoniella chlamydospora*, a key causal agent of Esca, was the most abundant taxa with a global representation of 28%. Alpha diversity of the fungal communities didn't reveal significant differences between the two pruning methods across most plant tissues. However, in graft union tissues, fungal diversity was higher for vines pruned with the non-virtuous method. For beta diversity, our results indicated the health status of the vine tissue (i.e., healthy vs. necrotic) was the main factor to differentiate the fungal communities (R^2 = 28%) followed by 9% for type of plant tissue and 0.3% for pruning method. Overall, our results suggest that pruning practices have limited influence on the fungal community in asymptomatic vines. However, the variations observed in the graft union communities may indicate that roots tend to respond more dynamically to environmental disturbances. These findings recommend that further research should be done about the presence of Esca-related pathogens in soil and how the roots are influenced by pruning practices, since it could facilitate their colonization through the aerial and underground parts of the vine.

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Characterization of the microbiome of spontaneous grapevines. Building a reference standard.

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The grapevine microbiome is crucial for plant health and may play roles in grapevine trunk diseases (GTD). Endophytes are believed to help grapevines cope with biotic stress, but defining a “healthy” endophytic microbiome is challenging. Over a century of agricultural practices, including pruning, grafting, and fungicide application, have significantly altered the microbiome, with changes likely passed through clonal propagation. To identify a native microbiome, undisturbed grapevine stocks in natural environments, presumably originating from seedlings, provide an ideal model. Spontaneous grapevine populations, likely representing individuals of the crop progenitor (*Vitis vinifera* subsp. *sylvestris*), may offer these conditions. In this study, we used DNA metabarcoding, targeting the ITS gene (ITS1F2-ITS2 primer set; AVITI® sequencing), to investigate the microbial diversity of wood, rhizoplane and soil of spontaneous grapevines found in Portugal, Croatia, and Italy. Our data reveal that grapevines host significantly different microbial communities across sampling sites and plant materials. In wood, taxa *Cladosporium* spp. and *Alternaria alternata* were frequently observed, even though among the most abundant -across sampling sites- we found *Trichoderma lixii* and *Sebacina* sp., both rarely reported in the wood of cultivated grapevines. Several wood pathogens were detected, with *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., *Diaporthe ampelina*, and *Neofusicoccum parvum* ranking among the 20 most abundant taxa. These fungi were highly abundant in 20–30% of sampled grapevines, where they dominated the microbial community. Wood from these individuals also exhibited GTD-related symptoms, such as brown wood streaking and necrotic lesions. When comparing symptomatic and asymptomatic wood, significant differences were observed for multiple alpha diversity indices (Simpson, Shannon, Pielou’s; $P < 0.05$), suggesting microbial dysbiosis. Interestingly, white rot symptoms were never detected, and *Fomitiporia mediterranea*, a white rot agent associated with GTD-affected vineyards, was absent from spontaneous grapevines. This study provides valuable insights into the microbiome composition of wild grapevines, which may contribute to future efforts of microbiome manipulation in cultivated varieties.

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Impact of nursery-inherited microbiome and pathobiome on health and productivity of a young vineyard.

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This work focuses on the grapevine microbiome dynamic of the rhizosphere, root and wood endosphere from the nursery to the vineyard. The research goals were to determine the extent to which microbial inheritance from nursery shapes the microbiome of young grapevine and its impact on vine vigor and yield. We hypothesize that the microbial communities inhabiting the grapevine endosphere are relatively stable and shaped by cultural practices in nurseries. In contrast the communities composing the rhizosphere and root endosphere are highly dynamic and largely influenced by the vineyard soil. We used two Cabernet Sauvignon and Chardonnay cultivars grafted on 1103P rootstock from two nurseries that were planted in a commercial vineyard in California. We collected samples from the trunk above the graft union (scion), below the graft union (rootstock) and at the crown (rootstock), as well as from the root and the rhizosphere. Samples were collected on nursery vines prior to planting and in the vineyard every year for three consecutive years after planting. The fungal and bacterial taxa were profiled using an amplicon-based metagenomic Illumina sequencing approach. In parallel, trunk diameter (year 1-3), pruning weight (year 2-3), and yield (year 3) were recorded. Results show a strong imprint of the nursery vines on microbial communities that faded over time, although microbial turnover was much faster in the rhizosphere than in the trunk. Many grapevine trunk disease pathogens originated from the two nurseries and the incidence of some of them increased overtime, although it did not appear to significantly affect vine vigor or yield. Results also identified several beneficial organisms that that may correlate with yield. The outcome from this work underscores the importance of the nursery life stage on the core microbiome of grapevine and its implication on vineyard productivity outcome.

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Soil microbiota exerted plant opportunistic disease-suppressive function by integrating various beneficial strains counteracting different pathogens.

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Opportunistic infections caused by pathogenic microbes pose a serious threat to plant health and productivity. It is commonly recognized that soil microorganisms possess a strong potential to suppress the occurrence of plant diseases. However, it remains unclear whether the soil microbiome can exert disease-suppressive function in cases of complex opportunistic plant diseases caused by diverse pathogens. Grapevine trunk diseases (GTDs) represent a kind of typical opportunistic diseases caused by pathogenic fungi and are major threats to viticulture in most grapevine growing regions. Here, we elucidated the critical importance of microbiome homeostasis in grapevine roots for the development of GTDs through microbiome analysis, microbial isolation, and greenhouse inoculation experiments. Symptomatic vines exhibited reduced complexity in co-occurrence networks and were enriched in *Fusarium* spp. Inoculation experiments demonstrated that co-infection with *Fusarium* spp. and GTD pathogens led to increased abundance of the inoculated GTD pathogen and exacerbated disease symptom severity in grapevines. Asymptomatic samples were enriched with *Pseudomonas* spp. and *Bacillus* spp., and this supplementation could effectively suppress the activity of the pathogenic fungi and significantly reduce the pathogen abundance of grapevine roots and trunks. Syncoms constructed based on *Pseudomonas* spp. and *Bacillus* spp. exhibited higher capacity in pathogen inhibition than single bacterial strains. The symcoms composed of different strains displayed the highest inhibitory abilities counteracting *Fusarium* spp. and GTDs pathogens. Taking together, our findings highlight the role of soil microbes in fighting disease and supporting plant health, and indicate the potential of using microbial inocula to fight plant opportunistic diseases.

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Seasonal succession of *Cytospora* fungal communities in grapevine pruning wounds.

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Cytospora canker, a grapevine trunk disease (GTD) caused by various species within the genus *Cytospora*, is a significant threat to vineyard longevity and productivity. While it has been extensively studied in North America, its occurrence, species composition, and ecological dynamics remain poorly understood in European viticulture. This study aimed to investigate the ecology and temporal behavior of *Cytospora* spp. colonizing grapevine pruning wounds and to explore how interannual climatic variability influences their species composition and diversity. Over three consecutive growing seasons (2020–2023), pruning wounds were monitored in a commercial 'Tempranillo' vineyard planted in 2001 in Samaniego, Álava (Northern Spain). Each February, 1-year-old canes were spur-pruned, and after 12 months, symptomatic wood samples were collected and processed for fungal isolation. A total of 372 *Cytospora* isolates were obtained and identified through multilocus sequencing of the ITS, ACT, and TEF regions. Six species were detected, with *C. viticola* being the most prevalent (87% of isolates), followed by *C. ribis, C. ceratosperma*, and others. Alpha diversity (Shannon and Simpson indices) and species richness fluctuated across years, showing strong correlations with climatic conditions—particularly temperature and relative humidity—during the critical infection window (March–April). The 2022 season, characterized by warm temperatures and moderate rainfall, supported the most diverse and evenly distributed *Cytospora* community. In contrast, the colder and much drier 2023 season, despite high humidity, showed a marked decline in richness and community evenness, with a near-monodominant structure. These findings suggest that temperature and moisture availability play pivotal roles in mediating infection success and species interactions. Principal Component Analysis further revealed distinct annual shifts in community composition, reflecting ecological niche partitioning and adaptive responses to environmental variation. This study provides novel insights into the ecology and environmental responsiveness of *Cytospora* species in grapevine pruning wounds and highlights the importance of integrating climate factors into GTD management and pruning timing strategies.

Preliminary characterization of volatile compounds against *Neofusicoccum parvum* on *Vitis vinifera* under biocontrol conditions. D. CASTILLO-NOVALES[1,2,3], P. VEGA-CELEDÓN[1,2,3], A. LARACH[1,2], M. SEEGER[2,3], X. BESOAIN[1,3]. [1]*Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, San Francisco s/n La Palma, Quillota 2260000, Chile.* [2]*Molecular Microbiology and Environmental Biotechnology Laboratory, Department of Chemistry, Center of Biotechnology Daniel Alkalay Lowitt, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso 2390123, Chile.* [3]*Millennium Nucleus Bioproducts, Genomics and Environmental Microbiology (BioGEM), Avenida España1680, Valparaíso 2390123, Chile. E-mail: diyaniracastillonovales@gmail.com*

Using plant growth-promoting bacteria as biocontrol agents represents a sustainable strategy for managing grapevine stem diseases. In this study, the production of volatile organic compounds (VOCs) was characterized under confrontation between native bacterial strains and the pathogen *Neofusicoccum parvum*. These assays were conducted on solid media under controlled conditions, and VOCs were collected by solid-phase microextraction (SPME) and analyzed by gas chromatography- mass spectrometry (GC-MS). More than 40 compounds were identified, including monoterpenes such as α-pinene and β-pinene, alcohols such as phenylethyl alcohol, cyclic ketones such as isophorone, and sesquiterpenes such as γ-muurolene, δ-cadinene, and trans-calamenene. The chemical profiles varied significantly among treatments, with higher relative abundances of compounds such as 1-undecene, palustradiene A/B, and (+)-2-bornanone observed under the highest fungal growth inhibition conditions. The differential presence of these VOCs suggests an active role in the inhibition of *N. parvum* and lays the groundwork for their potential use as biomarkers.

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Grapevine Trunk Diseases in Greece: fungi involved in discrete geographical zones and wood microbiome analysis in symptomatic and asymptomatic vines. S.G. TESTEMPASIS[1], E.A. MARKAKIS[2,3], F. BEKRIS[4], S. VASILEIADIS[4], G.I. TAVLAKI[3], S. SOULTATOS[2,3], C. TSOUKAS[5], A. SAMARAS[1], D. GKIZI[5], A. TZIMA[5], E. PAPLOMATAS[5], D.G. KARPOUZAS[4], G.S. KARAOGLANIDIS[1]. [1]*Aristotle University of Thessaloniki, Department of Agriculture, 54124 Thessaloniki, Greece.* [2]*School of Agricultural Sciences, Hellenic Mediterranean University, Stavromenos 71004, Heraklion, Crete, Greece.* [3]*Institute of Olive Tree, Subtropical Crops and Viticulture, Hellenic Agricultural Organization "DIMITRA", Kastorias 32A, 71307, Heraklion, Crete, Greece.* [4] *University of Thessaly, Department of Biochemistry and Biotechnology, 41500 Larissa, Greece,* [5]*Agricultural University of Athens, School of Plant Sciences, Department of Crop Science, Iera Odos 75, Votanikos, 11855 Athens, Greece. E-mail: gkarao@agro.auth.gr*

Grapevine trunk diseases (GTDs) are caused by wood-inhabiting pathogenic fungi from a wide range of genera. This study aimed to investigate the incidence and etiology of GTDs in three distinct geographic regions of Greece (Crete, Nemea, and Amyntaio), and to characterize the fungal and bacterial microbiomes in the wood of symptomatic and asymptomatic vines using amplicon sequencing. A total of 310 vineyards were surveyed, and 533 fungal strains were isolated from diseased vines. Morphological and molecular analyses revealed that the isolates belonged to 35 distinct fungal genera. The composition of GTD-associated fungal populations varied significantly between geographic zones. *Phaeomoniella chlamydospore* was most prevalent in Heraklion, while *Diplodia seriata* predominated in Nemea and Amyntaio. Multi-gene sequencing (rDNA-ITS, LSU, tef1-α, tub2, and act), combined with pathogenicity tests, revealed several fungal species—*Neosetophoma italica*, *Seimatosporium vitis*, *Didymosphaeria variabile*, and *Kalmusia variispora*—as potential GTD agents newly reported in Greece. Amplicon sequencing analysis showed that the combined factor of cultivar and biogeography was the strongest determinant of the wood fungal microbiome

(*P* < 0.001, 22.7%), followed by GTD symptom status (*P* < 0.001, 3.5%). Several fungal Amplicon Sequence Variants (ASVs), including *K. variispora*, *Fomitiporia* spp., and *P. chlamydospora*, were positively associated with symptomatic vines. Random Forest analysis identified *P. chlamydospora*, *K. variispora*, *Alternaria alternata*, and *Cladosporium* sp. as highly accurate predictors of symptomatic vines (0% error rate). The bacterial wood microbiome exhibited similar patterns, with biogeography/cultivar being the primary driver of composition (*P* < 0.001, 25.5%), followed by GTD symptom status (*P* < 0.001, 5.2%). Differential abundance analysis revealed a consistent positive correlation (*P* < 0.001) between *Bacillus* and *Streptomyces* ASVs and asymptomatic vines. Network analysis identified a significant negative co-occurrence between these beneficial bacterial genera and the pathogens *Phaeomoniella*, *Phaeoacremonium*, and *Seimatosporium*. In conclusion, this study discusses the associations of GTD pathogens with vine age, cultivar, and prevailing climatic conditions across different Greek viticultural zones. It also highlights the potential protective role of *Bacillus* and *Streptomyces* against GTD pathogens in the grapevine wood microbiome.

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Exploring the table grape microbial community for the identification of new potential biocontrol agents to manage grapevine trunk diseases. A. AGNUSDEI[1], D. SALAMONE[1], P. TANCREDI[1], F. DALENA[1], D. GERIN[1], D. CORNACCHIA[1], G.L. BRUNO[1], F. MANNERUCCI[1], F. FARETRA[1], S. MAVICA[2], D. AIELLO[2], S. POLLASTRO[1]. *[1]Department of Soil, Plants and Food Sciences, University of Bari Aldo Moro, via Amendola 165/A, Bari, Italy. [2]Department of Agriculture, Food and Environment, University of Catania, via S. Sofia, 100, Catania Italy. E mail: francesco.faretra@uniba.it; dalia.aiello@unict.it*

The antagonistic activity of *Aphanocladium album, Clonostachys rosea*, *Trichoderma* spp., *Bacillus* spp., *Streptomyces* spp. and *Pseudomonas* spp., selected as representative of the microbial communities of table grapes varieties from southern Italy, was investigated against GTDs pathogens. In a dual culture assay, an inhibition of the growth of *Neofusicoccum parvum* (37-62%), *Eutypa lata* (34-63%), *Diplodia seriata* (50-64%), *Cylindrocarpon destructans* (22-79%), *Phaeomoniella chlamydospora* (37%) and *Diaporthe neoviticola* (47 71%) was determined by 16 different *Trichoderma* species. A deadlock at mycelial contact was observed during the interactions between *A. album* and *Fomitiporia mediterranea* or *Phaeoacremonium minimum*, while the growth of the pathogens was stopped by the contact with *C. rosea*. *Bacillus amyloliquefaciens* (D747) and *Bacillus subtilis* (QST 713) moderately limited the growth of *N. parvum* (34 and 33%, respectively) and *E. lata* (13% and 16%, respectively). The ability of the BCAs to produce toxicants was evaluated by growing them in liquid media under stress conditions. An inhibition in the growth of *D. seriata* (60%), *E. lata* (76%), *N. parvum* (50%), *C. destructans* (30%), *P. minimum* (15%) and *D. neoviticola* (71%) was observed when the culture filtrate of *C. rosea* was added to growth media. Furthermore, the antifungal effect of possible volatile organic compounds was evaluated. Using the sandwich system, an inhibition in the growth of *P. chlamydospora* (24-58%), *C. destructans* (2-49%) and *P. minimum* (4-46%) was determined by 16 *Trichoderma* spp. The growth of *C. destructans* and *P. chlamydospora* was affected by *A. album* (7 and 29%, respectively), *C. rosea* (36 and 26%, respectively), *B. amyloliquefaciens* (D747) (10 and 39%, respectively), B. subtilis (QST 713) (3 and 37%, respectively), while the growth of *P. minimum* was also affected by *B. amyloliquefaciens* (D747) (30%). These results highlight the potential of members of the table-grape microbial communities as a promising approach to control GTDs-associated fungal pathogens.

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Ten years of non-virtuous pruning on Ugni blanc: a decline acceleration? E. BRUEZ[1], C. CHOLET[*], T. MARTIGNON[2], M. GIUDICI[2], M. BOISSEAU[3], X. POITOU[3], P. COLL[3], P. REY[4], L. GENY[1]. *[1]UMR Oenologie, EA 4577, Université de Bordeaux, INRAE, BSA, Institut des Sciences de la Vigne et du Vin, 210 Chemin de Leysotte - CS 50008, 33882 Villenave d'Ornon, France.[2] Simonit&Sirch, Maîtres Tailleurs de Vigne, 1 Rue Porte des Benauges, 33410 Cadillac, France. [3] Hennessy Jas et Cie, 1 rue Richonne 16100 Cognac, France. [4] Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, Institut des Sciences Analytiques et de Physicochimie pour l'Environnement et les Matériaux - UMR 5254, IBEAS*

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Ever since the ban of sodium arsenite, researchers have been trying to find alternative solutions to deal with grapevine trunk diseases (GTDs). Amongst other methods, quality of pruning is currently being used in attempts to protect the vines against pathogen entry and thus prolong grapevine life. Utilising an Ugni blanc vineyard planted in 2006, conducted in double Guyot-Poussard, this research aimed to demonstrate the effects of different pruning methods commencing from 2016 on vine decline over 10 years. Virtuous pruning leaves a chicot and preserves the diaphragm, unlike non-virtuous pruning, which damages the diaphragm. The diaphragm is a natural internal partition within the node of a vine shoot that acts as a barrier, helping to isolate wounds, thus protecting the vine from pathogens entry. Our experiment, which focused on the zone of desiccation formation of the wood below the pruning wound. It examined the correlation between spur diameter and the depth of desiccation, and also measured the evolution of desiccation cones according to the pruning quality. To verify the impact of non-virtuous pruning on the sap flow, sensors were put on the cordons of non-esca-foliar symptomatic plants each year from 2021 to 2024 for measurements. During the summer, other physiological indicators, transpiration and gas exchanges, were monitored in the vines. No correlations were demonstrated between the diameter spur and the necrotic zone depth. The sap flow seemed to be lower for the non-virtuous pruning vines and particularly the warm season (in 2022). The results of the other physiological indicators did not show any differences between the two pruning methods. However, the grape yield was impacted with a lower production with the non-virtuous pruning. More vines expressed esca-foliar symptoms in the non-virtuous modality, after 5 years of pruning. In conclusion, the non-virtuous pruning promotes significant development of an internal desiccation cone which has a negative medium-term impact on sap flow. So, retaining the diaphragm helps to protect the sap flow and allows the sap flow path to function fully and longer, and avoid decline acceleration.

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Exploring grapevine microbial-based inoculants for trunk disease management: comparing a custom endophytic-based microbial synthetic community with a commercial microbial inoculum. W. CHITARRA[1,2], C. PAGLIARANI[2], L. MOFFA[1], NERVA[1,2]. *[1]Council for Agricultural Research and Economics, Research Centre for Viticulture and Enology (CREA-VE), Via XXVIII Aprile 26, 31015 Conegliano (TV), Italy. [2]National Research Council of Italy - Institute for Sustainable Plant Protection (CNR-IPSP), Strada delle Cacce 73, 10135 Torino (TO), Italy. E-mail: walter.chitarra@crea.gov.it*

Microbial inoculants for grapevine protection and growth promotion are commercially available, but only a few rely on microbial strains originally isolated from grapevine tissues. In this study, we developed a synthetic microbial consortium (SynCom) composed of seven endophytic bacterial strains isolated from grapevine woody tissues and selected based on their *in vitro* antagonism activity against key fungal pathogens associated with grapevine trunk diseases (GTDs). We hypothesized that a grapevine-specific SynCom would offer greater protective efficacy. To test this, we compared its effects with a commercial inoculant containing a mixture of arbuscular mycorrhizal fungi and a rhizospheric *Bacillus coagulans* strain (AMF+B). Grapevine cuttings were inoculated either with the SynCom or with the AMF+B product, while other vines served as uninoculated controls (CTRL). Following field transplant, gas exchange parameters were monitored on inoculated and CTRL plants, and stilbenes analyses were performed on leaf and root samples collected at the end of the experiment. Additionally, RNAseq in SynCom plants and root microbiome dynamics along treatments in root tissues were also investigated. Under the tested conditions, the customized SynCom successfully activated the plant's defence machinery at molecular level, leading to a significant accumulation of stilbenes in both leaves and roots. This protective effect was accompanied by a reduction in photosynthesis, indicating an energy shift towards defense. Conversely, the AMF+B treatment promoted more balanced physiological performance while still enhancing defence compounds. Notably, the metabarcoding analysis showed a reduced abundance of GTD-related pathogens in SynCom-treated plants. These findings highlight the potential of tailored SynComs, composed of biocontrol agents, for managing GTDs, particularly in vineyards under high disease pressure. This work highlights the value of microbiome-based practices in viticulture and encourages further development of SynCom strategies, including cross-kingdom designs, to support sustainable management of grapevine trunk diseases.

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Ministry of Environment and Energy Security, EC-DEC n. 86 of 07 September 2023). This study was also conducted within the Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, CN00000022) and Shield4Grape project in the frame of Horizon Europe program (Grant agreement 101135088). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Evaluation of the retroactive effect of Mamull® in the control of *Diplodia seriata* in grapevines in Chile. W. HETTICH[1], E. DONOSO[1], L. ROMERO[1], M. PACHECO[2], J. CABALLERO[2]. *[1]Bio insumos Nativa, parcela antilhue lote 4b2, Maule. [2] Fitonova SPA, parcela antilhue lote 4b2, Maule. E-mail: edonoso@bionativa.cl*

The implementation of biological control in the management of wood diseases raises critical questions about optimal application timing during pruning. Previous trials in Peru using *Lasiodiplodia theobromae* demonstrated a retroactive effect of Mamull (a formulation of *Trichoderma spp.* and *Bionectria ochroleuca*) up to 48 hours post-inoculation. This study aimed to assess the effectiveness of Mamull application timing under natural infection conditions. The trial was conducted in Talca, Chile, on 35-year-old Semillón grapevines, trained on Spanish trellises with a high *Diplodia seriata* pressure. A 2x5 factorial randomized design was employed: two treatments (Control and Mamull®) at 100 g hL^{-1} + Break at 10 cc hL^{-1}, and seven post pruning applications intervals (0, 24, 48, 72, 96, 120, and 144 hours). Treatments were applied using a turbo-fogger at 600 L ha^{-1}. Each replicate covered 0.5 hectares, with 100 marked pruning wounds. Ten months later, incidence (lesion cuts) and severity (lesion length) were assessed. Control vines showed no differences across timepoints (14.7% incidence; 3.9 cm severity). Mamull® significantly reduced both incidence ($P < 0.001$) and severity ($P < 0.05$) up to 48 hours post pruning. From 72 to 120 hours, a significant reduction in severity was observed compared to the control (0.5, 0.7, and 1.6 cm, respectively), though incidence was not affected. After 120 hours, no differences were observed between Mamull® and the control. These results confirm that Mamull® maintains preventive efficacy for up to 48 hours post-pruning. The findings align with Peruvian data and highlight a logistic advantage of Mamull® over traditional protectants require immediate application.

Effect of an organic amendment made of grapevine pruning wood and kitchen waste on the endophyte microbiome structure and the latent Lasiodiplodia brasiliensis infection in grapevine cuttings. L. GUERRERO-CABRERA[1], M. PAOLINELLI[2,3], C. VALENZUELA-SOLANO[4], R. HERNANDEZ-MARTINEZ[1]. *[1]Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California, México. [2]Estación Experimental Agropecuaria de Mendoza, Instituto Nacional de Tecnología Agropecuaria, Argentina. [3]Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina. [4]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Campo Experimental Costa de Ensenada, Ensenada, Baja California, 22880, México.* E-mail: *luisgc@cicese.edu.mx*

Botryosphaeria dieback affects grapevine and other crops worldwide. One of the causal agents is *Lasiodiplodia brasiliensis*, which has been frequently found in grapevines from Baja California and Sonora in Mexico, mainly in plants exhibiting wood cankers. Several control strategies have been tested, including biological control agents such as bacteria and fungi; however, alternatives like organic amendments (OAs) have not been explored. Therefore, this study aimed to evaluate the effect of OA on the co-occurrence of latent *L. brasiliensis* infection and the endophytic community in grapevines through qPCR and metabarcoding analysis. Three concentrations of the extract of the OA sludge KWW (kitchen waste and grapevine wood) were applied to grapevine cuttings inoculated with *L. brasiliensis.* The KWW extract at 10% applied on both infected and non-infected cuttings, performed the highest values of plant growth parameters on the development of buds' number, bud length, flower number, flower length, root number, and a suppressive effect of 70%. The abundance of endophyte genera *Acinetobacter, Hymenobacter, Pseudomonas, Flavobacterium*, were enhanced by KWW and significantly associated with antagonist activity. Meanwhile, *Sphingomonas*, *Ochrobactrum*, *Azospirillum*, and *Rheinheimer* were possibly involved in plant-induced systemic resistance mechanisms. Additionally, the dominant genera *DMER64* and *Orbiblia* present in KWW were recruited to colonize tissues with possible growth-promoting activity and other physiological processes. Organic amendments can be an effective strategy for controlling grapevine trunk diseases. In particular, the KWW sludge demonstrated multiple suppressive actions, such as providing humic substances and biochemical compounds. This allowed the plant to recruit beneficial bacteria and fungi across the rhizosphere interface in response to the latent *L. brasiliensis* infection. Additionally, KWW enhanced the relative abundance of endophyte species capable of stabilizing the interaction network of the endophytic microbiome around the pathogen.

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Integrative approaches to Petri and esca disease mitigation in grapevine: from molecular interference to field applications. L. NERVA[1,2], F. FAVARETTO[1], M. GUASCHINO[1], W. CHITARRA[1,2]. *[1]Council for Agricultural Research and Economics - Research Centre for Viticulture and Enology (CREA-VE). Via XXVIII Aprile, 26, 31015 Conegliano (TV), Italy.[2]National Research Council of Italy - Institute for Sustainable Plant Protection (IPSP-CNR). Strada delle Cacce, 73, 10135 Torino (TO), Italy. E-mail: luca.nerva@crea.gov.it*

Along with grapevine trunk diseases (GTDs), esca poses increasing threats to viticulture due to its complex aetiology and lack of curative treatments. This study integrated fundamental and applied research to investigate Petri and esca-associated plant-pathogen interactions and to develop sustainable management strategies. Exogenous application of double-stranded RNA (dsRNA) targeting vital genes of *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum* was evaluated for grafted grapevine plants in nurseries. The dsRNA treatments significantly reduced lesion length and pathogen isolation frequency, although full suppression of fungal colonization was not achieved. Additionally, grafting combinations influenced disease development and treatment efficacy, with the scion genotype modulating defence responses and rootstock microbial community. Metabarcoding revealed that both pathogen inoculation and dsRNA applications significantly altered the fungal endophytic community composition, suggesting a broader impact on plant-associated microbiota. In parallel, a four-years field trial assessed the effectiveness of a foliar fertilizer formulation in reducing esca incidence and severity under vineyard conditions. Three different pedoclimatic regions and two cultivars (Glera and Cabernet Sauvignon) with at least 1 hectare per site were monitored. The treatment consistently reduced disease incidence by 26–40% and severity by 35–50% relative to conventional protocols. Treated symptomatic vines exhibited improved stomatal conductance and transpiration, indicating reduced esca-related water stress. Furthermore, transcriptomic analysis revealed treatment-induced expression of genes involved in plant immunity and oxidative stress regulation, particularly in symptomatic plants. These findings offer new insight into grapevine defence mechanisms and demonstrate the potential of combining RNA-based biotechnological tools with novel field level approaches. Integrating dsRNA interference, scion-rootstock dynamics, eco-physiological measurements and transcriptomic profiling can stimulate the development of more effective and sustainable esca disease management strategies.

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Evaluation of commercial bioinoculant products on the health of potted nursery vines. M. PUEBLA, P. ROLSHAUSEN. *University of California, Riverside. Department of Botany and Plant Sciences, Riverside CA, USA. E-mail:* philrols@ucr.edu

Our study investigated the effect of commercial bioinoculants used by nurseries on vine health and wood endophytic microbial communities. We partnered with two California nurseries that applied bioinoculants at different stages of vine propagation. Chardonnay plants grafted onto SO4 rootstock were treated during the callusing stage at the first nursery, whereas at the second nursery Cabernet Sauvignon plants grafted onto 110R rootstock were treated post-planting. Green vines were collected from both nurseries and kept in a lath house at UC Riverside for an additional 10 weeks. We measured shoot mass, root mass, and trunk diameter to compare vine vigor between treatments. Our results showed that vines treated with bioinoculants during callusing displayed significantly higher shoot and root biomass. We also measured internal tissue necrosis using imaging software and found that the bioinoculants significantly reduced wood necrotic area. Finally, next generation sequencing of the fungal ITS and bacterial 16S regions yielded at least 50,000 quality reads. We recorded a higher relative abundance of specific beneficial endophytic bacteria (*Bacillus*, *Paenibacillus*, *Streptomyces*) and fungi (*Trichoderma*) listed on the bioinoculant commercial labels, along with a lower relative abundance of GTD fungi (*Phaeoacremonium*) when treated at the callusing stage. In contrast, we observed no differences in all these parameters when vines were treated post-planting. These data suggest that the timing of application of bioinoculant treatments during vine propagation is critical for maximum effects on plant growth and GTD management in nurseries.

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Implementing remedial surgery to extend the lifespan of vineyards infected with grapevine trunk diseases in Canada. J.R. ÚRBEZ-TORRES, J. BOULÉ. *Agriculture and Agri-Food Canada, Summerland Research and Development Centre, Summerland, BC, Canada. E-mail: joseramon.urbeztorres@agr.gc.ca*

Grapevine trunk diseases (GTD) are the main biotic factor reducing vineyard lifespan worldwide. No systemic product is available to stop the infection caused by GTD fungi when inside the grapevine vascular system. Remedial surgery is a cultural practice consisting of cutting and removing visibly infected parts of the vine and retraining from canes and/or suckers left below the cut. It is the only technique currently available to eliminate GTD pathogen infection. A recent survey in British Columbia showed 100% of sampled vines (n=1,590) from different ages from 159 vineyard blocks to be infected (canker observed) at the lower part of the trunk (10 cm above ground). Accordingly, the main objective of this study was to determine whether remedial surgery can be used to expand the lifespan of vineyards when no healthy tissue can be found below the last possible cut. A total of 30 vines (3 vines in each of 10 panels) in two vineyards ('Pinot Gris' and 'Pinot Noir') planted in 1996 were cut and retrained in the winter of 2017 and their survival and performance compared against 30 uncut vines during six growing seasons. At the end of the experiment, 100% survival rate of retrained vines was recorded. Both 'Pinot Gris' and 'Pinot Noir' retrained vines showed significantly higher yields than the uncut control vines after two growing seasons. After six years, total yield estimated in the retrained 'Pinot Gris' and 'Pinot Noir' increased by 60% and 160%, respectively. Fruit quality (brix, pH, TA) was not statistically different between retrained and uncut vines. This study shows remedial surgery as an effective technique to extend the lifespan of vineyards even when infection has reached beyond the last possible cut allowed for retraining. Extending vineyard lifespan can provide growers with needed cash flow while preparing for vineyard replant.

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Early infection, lasting impact: trunk diseases reduce cane quality and spread via rootstock material. W.J. VAN JAARSVELD[1,2], L. MOSTERT[1], J.J. HUNTER[2], M. HAVENGA[2], M. VAN DER RIJST[2], F. HALLEEN[1,2]. *[1]Stellenbosch University, Department of Plant Pathology, Matieland, 7602, South Africa. [2]ARC Infruitec-Nietvoorbji, Stellenbosch, 7599, South Africa. E-mail: wynand@vititec.com*

Infection of rootstock mother vines with grapevine trunk disease pathogens leads to reduced yields and compromised phytosanitary quality of propagation material. This poses a serious threat to the sustainability of the South African grapevine industry. The age for replacing rootstock mother vines to minimize these risks are unknown. Therefore, this study aimed to investigate how the age of rootstock mother blocks influences the physiological, morphological and infection status of both the mother vines and one-year-old canes. Thirty-nine rootstock mother blocks of different ages of 10 varieties were surveyed over three seasons. Morphological dimensions including cane length, thickness and fresh mass were determined. Physiological analyses determined the water and starch content, macro- and micronutrients and total phenolic index. Fungal isolations were made from one-year-old canes and heads of mother vines, and *Diplodia seriata* and *Phaeomoniella chlamydospora* were quantified in one-year-old canes using qPCR. The effects of block age and season varied across different rootstock varieties, without conclusive association with block age. Interactions between certain minerals and cane morphology were both synergistic or antagonistic. *Botryosphaeriaceae* and *Celotheliaceae* (*P. chlamydospora*) were the most prevalent grapevine trunk disease pathogens found in one-year-old canes (incidences up to 28% and 2% and DNA concentrations reaching 3171 and 1055 ng μL^{-1}, respectively) and in the mother vine heads (incidences up to 84% and 90%, respectively), generally regardless of vine age. *Diplodia seriata* infections in the vine heads resulted in contamination in one-year-old canes. *Botryosphaeriaceae* in mother vine heads negatively affected cane morphology, while *Celotheliaceae* and *Diaporthaceae* were associated with reduced starch levels in canes. Infections by *Botryosphaeriaceae* and *Hymenochaetaceae* resulted in an elevated total phenolic index. Rootstock mother blocks, the heads as well as one-year-old canes, were infected with GTD pathogens from as young as three years-old, highlighting the need for starting with clean mother vines and ensuring that infections do not occur.

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Field evaluation of biocontrol and integrated strategies for managing grapevine trunk diseases in California.

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Grapevine trunk diseases (GTDs) are among the most damaging and persistent threats to viticulture worldwide. Over three consecutive years, we evaluated three integrated strategies involving biocontrol agents and fungicides for GTD management in both nursery and vineyard settings. Treatments were applied as (i) pre-grafting soaking of propagation material in nurseries, (ii) pruning wound sprays, and (iii) soil drenches in a mature experimental vineyard of *Vitis vinifera* cv. Cabernet Franc in Davis, CA. In nursery trials, both fungal and bacterial biocontrol agents improved callus formation at the graft union and rootstock. Lesion lengths and infection levels caused by *Neofusicoccum parvum* were significantly reduced by both fungicide and biocontrol treatments, while only infection levels (not lesion length) of *Eutypa lata* were lowered. No significant control was observed for *Phaeoacremonium minimum*. In vineyard trials, pruning wound protectants, especially synthetic fungicides and selected biocontrol agents significantly reduced infections by *N. parvum*. Soil drench applications also reduced lesion lengths and infection levels caused by *N. parvum*, *E. lata* and *P. minimum*. These findings demonstrate that integrating biocontrol agents across multiple application points (propagation, pruning, and soil) can contribute significantly to GTD management under California field conditions. Further validation in commercial vineyards is required for broad adoption of these strategies into IPM programs.

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The occurrence and control strategies of grapevine black foot disease in China.

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Grapevine black foot disease (BFD) is one of the major grapevine trunk diseases (GTDs) threatening Chinese viticulture, with its incidence and severity increasing annually. First reported in China in 2017, initial surveys conducted between 2017 and 2019 across five provinces revealed incidence rates ranging from 0.1% to 1%. Systematic surveys carried out from 2020 to 2023 showed that BFD has now been detected in 11 provinces, with pathogen carrier rate exceeding 20% in seven of them. Consequently, BFD has become the second most important GTD in China, following Botryosphaeria dieback. In addition, BFD-associated fungi are frequently found co-infecting grapevine alongside other GTD pathogens. Currently, effective management strategies for BFD are lacking, underscoring the need for early and accurate diagnosis to support timely cultural and preventive interventions. This study aimed to (i) develop a triplex droplet digital PCR (ddPCR) assay for the early detection of major BFD-associated species in China, and (ii) evaluate the efficacy of bacterial biocontrol agents for BFD management. A total of 193 fungal isolates associated with BFD were obtained and identified based on morphological and molecular analyses. Of these, 66.3% were *Dactylonectria* spp., followed by *Ilyonectria* (23.3%), *Cylindrocladiella* (7.8%), and *Campylocarpon* (2.6%). A triplex ddPCR assay was developed, enabling the simultaneous detection of seven major BFD-associated species, with a detection limit of 1×10^6 ng μL^{-1}, providing a sensitive tool for early diagnosis. Six bacterial strains exhibited over 70% mycelial inhibition against BFD-associated isolates, with strain O-C9 (*Pseudomonas aeruginosa*) showing the highest efficacy. A fermentation broth of O-C9 at 8.19×10^9 CFU mL^{-1} significantly reduced pathogen colonization by 76% in roots, 78% in trunks, and 43% in new shoots of one-year-old potted grapevine seedlings. Notably, O-C9 also showed broad-spectrum mycelial growth inhibition (48%–76%) against other GTD pathogens, presenting a promising strategy for integrated control of BFD co-infected with other GTDs in the field. Field trials of O-C9 are currently underway. This study provides an updated overview of BFD in China, and advances both diagnostic and management tools, contributing a scientific basis for the integrated control of this increasingly important disease.

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POSTER PAPERS

Production of *Cryptovalsa ampelina* spore suspension and optimal inoculum dose for wound infection. M. ANDRES-SODUPE[1,2], M.R. SOSNOWSKI[3,4], J. HRYCAN[2], C.C. STEEL[1,2], S. SAVOCCHIA[1,2]. *[1] Faculty of Science and Health, School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, New South Wales, 2678, Australia.[2] Gulbali Institute, Charles Sturt University, Wagga Wagga, New South Wales, 2678, Australia. [3]South Australian Research and Development Institute, Adelaide, SA, 5001, Australia.[4] School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, Adelaide, SA 5005, Australia. E-mail: mandressodupe@csu.edu.au*

Eutypa lata and *Cryptovalsa ampelina* are the primary diatrypaceous species implicated in Eutypa dieback (ED) in Australia. These ED pathogens propagate through sexual reproduction by developing perithecia containing eight to multiple-spored asci in dead stroma tissues. Asexual reproduction produces conidia, but they are unable to germinate and cause disease, limiting the ability to use them for experiments to infect pruning wounds for the evaluation of control strategies. Additionally, ascospores cannot be produced *in vitro*. To overcome these challenges, a spore suspension protocol originally developed for *E. lata* was validated for *C. ampelina*. Firstly, perithecia in dead wood were dissected and observed under the microscope. When 32-spored asci were found, spores were transferred to potato dextrose agar amended with chloramphenicol (PDA-C). Cultures were morphologically identified and confirmed by loop-mediated isothermal amplification (LAMP) with species-specific primers. Small pieces of dead grapevine wood containing perithecia were fixed to a container lid and submerged in sterile distilled water for 1 hour. After soaking, the lid was screwed onto a clean container and left overnight at room temperature. The next day, the wood was removed, and the walls of the container were rinsed with sterile distilled water to collect spores. The identity of *C. ampelina* was confirmed morphologically by plating the suspension onto PDA-C and by LAMP analysis 3 days after spore release, while spores were kept in cool storage at 4°C. To determine the optimal spore dose for wound inoculation, a detached cane assay was conducted by applying 10 to 500 spores to fresh pruning wounds. After one-month, successful colonisation by *C. ampelina* was assessed by pathogen recovery on PDA-C. This spore liberation protocol provides a sound, reproducible method for future pathogenicity and wound protection studies.

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Comparing qPCR and isolation methods to assess *Eutypa lata* infection in grapevine wounds. T. FURLAN[1,2], N. CAPOTE[3], K. HILL[1,2], D. GIBLOT-DUCRAY[1,2], I. DUMITRESCU[1], H. HERDINA[1], M.R. SOSNOWSKI[1,2]. *[1]South Australian Research and Development Institute, Adelaide SA 5001, Australia. [2]School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, SA 5005, Australia. [3]Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Seville, Spain. E-mail: mark.sosnowski@sa.gov.au*

Eutypa lata infects grapevines through wounds and colonises vascular tissue, causing Eutypa dieback. The presence of *E. lata* is traditionally assessed by isolating from wood pieces on potato dextrose agar (PDA). A widely used technique, it is time consuming and can be hindered by contamination or misidentification. To develop a more sensitive and consistent technique, a TaqMan qPCR assay for the specific detection of *E. lata* was evaluated. The assay, targeting the ITS region of the genome, was previously shown to be sensitive and mostly specific, only cross reacting with *Diatrypella vulgaris*, a related pathogen. The qPCR assay was compared with isolation using an existing pruning wound protection trial where wounds were inoculated with approximately 200 spores of *E. lata*. The trial included various treatments and positive and negative controls. One year later, treated canes were collected and cut in half longitudinally after removing the bark and desiccated stub. Half of the canes were assessed by isolation on PDA and the other half via qPCR. Plant crude extracts were prepared by grating 100 mg of tissue below the margin of discoloured wood into an extraction buffer and beating in a tissue lyser. Dilutions (1:5) of plant crude extracts were directly analysed in triplicate using qPCR. Of 638 samples analysed, 59% corresponded for both methods, with the Cohen's kappa index indicating 'fair agreement' between the two methods. Thirty-nine percent of samples were positive for qPCR only, and 2% positive for isolation only. qPCR was considerably more sensitive than isolation for detecting *E. lata* in canes, offering a more rapid and sensitive method. qPCR assessment was estimated to cost 1.5 times more than the isolation method. Further evaluation is planned to investigate reducing replication and confirming accuracy of the method for wound susceptibility experiments.

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On the hunt for grapevine trunk diseases: A preliminary survey of vineyards in Ontario, Canada. W. MCFADDEN-SMITH[1], J. RAMÓN ÚRBEZ-TORRES[2], O. ELLOUZ[3]. *[1]CCOVI, Brock University, 1812 Sir Isaac Brock Way, St. Catharines, Ontario, L1S2B1, Canada. [2]Summerland Research and Development Centre, 4200 Highway #97 Summerland, BC V0H 1Z0, Canada. [3]Agriculture and Agri-Food Canada, London Research and Development Centre, 4902 Victoria Avenue North, Vineland Station, ON L0R 2E0, Canada. E-mail:* mcsmith58@gmail.com

Until recently, grapevine trunk diseases (GTDs) have not been considered a major issue in the cool climate growing region of Ontario, the largest grape production area in Canada. To determine the prevalence of GTDs and their causal agents, randomly selected vineyards cultivar Chardonnay and Cabernet franc were surveyed in early and late summer during the 2024 growing season. The age of vines ranged from 2 to 30 years. From each selected vineyard, trunks were collected from 5 symptomatic vines, including poor vigor, dieback, lack of spring growth, and/or characteristic Eutypa dieback symptoms. Young vine decline symptoms were not observed. Fungal pathogens were first identified based on morphological characteristics and subsequently by multi-gene DNA analyses. Species within the *Botryosphaeriaceae* family were the most prevalent, specifically *Diplodia seriata* and *Neofusicoccum* spp., followed in number by *Phaeoacremonium minimum, Phaeomoniella chlamydospora* and *Eutypa* spp. This study represents the first attempt to demystify the status of GTD in Ontario, a grape-growing region with unique climatic conditions. Identifying the main GTDs pathogens in Ontario will assist to better understand their epidemiology and develop proper management strategies.

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Identification and characterization of *Cytospora* species isolated from grapevine cankers in British Columbia, Canada. J.R. ÚRBEZ-TORRES, A. ROBERTS, J. BOULÉ. *Agriculture and Agri-Food Canada, Summerland Research and Development Centre, Summerland, BC, Canada. E-mail: joseramon.urbeztorres@agr.gc.ca*

Grapevine trunk diseases (GTDs), one of the major threats to the industry's future economic sustainability, are caused by a wide range of taxonomically unrelated fungi. Recent studies have reported species in the genus *Cytospora* to be isolated from GTDs symptomatic vines in different parts of the world. Members of this genus are long known to cause cankers on a wide range of perennial woody hosts, primarily fruit trees. However, their role as pathogens in grapevines is still not fully understood. The objective of this study was to identify by means of morphological and molecular studies *Cytospora* isolates obtained from GTDs symptomatic vines in British Columbia (BC), Canada and complete pathogenicity studies to determine their role as GTDs causing fungi. Results from field surveys showed *Cytospora* isolated from 25% of surveyed vineyards (49 out of 194). A total of 107 *Cytospora* isolates were obtained primarily from cankers observed in spurs, cordons and/or trunks. Among these, 65 isolates were selected for DNA analyses of four genes (ITS1-5.8S-ITS2, β-tubulin, TEF-1α, ACTIN). DNA sequencing showed *Cytospora viticola* to be the most prevalent species isolated from grapevine cankers in BC. Other identified species were *C. chrysosperma*, *C. parasitica*, *C. populicola*, *C. pruniopsis*, and a *Cytospora* sp. Eight isolates were selected to complete pathogenicity studies in both Chardonnay and Merlot cultivars in a detached cane assay using mycelium plugs as inoculum. Results showed all *Cytospora* isolates from the different species to cause vascular necrosis significantly larger than the non-inoculated controls in both cultivars. Pycnidia from the inoculated isolates was observed in the inoculated canes. The small necrosis length recorded from the different species (average 6.25 mm) suggests *Cytospora* spp. identified in this study to be weak pathogens of grapevines. Further studies are in progress to better understand the role that *Cytospora* spp. may play on GTDs development.

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Characterization of *Diaporthe* spp. associated with grapevine trunk diseases in the northwest of Mexico. C.S. DELGADO-RAMÍREZ[1], E.A. RANGEL-MONTOYA[1,3], E. SEPÚLVEDA[1], C. VALENZUELA-SOLANO[2], R. HERNANDEZ-MARTINEZ[1]. [1]*CICESE. Departamento de Microbiología. Ensenada, Baja California.* [2] *INIFAP Sitio experimental costa de Ensenada.* [3]*Facultad de ciencias químicas, UABC, Tijuana. E-mail: cdelgado@cicese.edu.mx*

Phomopsis dieback is a grapevine trunk disease caused by *Diaporthe* spp. Symptoms in plants include small clusters, fruit rot, and dieback and cankers. Grapevine cultivation has significant socioeconomic importance in Mexico. In the wine-producing regions of the country, there are reports of trunk disease fungi causing dieback and cankers, but none involve *Diaporthe* species. Therefore, the aim of this work was to characterize *Diaporthe* species associated with grapevines in vineyards of Sonora and Baja California, Mexico. Isolates with morphological characteristics like *Diaporthe* spp. were obtained from grapevine plants showing dieback and cankers. Ten isolates were identified through morphological characterization and molecular analysis using ITS and EF1-α markers. Phylogenetic analysis allowed the identification of strains belonging to the species *D. ampelina*, *D. eres*, and *D. foeniculina*. The optimal growth temperature ranged between 25 and 28°C, and they exhibited higher growth when inoculated in media supplemented with cellulose, pectin, and grapevine wood as carbon sources. Pathogenicity tests confirmed that all strains could produce necrotic lesions. These findings expand the current understanding of grapevine trunk disease etiology in Mexico and highlight the need for further studies on the epidemiology and management of *Diaporthe* species in local vineyards.

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Morphophysiological and phylogenetic characterization of Chilean isolates of *Eutypa lata* obtained from vineyards in Central Chile. Y. RUIZ[1], M. VALENZUELA[2], C. PACHECO[1], P. GONZÁLEZ[1], F. NUÑEZ[1], C. MUÑOZ[1], G. DÍAZ[1], M. LOLAS[1]. [1]*Laboratorio de Patología Frutal, Facultad de Ciencias Agrarias, Universidad de Talca, Talca, Chile.* [2]*Facultad de Agronomía, Universidad de Concepción, Chillán, Chile. E-mail:* mlolas@utalca.cl

Grapevine trunk diseases (GTDs) have become one of the most critical phytosanitary problems affecting this crop globally over the past three decades. Among them, Eutypa dieback, caused by the fungus *Eutypa lata* (family Diatrypaceae), is highly aggressive and widely distributed, with reports from vineyards across Africa, Europe, Asia, Oceania, and America. In Chile, *E. lata* was recently identified in grapevines and cherry trees, representing the only documented evidence of its presence in the country to date. The objective of this study was to characterize, from cultural, morphophysiological, and phylogenetic perspectives, the first Chilean isolates of *E. lata* obtained from vineyards located in central Chile. Five isolates were evaluated for growth rate and optimal growth temperature on four culture media (PDA, V8, corn agar, and tomato agar), incubated at 0, 5, 10, 15, 20, 25, 30, and 35 °C for 0, 7, 14, 21, and 28 days. Mycelial growth curves and temperature response models were developed using R Studio. Phylogenetic analysis was performed using ribosomal DNA (ITS region) and β-tubulin gene sequences, comparing the Chilean isolates with *E. lata* accessions from GenBank representing diverse global regions. Sequence alignment, phylogenetic inference, and visualization were conducted using MEGA 11, IQ-TREE, and iTOL. Results showed that PDA and corn agar supported the highest mycelial growth, with optimal temperatures ranging from 19.97 to 21.62 °C. Growth curves were similar across all isolates. Phylogenetic analysis revealed significant global genetic diversity of *E. lata*, and the Chilean isolates clustered with strains from Europe, the Americas, and Oceania. To date, the sexual stage of *E. lata* has not been reported in Chilean vineyards, highlighting the need for further studies aimed at its detection to better understand the epidemiology and management of this pathogen in local viticulture.

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Portable vineyard fungal diagnostics: a low-cost system for in-field *Lasiodiplodia* species identification. A. CRESPO-MICHEL[1], M. A. ALONSO-ARÉVALO[1], R. HERNANDEZ-MARTINEZ[2]. [1]*Department of Electronics & Telecommunications, Center for Scientific Research and Higher Education of Ensenada (CICESE), Ensenada, B.C, Mexico.* [2]*Department of Microbiology, Center for Scientific*

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A diagnostic system for *Lasiodiplodia* species classification was developed to address the challenge of accurately identifying these fungal pathogens, which pose a significant threat to grapevine and crop health. The system is designed to operate on a compact, low resource computing platform, making it suitable for deployment in field conditions. The system combines a bright-field microscope with a camera to capture high-resolution images of fungal spores. These images are processed through two parallel analysis pipelines. The first pipeline uses a MobileNetV2-based convolutional neural network (CNN) to directly classify species from raw images, achieving an accuracy of 96.25%. The second pipeline employs a fine-tuned Mask R-CNN model for segmenting individual spores, which are then analyzed using a feature extraction module that computes morphological traits such as area, shape descriptors, and color metrics. These extracted features are classified using an XGBoost model, which achieves 76.81% accuracy. The system was trained and tested on a dataset of 2,400 images representing eight different species of *Lasiodiplodia*. t-SNE visualization of the CNN extracted features revealed distinct species-level clustering, supporting the biological relevance of the extracted characteristics. Despite the lower accuracy of the feature-based approach, it provides interpretable insights into the morphological variability between species, which can be valuable for understanding phenotypic differences. All processing was performed on a low-cost, integrated computing unit, demonstrating the feasibility of deploying advanced fungal diagnostic tools in resource-limited environments. This system represents a promising step toward developing scalable, portable solutions for fungal identification and plant disease management, combining deep learning and interpretable models for more accessible and efficient monitoring of agricultural pathogens.

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Persimmon is a new host of the black foot disease pathogen *Ilyonectria liriodendri*.

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Several members of the fungal genus *Ilyonectria* infect plants through the roots and basal stem, causing 'black foot' diseases, predominantly in woody plants such as grapevine and walnut. In 2021, four *Ilyonectria liriodendri* isolates were cultured from the necrotized roots of *Diospyros virginiana* (persimmon) plants in Eger, Hungary. The isolates were identified by sequencing the ITS, β-tubulin, and partial histone H3 genes. The obtained sequences were used for phylogenetic analysis through multiple sequence alignment and the construction of a Maximum Likelihood tree, which revealed that all four isolates aligned with sequences of *Ilyonectria liriodendri*. The macro- and micromorphological deviations, such as conidial size and morphology, as well as coloration and relative growth differences between the isolates, suggested that they represent a somewhat diverse set of isolates within *I. liriodendri*. To prove their association with the symptoms observed in the host plants, the roots of one-year-old *D. virginiana* cuttings were artificially inoculated by dipping in 10^5 spore/ml conidial suspensions of the isolates for 30 minutes. Five replicates were set up for each isolate and the mock-inoculated control which were inoculated with water. After 90 days of incubation in a greenhouse, 3 to 5 plants out of the five replicates for each fungal isolate (total of 16 symptomatic plants out of 20 inoculated cutting) showed necrosis in the taproots, while the mock-inoculated cuttings remained symptomless. Necroses developed in the roots of the infected plants, and the inoculated fungi were reisolated, confirming their pathogenicity to *D. virginiana*. To the best of our knowledge, this is the first report of *Ilyonectria liriodendri* causing disease in persimmon.

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Evaluation of the susceptibility period of grapevine pruning wounds to natural infection by trunk disease fungi.

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Grapevine trunk diseases (GTDs) primarily infect through pruning wounds, which previous studies suggest remain susceptible for up to two months. Pruning timing may also influence the development of GTDs. Three GTD pathogens of importance in Spain are *Diaporthe ampelina*, *Diplodia seriata*, and *Cytospora viticola* responsible for Phomopsis dieback (PD), Botryosphaeria dieback (BD), and Cytospora canker (CC), respectively. In a vineyard plot located in Logroño (northern Spain), 80 grapevines were pruned in November (early pruning) and another 80 in February (late pruning). From each pruning group, wood samples were collected weekly over eight weeks from ten different vines. A total of 2 wood fragments per plant were processed for fungal isolation, and pathogens were identified by sequencing the ITS rDNA region. The incidence and severity of PD, BD, CC, and other GTD pathogens were evaluated using Kruskal-Wallis tests and correlation matrices in relation to climatic and temporal variables. There were no significant differences in GTD incidences across all weekly early and late pruning samples, supporting the hypothesis that pruning wounds remain vulnerable to infection for up to two months. However, GTD severity was overall significantly higher in late-pruned grapevines compared to early-pruned. Across climactic variables, temperature and precipitation had the most significant relationships with GTD pathogens. Overall, GTD pathogens incidence was positively correlated with temperature, while severity was positively correlated with precipitation. Individual pathogens responded differently: PD incidence positively correlated with temperature in fall, whereas BD incidence showed a negative correlation with temperature in spring. CC was only detected in spring samples, and its severity was negatively associated with humidity. These results highlight the importance of environmental conditions in disease development and suggest that pruning timing and climate affect both infection likelihood and disease progression.

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Dispersal of grapevine trunk disease spores from an infected vineyard. T. FURLAN[1,2], J. HRYCAN[3], M. LIU[3], S. SAVOCCHIA[3] and M.R. SOSNOWSKI[1,2]. [1]*South Australian Research and Development Institute, Adelaide SA 5001, Australia.* [2]*School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, SA 5005, Australia.* [3]*Gulbali Institute, School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, NSW, Australia. E-mail: mark.sosnowski@sa.gov.au*

The grapevine trunk diseases Eutypa (ED) and Botryosphaeria dieback (BD) are caused by fungal species of the Diatrypaceae and *Botryosphaeriaceae*, respectively. Perithecia of *Eutypa lata* on infected wood release ascospores during rain events, which are reported to be carried up to 50 km in wind. Pycnidiospores of BD pathogens are primarily dispersed by rain splash, and have been reported to only travel up to 20 m. For both ED and BD, spores land on fresh pruning wounds and infect the exposed vascular tissue to cause dieback and eventually vine death. It is important to understand the distance and quantity of spores that can be dispersed from an infected vineyard to adjacent blocks and neighboring vineyards. In this study, isolated old Shiraz blocks with dieback symptoms in South Australia were selected as source blocks. During August 2023 in the Barossa Valley, six Rotorod spore traps were positioned from 0 to 400 m from the source block in line with prevailing winds. During August 2024 in the Eden Valley, the spore traps were positioned from 0 to 4 km from the source block. At both sites, spores were repeatedly collected for 24-hour periods following rain events. DNA was extracted from spores on the rods and analysed using multi-species qPCR to quantify the number of ED and BD pathogen spores deposited at each position. Spores were detected at least 400 m away from the source with no reduction in quantity. When the distance was increased, ED and BD spores were detected up to 4 km away from the source during strong winds and heavy rainfall. Further research is planned for 2025 to increase intensity of sampling and at greater distances from the source. These results highlight the potential of trunk disease spread between vineyards and across a region, reinforcing the importance of proactive disease management.

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Dissecting plant-microbe interactions in grapevine trunk disease: a genotype-focused approach. A. NARDUZZO[1,2], F. FAVARETTO[1,2], F. VILLANO4, M. GUASCHINO[1], W. CHITARRA[1,3], L. NERVA[1,3]. [1]*Council for Agricultural Research and Economics - Research Centre for Viticulture and Enology (CREA-VE). Via XXVIII Aprile, 26, 31015 Conegliano (TV), Italy;* [2]*University of Padua, Department of Agronomy, Food, Natural Resources, Animals and Environment, Agripolis, Viale dell'Università 16 - 35020 Legnaro (PD), Italy;* [3]*National Research Council of Italy - Institute for Sustainable Plant Protection (IPSP-CNR). Strada delle Cacce, 73, 10135 Torino (TO), Italy;* [4]*University of Turku, 20014 Turku, Finland. E-mail: anna.narduzzo@crea.gov.it*

The delicate balance between plants and their microbial communities is vulnerable to environmental and biological disruptions, increasing plant susceptibility to disease. Within this context, Esca disease is a particularly challenging trunk disease of grapevine, involving a consortium of fungi whose interaction with the host, under partly unknown conditions, results in variable impacts on plant health. Our investigation started with an assessment of the resident endophytic fungal communities across a panel of 12 grapevine genotypes. This initial phase aimed to determine the profile of microbial composition within a large, randomized sample set by isolating from grapevine propagation material. After the assessment of diversity, we initiated a systematic evaluation of genotype-specific susceptibility to the primary etiological agents implicated in Grapevine Trunk Diseases (GTDs): *Phaeomoniella chlamydospora*, *Phaeocremonium minimum*, and *Diplodia seriata*. This involved inoculating 16 grapevine genotypes, selected among the most commercially important and traditional varieties known for their susceptibility or resistance to Esca-related fungi. These preliminary findings pave the way for future research, aimed at elucidating the holobiont changes brought by GTDs presence across different grapevine genotypes, wither classified as resistant or widely adopted commercially. In vivo analysis of microbial community dynamics during pathogenesis, through imaging of tagged reporter microbes or through metagenomic analysis, can significantly enhance our understanding of fungal communities associated with disease resistance. Future studies should prioritize identifying and characterizing, in one model genotype, resistance genes that are crucial in the plant-fungal interaction. Complementary, metabolomic profiling of both grapevine tissues and fungal communities, in healthy and diseased states, will be critical for pinpointing metabolic pathways involved both in plant defense and fungal protection/virulence. Ultimately, integrating the results of these analyses can lead to novel disease management strategies, such as selecting resilient grapevine varieties or manipulating the vineyard microbiome to enhance natural tolerance to diseases.

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Comparative cellular morphology and stress tolerance of *Botryosphaeriaceae* fungi affecting grapevines in Mexico. G. PANIAGUA-PEREZ[1], E.A. RANGEL-MONTOYA[1,2], R. HERNANDEZ-MARTINEZ[1]. *[1]Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE), Ensenada 22860, Mexico. [2]Facultad de Ciencias Químicas, Universidad Autónoma de Baja California, Tijuana, Mexico. E-mail: grecia@cicese.edu.mx*

Fungi from the *Botryosphaeriaceae* are associated with a wide range of diseases in numerous woody crops, including grapevine (*Vitis vinifera*). These pathogens significantly impact vineyard productivity and longevity by causing vascular necrosis, cankers, dieback, and, in severe cases, plant death. In Mexico, species from the genera *Lasiodiplodia*, *Neofusicoccum*, *Diplodia*, and *Botryosphaeria* have been identified in Baja California, Sonora, and Coahuila, exhibiting varying levels of virulence. Given their impact and the limited understanding of their cellular biology, this study aimed to analyze the cellular structures and stress tolerance of *Botryosphaeriaceae* strains with different levels of virulence. The strains used were *L. brasiliensis* MXBCL28, *N. parvum* MX14P4, and *B. dothidea* MXRJM25, all isolated from Mexican vineyards. Cellular analysis was performed using confocal laser scanning microscopy. Samples were stained with Calcofluor White (0.02%) for cell walls, FM4-64 for membranes, vesicles, and the Spitzenkörper, and DAPI/Hoechst 33258 for nuclei. No significant differences in cell wall structure were observed among the three species. However, variations in nuclear quantity were noted with *L. brasiliensis* displaying a higher number of nuclei compared to the other species. FM4-64 staining confirmed the presence of the Spitzenkörper in all three species. Additionally, fungal growth was evaluated under stress conditions induced by phenolic compounds (salicylic acid and benzoic acid at 10 and 15 mM) and oxidative stress (hydrogen peroxide at 1 and 5 mM). *Lasiodiplodia brasiliensis* was able to utilize both phenolic compounds as carbon sources at all tested concentrations. *Neofusicoccum parvum* and *B. dothidea* grew in the presence of salicylic and benzoic acids up to 10 mM. Under oxidative stress, all species showed growth comparable to the control. These findings provide a cellular level comparison of *Botryosphaeriaceae* and contribute to a better understanding of mechanisms and stress tolerance.

Comparative colonization and tissue degradation of grapevine wood by *Lasiodiplodia brasiliensis*, *Neofusicoccum parvum*, and *Botryosphaeria dothidea*. E.A. RANGEL-MONTOYA[1,2], I. CÓRDOVA-GUERRERO[1], R. HERNANDEZ-MARTINEZ[2]. *[1]Facultad de Ciencias Químicas e Ingeniería, UABC. [2]Centro de Investigación Científica y de Educación Superior de Ensenada. E-mail: erangel@cicese.mx*

Botryosphaeria dieback is one of the most aggressive grapevine trunk diseases caused by fungi from the *Botryosphaeriaceae* family. These pathogens typically infect the plant through pruning wounds and colonize the xylem, leading to necrosis and vascular dysfunction. In Mexico, *Lasiodiplodia brasiliensis* has been identified as highly virulent, followed by *Neofusicoccum parvum*, whereas *Botryosphaeria dothidea* exhibits low virulence. This study aimed to compare the colonization behavior of *Botryosphaeriaceae* fungi with varying levels of virulence in grapevine. 'Cabernet Sauvignon' plants were mechanically wounded and inoculated with *L. brasiliensis* MXBCL28, *N. parvum* 14P4MX, and *B. dothidea* RJM25MX, and maintained under greenhouse conditions for two months. Half of the samples were used for histological analysis to observe structural changes in the grapevine tissue. Plants inoculated with *L. brasiliensis* and *N. parvum* showed reduced levels of starch, cellulose, hemicellulose, and lignin compared to control plants, whereas those inoculated with *B. dothidea* exhibited profiles like the control. The other half of the samples were analyzed to quantify starch and total phenolic content. Results indicated that plants inoculated with *L. brasiliensis* had significantly lower starch levels and higher phenolic content than the control. Additionally, fungal growth was assessed on various carbon sources related to grapevine tissue components. *L. brasiliensis* showed the highest growth rate on pectin, *N. parvum* on glycogen, and *B. dothidea* on tannic acid. These findings improve our understanding of the colonization strategies employed by these fungi and may contribute to the development of more effective disease management strategies.

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Evaluation and characterization of the biological activity of secondary metabolites from actinobacteria of the genus *Streptomyces*. Y. OSORIO-SÁNCHEZ, C.S. DELGADO-RAMÍREZ, L. M. DURÁN- RIVEROLL, R. HERNANDEZ-MARTINEZ, E. SEPÚLVEDA. *Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Baja California, México. E-mail: yessica.osorio@cicese.edu.mx*

The increasing global demand for food, intensified by climate change, has led to a greater reliance on agrochemicals to manage phytopathogenic fungi, bacteria, and uncontrolled weed proliferation. However, such dependence poses significant environmental and health concerns. As a sustainable alternative, biocontrol agents, particularly actinobacteria, have demonstrated considerable potential due to their ability to synthesize diverse secondary metabolites with herbicidal, antifungal, and antibacterial activities. In this study, four actinobacterial isolates (rbES158, rbES173, rbES339, and rbES331) were evaluated for their in vitro antifungal activity against *Lasiodiplodia brasiliensis* strain L28BCMX, one of the causal agents of dieback of grapevines in Baja California, Mexico. Among them, isolate rbES331 exhibited the highest antifungal activity, reaching 35% fungal growth inhibition under optimized temperature conditions (40 °C). To enhance the biosynthesis of bioactive secondary metabolites, rbES331 was cultured in the presence of filtered supernatants from *F. oxysporum* (iFOL) and *L. brasiliensis* (iL28) as elicitors. Induction with iFOL significantly increased antifungal efficacy, resulting in 50% inhibition against *F. oxysporum* and up to 70% against *L. brasiliensis*, whereas iL28 reduced antifungal activity to below 10% for both pathogens. These results suggest that the production of secondary metabolites in rbES331 is strongly influenced by specific biotic stimuli, underscoring the relevance of elicitor-based strategies for modulating secondary metabolism in actinobacteria for biocontrol applications.

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Antibacterial activity of *Phaeomoniella chlamydospora* is regulated by acetate-mediated quorum sensing. Á. NOVÁK[1], D. SZABÓ[1], N. MOLNÁR[1], A. GOMBA-TÓTH[1], X. PÁLFI[1], K.Z. VÁCZY[1], Z. KARÁCSONY[1]. *[1]Eszterházy Károly Catholic University, Food and Wine Research Institute, Leányka str. 8/G, Eger, H3300, Hungary, E-mail: karacsony.zoltan@uni-eszterhazy.hu*

Phaeomoniella chlamydospora is regarded as a "pioneer" pathogen among the fungal agents responsible for Esca disease, playing a key role in the initiation and progression of symptoms. The disease has an unusually long asymptomatic phase, suggesting that these pathogens may persist as endophytes within the host for extended periods. Their transition to pathogenic behavior appears to be triggered by rarely occurring factors, such as severe host stress or the accumulation of a threshold level of fungal biomass. Our previous findings demonstrated that *P. chlamydospora* secretes acetate, which positively affects biofilm development through quorum sensing mechanism. In this study, we investigated how exogenously applied acetate influences the expression of antibacterial activity by *P. chlamydospora*. Four bacterial strains belonging to the genus *Pseudomonas*, isolated from the grapevine xylem, were used in the experiments. All three examined *P. chlamydospora* strains exhibited antibacterial activity against these isolates in a preliminary confrontation assay. To assess the effect of acetate on this phenomenon, liquid culture assays were conducted with the fungi using an increasing acetate supplement (6.25-100 mM; five-step, two-fold dilution series). Bacterial growth in the presence of fungal culture filtrates was assayed by spectrophotometry. Our results indicated that acetate inhibits the antibacterial capacity in all bacterium-fungus strain combinations, especially at high concentrations. These results suggest that acetate-mediated quorum sensing regulates antibacterial activity of *P. chlamydospora* by permitting this process at lower fungal cell densities, whilst inhibiting antibacterial activity at higher cell densities.

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Wood microbiome analysis in GTDs-symptomatic and asymptomatic table grape vines across Greece.

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Grapevine Trunk Diseases (GTDs) are among the most destructive diseases affecting grapevines worldwide. This study examined the structure and diversity of microbial communities inhabiting grapevine wood, with a particular focus on the table grape cultivar Sultanina, to explore their potential roles in GTDs. Sampling was conducted in three geographically distinct viticultural regions of Greece (north, central, and south). Amplicon sequencing targeting the V4 region of the 16S rRNA gene and the ITS2 region was performed to characterize bacterial and fungal communities, respectively. Non-metric multidimensional scaling (NMDS) and permutational multivariate analysis of variance (PERMANOVA) were used to evaluate differences in microbial community composition between symptomatic and asymptomatic vines, as well as among geographic regions. The analysis identified biogeography as the strongest determinant of the fungal microbiome ($P < 0.001$, 18.5%), followed by vine age ($P < 0.001$, 7.3%). GTD symptom status also had a statistically significant, albeit smaller, effect ($P < 0.001$, 1.3%). The bacterial microbiome exhibited similar but weaker patterns compared to the fungal communities. Co-occurrence network analysis between GTD-associated fungal pathogens and bacterial taxa revealed interactions that may guide the isolation and development of novel biocontrol agents against GTDs.

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Unraveling the interplay of soil health, water quality, and fungal pathogens in a Mexican vineyard: A multiyear assessment.

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Grapevine trunk diseases (GTDs) represent a major constraint to global viticulture, compromising vine longevity and productivity. This study aimed to identify environmental stressors that contribute to GTD development and to propose integrated vineyard management strategies to improve vine health and sustainability. A multi-year investigation (2014–2023) was conducted in a *Vitis vinifera* vineyard located in San Miguel de Allende, Mexico, examining the complex interactions between soil health, irrigation water quality, and the emergence of trunk-associated fungi. Physicochemical analyses consistently revealed alkaline pH in both soil and water, along with variable salinity and sodicity. Microbial plate counts indicated reduced populations of aerobic bacteria, suggesting soil compaction and impaired nutrient cycling. These environmental stressors likely induced chronic vine stress, creating conditions favorable for GTD development. The presence of *Aspergillus* spp. and *Lasiodiplodia* spp. was documented, potentially acting as opportunistic pathogens in weakened vines. The findings underscore the urgent need for integrated vineyard management strategies that simultaneously improve soil health, ensure water quality, and enhance plant defense mechanisms to mitigate GTD incidence and support long-term vineyard sustainability.

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Effects of virtuous and non-virtuous pruning types on Charente Ugni blanc grape and wine quality. E. BRUEZ[1], C. CHOLET[1], M. BOISSEAU[2], X. POITOU[2], P. COLL[2], S. LACAMPAGNE[1], L. GENY[1]. *[1]UMR Oenologie, EA 4577, Université de Bordeaux, INRAE, BSA, Institut des Sciences de la Vigne et du Vin, 210 Chemin de Leysotte - CS 50008, 33882 Villenave d'Ornon, France. [2] Hennessy Jas et Cie, 1 rue Richonne 16100 Cognac, France. E-mail: emilie.bruez@u-bordeaux.fr*

Since the use of sodium arsenite was banned in 2001, Grapevine Trunk Diseases (GTDs) have become more widespread. Different methods are used to control the disease. As a preventive method to avoid pathogen entry and reduce disease expression, the question of pruning quality has come to the fore. Our trial concerned two Ugni blanc parcels planted in 2006 and 2015, in vineyards managed in double Guyot-Poussard,by JAS HENNESSY & CO, at Juillac-le-Coq and Saint-Preuil in Charente. The parcels were established as double Guyot-Poussard. Two different types of quality pruning were applied in each of the two parcels. Non-virtuous pruning damaged the diaphragm, whereas virtuous pruning ensured a desiccation cone to maintain the diaphragm. The diaphragm is a natural internal partition within the node of a vine shoot that acts as a barrier, helping to isolate wounds, thus protecting the vine from pathogens entry. The desiccation cone generated by the pruning is deeper. The aim of our work was to measure over two vintages, 2023 and 2024, the different impacts of these two types of pruning quality on grape and wine quality on non-esca-foliar symptomatic vines, before the appearance typical foliar symptoms. Weight and quality of grapes were analysed at harvest. Microvinifications were then carried out without sulphur use. The wines were distilled using the Cognac process. On musts, wines and distillates, chemical analyses were performed: ester and higher alcohol aroma wine markers. Triangular wine tasting was carried out on six-month-old wines. Although the wine tasting analyses did detect differences (such as vegetal flavour more detected in young vines) between virtuous and non-virtuous pruning for the young parcel, it was more difficult to do so for the older one. The present study confirms the interest of applying virtuous pruning on a long-term basis. It can have an indirect effect on the plant's physiological functioning, keeping the vines alive longer and preserving the grape quality.

Utilizing native biocontrol agents from Pacific Northwest vineyards for grapevine trunk disease management. J.B. DESHIELDS[1,2], A. HOWARD[2], J. WOODHALL[3], A. N. KC[1,2]. *[1]Southern Oregon Research and Extension Center, 569 Hanley Rd, Central Point, OR 97502. [2]Department of Botany and Plant Pathology, Oregon State University, 2082 Cordley Hall, Corvallis, OR 97331. [3]Department of Entomology, Plant Pathology and Nematology, University of Idaho, Parma, ID 83660. E-mail: achala.kc@oregonstate.edu*

Grapevine trunk diseases (GTDs) pose a significant threat to vineyard productivity due to yield losses and increased management costs. Chemical fungicides are commonly used; however, few products are labeled for GTDs. Biological control agents (BCAs) offer a potential alternative, but their efficacy varies based on regional and environmental conditions. This study aims to identify native microbial populations in Oregon vineyards with potential biocontrol properties against GTDs, focusing on their *in vitro* antagonistic effects against GTDs fungi. Two hundred spur samples were collected from eight vineyards across Oregon's Willamette and Rogue Valleys, from which microorganisms were isolated to assess their potential as BCAs. Samples were plated on selective media targeting suspected BCA genera, and distinct cultures were isolated based on morphology. Each isolation originated from a single fungal spore or bacterial colony and was characterized via PCR and sequencing. Potential BCA genera identified include *Aureobasidium* (95%), *Bacillus* (62%), *Pseudomonas* (95%), *Rhodotorula* (62%), and *Trichoderma* (6%), along with 97 isolates of potential GTD pathogens. The Willamette Valley exhibited significantly higher recovery rates for *Bacillus* (P = 0.003) and *Rhodotorula* (P = 0.044), highlighting regional differences. In vitro inhibition assays are ongoing to evaluate the antagonistic effects of isolates on GTD pathogens. Assays are performed at controlled temperatures (10°C, 15°C, 20°C, and 25°C) to assess the temperature-dependent efficacy of BCAs, addressing concerns about diminished performance in Oregon's cooler climate. Preliminary results suggest *Bacillus velezensis*, collected from Oregon vineyards, has a significant effect (-23.4%; $P = 0.0261$), at 25°C, on the reduction of *in vitro* growth of *Dothiorella iberica*, a causative agent of Botryosphaeria dieback. These findings highlight the potential for GTD management in PNW vineyards. Further research with temperature-specific trials will help refine application recommendations for optimal performance in cooler climates where BCA efficacy has historically been limited.

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Biological control of *Diplodia* spp. inoculum in dormant *Vitis vinifera* using *Trichoderma* spp. E. DONOSO[1], W. HETTICH[1], L. ROMERO[1]. [1]*Bio insumos Nativa, parcela antilhue lote 4b2, Maule. E-mail: edonoso@bionativa.cl*

This study investigated the efficacy of *Trichoderma* spp. (Mamull®) formulations in controlling *Diplodia seriata* inoculum on dormant *Vitis vinifera* following pruning. A field trial conducted in Talca, Central Chile, used a randomized block design with 10 replicates per treatment. Pruning debris inoculated with *Diplodia seriata* pycnidia was placed under Semillon grapevine. Treatments included: T0 (inoculated control), T1 (non-inoculated control), T2 (chemical pruning paste: pyraclostrobin), T3 (foliar Mamull®), and T4 (Mamull® soil application). Pycnidia parasitism and viability were assessed 15 days post-pruning while wood damage incidence and severity were evaluated 180 days. T3 (foliar Mamull®) had the lowest incidence (1.5%), significantly different from T0 (15.8%), and comparable to T2 (3.5%). T4 soil Mamull® (8.4%), while T1 was 5.8%. Severity followed a similar trend ($P<0.05$): T0 (3.23 cm), T3 (0.4 cm), T2 (0.5 cm), T4 (1.4 cm), and T1 (0.9 cm). Pycnidia parasitism was highest in T4 (63.4%) with lowest viability (10.5%), significantly different from all other treatments. T3 had 10.4% parasitism and 24.5% viability. T2 and T0 showed no significant difference (4.5% parasitism, 45.4% viability). These results support the potential of foliar and soil applications of Mamull® as effective biological control tools for managing *D. seriata* inoculum in pruning debris.

Evaluation of fungicides and biocontrol agents against *Aspergillus* Vine Canker. E.A. RANGEL-MONTOYA[1,2], M. SÁNCHEZ-SÁNCHEZ[2], R. HERNANDEZ-MARTINEZ[2]. [1]*Facultad de Ciencias Químicas e Ingeniería, Tijuana UABC.* [2]*Centro de Investigación Científica y de Educación Superior de Ensenada, CICESE. E-mail: erangel@cicese.mx*

Aspergillus Vine Canker (AVC) is an emerging disease affecting grapevine shoots, particularly during the training phase. Symptomatic plants show reddish sap exuding from infection sites, discolored, spongy vascular tissue, and black cankers. Recent reports from Mexico have identified highly virulent isolates of *Aspergillus niger* and *Aspergillus tubingensis* in vineyards in Sonora and Baja California. Currently, no effective management measurements are available for AVC. This study evaluated the efficacy of various fungicides and biocontrol agents against *Aspergillus* spp. A total of 18 commercial fungicides were tested using poisoned PDA media at recommended concentrations. Additionally, dual culture assays were conducted with five *Bacillus* spp. and six *Trichoderma* spp. strains. All assays were performed in triplicate, and data were analyzed using one-way ANOVA followed by Fisher's LSD test ($\alpha < 0.05$). Results showed that fungicides containing benzimidazole, myclobutanil, and hymexazol achieved nearly 100% inhibition of fungal growth. Among the biocontrol agents, *Bacillus amyloliquefaciens* isolates BEPVP26BCMX and BEPVP31BCMX, along with *Trichoderma harzianum* T06BCMX and *Trichoderma asperellum* EF09BCMX, inhibited fungal growth by 40–60%. Further analysis assessed the effect of the most effective treatment on conidial germination. Myclobutanil and benzimidazole completely inhibited germination, while biocontrol agents disrupted the fungal cell wall, thereby impeding germination. Finally, the efficacy of these treatments was assessed in planta. Grapevines inoculated with *Aspergillus* and treated with benzimidazole and myclobutanil-based fungicides, as well as *B. amyloliquefaciens* and *T. asperellum*, showed no necrotic lesions, indicating their potential to suppress *Aspergillus* growth in grapevines. These findings support the development of integrated and effective control strategies for managing AVC in Mexican vineyards.

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Improving and extending the productive life of grapevines affected by trunk disease using remedial surgery. M.R. SOSNOWSKI[1,2], E. VAN ZIJLL DE JONG[3]. [1]*South Australian Research and Development Institute, Adelaide SA 5001, Australia.* [2]*School of Agriculture, Food and Wine,* *Waite Research Institute, The University of Adelaide, SA 5005, Australia. [3]Linnaeus Limited, PO Box 1199, Gisborne 4040, New Zealand. E-mail: mark.sosnowski@sa.gov.au*

Grapevine trunk diseases such as Botryosphaeria and Eutypa dieback undermine the economic viability of vineyards. Causal pathogens typically enter vines through pruning wounds, causing wood decay, reduced productivity, and eventually vine death. Remedial surgery is the only curative option for managing GTDs and involves removing diseased cordons and trunks and training a new watershoot. The objective of this research was to evaluate the benefits of remedial surgery to vine health, productivity and longevity in grafted vines. Trials were established in three mature commercial vineyard blocks using approximately 4000 vines each of ‘Sauvignon Blanc’, ‘Cabernet Sauvignon’ and ‘Merlot’. Remedial surgery was undertaken over 5 years in winter, and spring and severity of disease was recorded prior to surgery and their performance following treatment was compared to untreated controls. Significant changes were recorded in incidence and severity of disease in both the canopy and trunk. Internal trunk staining did not correspond with canopy symptom severity. Multiple species of *Botryosphaeriaceae* pathogens were frequently detected in the trunks >200 mm in advance of the staining, occasionally at shoot removal wound sites and often at the bottom of trunks. *Eutypa lata* was occasionally detected together with these pathogens. Vine recovery after remedial surgery varied between cultivars, declined over time and with increased disease severity, but did not differ between winter and spring surgery. Over the 5-year study, yields in control vines declined significantly. Remedial surgery improved vine productivity and the yields from reworked vines often surpassed target levels for the blocks. Remedial surgery appeared to slow the trunk disease progression, with no obvious dieback observed in the canopies of reworked vines, though occasional vine deaths occurred. This is the most comprehensive study on remedial surgery worldwide, and ongoing monitoring of these blocks will elucidate the impact of remedial surgery on vineyard longevity.

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Efficacy of Tachigaren® (hymexazol 360 g/L) SL in plant recovery of grapevine associated with *Lasiodiplodia theobromae* in Peru. L.A. ALVAREZ[1], G. ESPINO[2]. *[1]Facultad de Ciencias Agrarias. Universidad Nacional de Cañete. Cañete, Lima. [2] Summit Agro South America SpA, Sucursal Perú, E-mail: lalvarez@undc.edu.pe*

Peru is currently the leading exporter of table grapes in the world. Grapevine trunk pathogen such as *Lasiodiplodia theobromae* (teleomorph: *Botryosphaeria rhodina*) causes severe losses in the main grapevine producing areas of the country. Field studies were conducted to determine the efficacy of hymexazol in the control of infections by *L. theobromae* and its role as root growth promoter. Five doses of hymexazol were evaluated: 0.0, 1.5, 2.0, 2.5, and 3.0 L ha-1, which were applied via irrigation systems at the berry setting stage. Healthy vines were distributed among five treatments in a randomized complete block design. Canes from these vines were inoculated by wound using a plug colonized by an isolate of *L. theobromae* five days before hymexazol application. For evaluation of hymexazol as root promoter, five galvanized steel boxes were placed randomly around vines in each experimental unit to evaluate root development. Sixty days after hymexazol application, the galvanized steel boxes were collected to evaluate the weight of new roots per treatment, and the inoculated canes were also collected to evaluate the lesion expansion. Lesions registered in vines treated with hymexazol were significantly lower than the nontreated control. The lesion length was reduced compared to nontreated control by 68%, 77%, and 81% for 2.0, 2.5, and 3.0 L ha^{-1}, respectively. The doses of 2.5 and 3.0 L ha^{-1} had 28 % and 36 %, respectively, more root development than the control treatment. The use of hymexazol in an integrated management program may represent an interesting tool to control *L. theobromae* in grapevine plants.

Compost as a source of beneficial *Trichoderma* for the biocontrol of Grapevine Trunk Diseases. J. ANGUIANO[1], C. DELGADO-RAMÍREZ[1], L. GUERRERO[1], C. VALENZUELA-SOLANO[2], R. HERNANDEZ-MARTINEZ[1]. *[1]CICESE. Departamento de Microbiología. Ensenada, Baja California. [2]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Campo Experimental Costa de Ensenada, Ensenada, Baja California, 22880, Mexico. E-mail: anguiano@cicese.edu.mx*

In northwestern Mexico, the main grapevine trunk diseases are caused by *Lasiodiplodia* spp. and *Cylindrocarpon*-like fungi, representing an increasing problem due to the scarcity of effective control strategies. Grapevine wood compost may serve as a promising source of beneficial microorganisms for managing these diseases. This study aimed to isolate and identify *Trichoderma* strains from grape compost with biocontrol activity against grapevine trunk disease fungi. A total of twenty-five *Trichoderma* strains (TL01-TL25) were obtained from a four-month-old compost pile. All strains produced chitinases, and twenty-three also produced siderophores. Most strains showed optimal growth between 25°C and 30°C, but TL15 exhibited the highest adaptability to higher temperatures, maintaining growth across a broader range from 25°C to 37°C. Although none of the strains grew at 45°C, all resumed growth when returned to room temperature. Dual culture assays were conducted to assess antagonistic activity against *Lasiodiplodia brasiliensis* isolate MXBCL28, *Nectria* sp. isolate RCCM9 and *Dactylonectria* sp. isolate MGN2. Four strains of *T. asperellum* (TL06, TL09, TL11 and TL16) were selected, based on their consistent performance, inhibiting the growth of *Nectria* and *Dactylonectria* by over 70%. These four strains were further evaluated for their production of volatile and non-volatile compounds, as well as their mycoparasitic activity using a pre-colonized plate method. None inhibited *L. brasiliensis* through production of volatile compounds, although they inhibited both *Nectria* sp. and *Dactylonectria* sp. by up to 40%. In contrast, non-volatile compounds inhibited the growth of all tested fungi by up to 73%, and in the mycoparasitism assays, pathogens were eliminated from 23% to 100% of the agar plugs from the precolonized plates. In conclusion, *Trichoderma asperellum* strains capable of suppressing grapevine trunk pathogens through multiple mechanisms of action were identified. Further *in planta* studies are needed to confirm their effectiveness under field conditions.

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Evaluating the biocontrol potential of *Fusarium* spp. against the grapevine trunk disease pathogen *Lasiodiplodia brasiliensis*.

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Trunk diseases caused by fungal pathogens represent one of the most critical phytosanitary concerns in grapevine production worldwide. *Lasiodiplodia brasiliensis* is a highly virulent species responsible for necrotic lesions, cankers, dieback, and premature plant death, leading to substantial economic losses in the northwestern region of Mexico. Non-pathogenic strains of *Fusarium* have the potential to be effective biocontrol agents against plant diseases through various mechanisms, including competition, induced resistance, and the production of antifungal metabolites. Given the limitations of chemical control and the need for sustainable alternatives, this study aimed to characterize *Fusarium* strains and assess their potential as biological control agents against *L. brasiliensis*. *Fusarium* spp. isolates were obtained from citrus trees and tested to be non-pathogenic after one year of inoculation. Sixteen isolates of *Fusarium* were identified through molecular analysis using ITS and EF-1α markers as *F. equiseti* (4), *F. nanum* (1), *F. citri* (1), *F. incarnatum* (7), *F. brachygibbosum* (1), and *F. denticulatum* (1). Enzymatic activity assays showed that *F. equiseti* isolate SCT04-5 had the highest amylase production, while *F. brachygibbosum* isolate SCT33-3 demonstrated the most potent protease activity. Antagonism, evaluated through in vitro dual culture assays, found that *F. brachygibbosum* isolate SCT33-3 showed the most significant inhibition (38.7%), followed by *F. denticulatum* isolate SCT58-3 (33.6%). Inhibition halos were also observed for *F. equiseti* (isolates SCT04-5 and SCT51-5) and *F. incarnatum* isolate SCT16-3, indicating the possible secretion of antifungal metabolites. Although in planta assays are underway, these findings suggest the potential use of *Fusarium* isolates as biocontrol agents for grapevine trunk diseases.

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Nanoparticles and selected chemical compounds significantly inhibit grapevine trunk disease pathogens.

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Grapevine is among the most important cultivated crops globally, but is increasingly threatened by the grapevine trunk disease (GTD) complex. Due to the lack of effective curative treatments and growing restrictions on chemical pesticide use, alternative control methods—such as nanoparticle applications—are gaining attention. We hypothesized that the selected chemical compounds and nanoparticles would exhibit antifungal activity against GTD pathogens. In a two-year *in planta* study, the inhibitory effects of four chemical agents (sodium arsenite, silver nitrate, silver thiosulfate complex and 8-HCH), and one nanoparticle formulation (AgSe at 100 % concentration, containing 2.59 g l^{-1} of Ag and 0.90 g l^{-1} of Se) were tested against three key GTD pathogens: *Diaporthe eres* Nitschke, *Diplodia seriata* De Not., and *Eutypa lata* (Pers.) Tul. & C. Tul. The pathogens were artificially inoculated using fungal discs (3 mm in diameter). The percentage of inhibition was calculated using the formula: $I = ((r_c - r_t)/r_c) \times 100$, where I is the inhibition percentage, r_c is the average percentage of necrosis in the control group, and r_t is the average percentage of necrosis in the treated group. All tested chemical agents (excluding nanoparticles) demonstrated inhibitory activity, ranging from 33.64% to 93.65%, significantly different from the untreated control. The silver-selenium nanoparticle formulation showed inhibitory effects specifically against *D. eres* and *E. lata*, with inhibition ranging from 55.01% to 86.85%. These findings highlight the potential of nanoparticle-based treatments as a promising alternative in integrated GTD management strategies.

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Evaluation of selected fungal biological control agents for the protection of grapevine pruning wounds against *Diplodia seriata*. A. FLOUDAS[1], S. TESTEMPASIS[1,2], A. FLARI[1], E. DIMOU[1], A. ELEFTHERIADOU[1], G. S. KARAOGLANIDIS[1]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, Plant Pathology Laboratory, Thessaloniki, Greece.* [2]*Department of Agriculture, School of Agricultural Sciences, University of Western Macedonia, 53100 Florina, Greece. E-mail:* afloud@agro.auth.gr

Grapevine Trunk Diseases (GTDs) are among the most destructive grapevine diseases, causing significant economic losses due to vineyard decline and replanting costs. The lack of effective chemical measures highlights the urgent need for environmentally and consumer-friendly alternatives. Biological Control Agents (BCAs) offer a promising, sustainable approach, targeting multiple GTD-associated fungi. This field study was conducted in the viticultural region of Nemea to evaluate the efficacy of four BCAs - *Trichoderma atroviride*, *T. citrinoviride*, *T. ghanense*, *Talaromyces pinophilus*—and a mixture (*T. ghanense* + *T. pinophilus*) in controlling infections by *Diplodia seriata*, a key GTD pathogen in Greece. Two Greek grapevine cultivars with contrasting susceptibility - Roditis (susceptible) and Limnio (tolerant) were used. BCAs were applied as conidial suspensions on fresh pruning wounds, followed 24 hours later by artificial inoculation with *D. seriata*. After six months, samples were collected and analyzed using a culture-based method. Pathogen re-isolation was conducted on PDA medium, and all obtained fungal isolates were identified molecularly. The results revealed that *T. pinophilus* and its mixture with *T. ghanense* provided strong protection in the susceptible cultivar Roditis, substantially reducing pathogen recovery. Conversely, *T. atroviridae* was more effective in the tolerant cultivar Limnio, indicating a potential cultivar-specific interaction. All BCAs demonstrated the ability to colonize and persist on pruning wounds. These findings reinforce the potential of BCAs as effective, sustainable tools for GTD management and support their integration into future vineyard protection strategies.

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Ozonated Water: A novel strategy for controlling grapevine trunk pathogens during grapevine's propagation. N. CHRISTOU[1], S. TESTEMPASIS[1,2], A. FLOUDAS[1], S. KARATSALOU-LEGAKI[3], K. BAKASIETAS[3], G.S. KARAOGLANIDIS[1]. [1]*Laboratory of Plant Pathology, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece;* [2]*Vine Nurseries Bakasietas, Nemea, Greece;* [3]*Department of Agriculture, School of Agricultural Sciences, University of Western Macedonia, 53100 Florina, Greece E-mail:* gkarao@agro.auth.gr

Grapevine Trunk Diseases (GTDs), caused by various fungal pathogens, represent a major threat to viticultural production worldwide. Effective management of GTDs depends on multiple factors, including the pathogen species, geographic location, climate, grapevine cultivar, and agricultural practices. Notably, the propagation phase in nursery production has been identified as a critical point for the dissemination of GTD pathogens. This study evaluates the efficacy of ozonated water (2.5 ppm) as an alternative to a conventional fungicide (Switch 25/37.5 WG, Syngenta, Greece) in the grapevine propagation process. Scion and rootstock materials were artificially inoculated with a conidial suspension (2×10^4 conidia mL^{-1}) of *Phaeomoniella chlamydospora* (Pch) and *Dactylonectria torresensis* (DacT). The commercial propagation protocol of VNB Bakasietas (Nemea, Greece) was followed. During the rehydration steps, plant materials were treated with either ozonated water or the conventional fungicide. The resulting grafted plants were established in commercial vineyards and assessed six months later for plant growth parameters (dry matter, stem and root length) and disease severity, the latter via RT-qPCR-based quantification of pathogen DNA. Overall, plants treated with ozonated water showed enhanced growth performance, with significantly longer roots and stems compared to those treated with fungicide. In artificially inoculated material, significantly lower levels ($P < 0.05$) of Pch and DacT were detected in ozonated water treatments. Specifically, the mean concentrations of Pch and DacT in ozonated plants were 3.94 and 4.14 copies µL^{-1}, respectively, versus 10.73 and 6.23 copies µL^{-1} in fungicide-treated plants. These results demonstrate that ozonated water is a promising, eco-friendly alternative to chemical fungicides, offering both improved vine growth and reduced pathogen presence during the propagation phase.

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Pruning cut position affects the susceptibility of grapevine pruning wounds to *Eutypa lata* and natural dieback. D. MARCH[1], S.P.B. PARKER[1], E.S. SCOTT[1], M.R. SOSNOWSKI[1,2]. *[1]School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, SA 5005, Australia. [2]South Australian Research and Development Institute, Adelaide SA 5001, Australia. E-mail:* mark.sosnowski@sa.gov.au

Eutypa dieback is caused by infection of pruning wounds by spores of Diatrypaceaeous fungi, primarily *Eutypa lata*. Recently popularised pruning techniques claim to influence grapevine pathology and physiology. More specifically, retaining long wood stubs above the node on canes may decrease pathogen colonisation into live tissue beyond the node and natural dieback or desiccation into permanent vascular tissue to disrupt sap flow. Similarly, crown cuts that retain basal buds to produce shoots and retain sap flow may limit colonisation and dieback into permanent wood, compared to flush cuts which remove basal buds. Furthermore, the diaphragm, a zone of hardened pith cells that divides the pith at the node, has been proposed to limit dieback. The objective was to investigate the effect of pruning cut position on *E. lata* colonisation and natural dieback. Trials were established on Shiraz vines in South Australia. In 2021, two trials on spur-pruned vines involved cutting canes to two nodes either directly above the distal node or just below the next node to leave long stubs. In a third trial in 2022 on cane-pruned vines, 1-year-old canes were removed from 2-year-old canes by either cutting flush so that basal buds were removed or making crown cuts that retained the buds and diaphragm. Wounds were inoculated with spores of *E. lata* or left uninoculated and the following winter spurs and canes were removed and assessed for extent of staining and recovery of *E. lata*. Retention of stubs or basal buds reduced the incidence of recovery of *E. lata* following pruning, which may be related to diaphragm integrity or basal bud influence on wound responses. Staining, or natural dieback, was also limited where stubs or basal buds were retained; causal mechanisms require further investigation. These findings support retention of wood stubs and basal buds when pruning.

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