12th INTERNATIONAL WORKSHOP ON GRAPEVINE TRUNK DISEASES

10. 7. - 14. 7. 2022
MIKULOV
CZECH REPUBLIC

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International Council on Grapevine Trunk Diseases

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12th IWGTD TECHNICAL PROGRAM

SUNDAY JULY 10th

15:00 - 19:00	**REGISTRATION**
15:00 - 18:00	**ICGTD Council Meeting**
18:00 - 21:00	**POSTERS SET UP**
19:00 - 21:00	**WELCOME RECEPTION**

MONDAY JULY 11th

08:30 - 09:30		**REGISTRATION**
09:30 - 10:00		**WELCOMING ADDRESS**
10:00 - 10:30		**MORNING COFFEE**
10:30 - 12:00		**Session 1: S1 Pathogen Detection and Identification**
		Chairpersons: Josep Armengol & Cecilia Rego
10:30 - 10:45	**1.1**	**Investigating the causal agents of Sudden Vine Collapse in California** A. ESKALEN, E. HARDY, K. ELFAR, M. BUSTAMANTE, M. AL-RWAHNIH, C. STARR, S. BOLTON, N. MCROBERTS, M. BATTANY, L. BETTIGA, K. ARNOLD, G. ZHUANG
10:45 - 11:00	**1.2**	**Aspergillus Vine Canker: An overlooked canker disease of grapevine in California** M. I. BUSTAMANTE, K. ELFAR, M. ARREGUIN, G. ZHUANG, A. ESKALEN
11:00 - 11:15 **VIRTUAL**	**1.3**	**Development of loop mediated isothermal amplification (LAMP) assays for rapid detection of Eutypa and Botryosphaeria dieback pathogens** R. BILLONES-BAAIJENS, M. LIU, M. R. SOSNOWSKI, S. SAVOCCHIA
11:15 - 11:30 **VIRTUAL**	**1.4**	**Species diversity of Diatrypaceous airborne spores in Australian vineyards** R. BILLONES-BAAIJENS, M. LIU, M. R. AYRES, M. R. SOSNOWSKI, S. SAVOCCHIA
11:30 - 11:45 **STUDENT** **VIRTUAL**	**1.5**	**Implementation of droplet digital PCR to determining the health status and abundance of grapevine trunk disease fungi in ready-to-plant nursery material imported into Canada** J. HRYCAN, J. THEILMANN, A. MAHOVLIC, J. BOULÉ, J. R. ÚRBEZ-TORRES
11:45 - 12:00	**1.6**	**Rapid detection of *Neofusicoccum parvum* using Loop mediated isothermal amplification (LAMP)** S. ECHEVERRÍA, J. PÉREZ, R. POLANCO, F. GAÍNZA-CORTÉS
12:00 - 13:30		**LUNCH**

13:30 - 15:00 Session 2: S1 Pathogen Detection and Identification

Chairpersons: Antonia Carlucci & Akif Eskalen

13:30 - 13:45	**2.1**	**Botryosphaeria dieback is a major component of grapevine trunk disease complex in Oregon vineyards** A. N. KC, M. HERNANDEZ
13:45 - 14:00 **STUDENT**	**2.2**	**Carbohydrate-active enzymes and secondary metabolites production using two lignocellulosic biomass on *Neofusicoccum parvum* Bt-67, a grapevine trunk pathogen** J. D. RESTREPO-LEAL, M. BELAIR, J. FISCHER, N. RICHET, F. FONTAINE, C. RÉMOND , O. FERNANDEZ, L. BESAURY
14:00 - 14:15	**2.3**	**Exploration of a non-enzymatic wood degradation pathway in *Fomitiporia mediterranea*, the main white rot agent in european Esca-affected vineyards** S. MORETTI, M. L. GODDARD, J. LALEVÉE, S. DI MARCO, L. MUGNAI, C. BERTSCH, S. FARINE
14:15 - 14:30	**2.4**	**Analyzing the presence of trunk diseases fungi in heritage grapevines of Baja California** C. S. DELGADO-RAMÍREZ, E. SEPÚLVEDA, C. VALENZUELA-SOLANO, R. HERNÁNDEZ-MARTÍNEZ
14:30 - 14:45	**2.5**	**The most important fungal pathogens responsible of grapevine trunk disease (GTD) in south Italy** A. CARLUCCI, M. L. RAIMONDO, F. LOPS
14:45 - 15:00	**2.6**	**4D imaging at the bedside of grapevines: towards non-destructive diagnosis of trunk diseases** R. FERNANDEZ, L. LE CUNFF, S. MÉRIGEAUD, J. L. VERDEIL, J. PERRY, P. LARIGNON, A. S. SPILMONT, P. CHATELET, M. CARDOSO, CH. GOZE-BAC, C. MOISY

15:00 – 15:30 AFTERNOON COFFEE

15:30 - 16:15 Session 3: S2 Epidemiology

Chairpersons: Christian Kraus & Erzsebet Karaffa

15:30 - 15:45 **STUDENT**	**3.1**	**Managing Esca in Bordeaux: An economic analysis of curative practices under appellation yield risk and uncertain disease severity** M. Y. E. KONAN, A. ALONSO UGAGLIA, J. KAPLAN
15:45 - 16:00	**3.2**	**Variation of mycorrhizal colonization in plants with canopy symptoms of Esca disease** L. LANDI, S. MUROLO, G. ROMANAZZI

16:00 - 16:15 **3.3** **Diversity and pathogenicity of fungi causing GTDs in Chilean patrimonial vineyards**
VIRTUAL
D. GRINBERGS, J. CHILIAN, M. REYES

16:15 - 17:00 Session 4: S3 Host-Pathogen Interactions

Chairpersons: Patricia Trotel-Aziz & Guzmán Carro

16:15 - 16:30 **4.1** **Establishment of a cell model to study molecular interactions between grapevine and Esca-associated pathogens**
F. RAKOTONIAINA, C. BRETON, L. TESSAROTTO, C. CHERVIN, A. JACQUES, O. RODRIGUES

16:30 - 16:45 **4.2** **Diversity of *Neofusicoccum parvum* for the production of the phytotoxic metabolites (-)-terremutin and (R)-mellein**
P. TROTEL-AZIZ, G. ROBERT-SIEGWALD, O. FERNANDEZ, C. LEAL, S. VILLAUME, J. F. GUISE, E. ABOU-MANSOUR, M. H. LEBRUN, F. FONTAINE

16:45 - 17:00 **4.3** **The beneficial effects of *Bacillus subtilis* PTA-271 and *Trichoderma atroviride* SC1 against Botryosphaeria-dieback pathogen *Neofusicoccum parvum* may vary with grapevine cultivar**
STUDENT
C. LEAL, N. RICHET, J. F. GUISE, D. GRAMAJE, J. ARMENGOL, F. FONTAINE, P. TROTEL-AZIZ

17:30 - 19:00 Wine. Poster Viewing with Presenters

P.1 **Diversity of *Dactylonectria* and *Ilyonectria* species causing black foot disease in grapevine nursery stock in Uruguay**
M. J. CARBONE, M. GELABERT, P. MONDINO, S. ALANIZ

P.2 ***Aspergillus* spp. causing Aspergillus Vine Canker on grapevine in Mexico**
E. A. RANGEL-MONTOYA, C. VALENZUELA-SOLANO, R. HERNÁNDEZ-MARTÍNEZ

P.3 **Molecular methods in detection and quantification of *Diplodia seriata* and *Phaeomoniella chlamydospora* in vine plants**
M. ACUÑA, R. ROA, P. R. DRIGUEZ, P. ARRAÑO, S. VARGAS, F. GAÍNZA-CORTÉS, G. DÍAZ, M. LOLAS

P.4 **Investigating the role of *Fusarium* spp. in young vine decline in California**
M. I. BUSTAMANTE, K. ELFAR, M. ARREGUIN, R. J. SMITH, L. J. BETTIGA, T. TIAN, G. A. TORRES, G. ZHUANG, A. ESKALEN

P.5 ***Diaporthe* spp. associated with dieback in Baja California vineyards**
C. A. DELGADO-RAMÍREZ, E. A. RANGEL-MONTOYA, J. C. LEE-CONTRERAS, C. VALENZUELA-SOLANO, R. HERNÁNDEZ-MARTÍNEZ

P.6 **Hymenochaetaceae fungus *Arambarria destruens* associated with Grapevine Trunk Diseases in Chilean patrimonial vineyards**
VIRTUAL
J. CHILIAN, D. GRINBERGS, M. REYES

P.7 **Fungal species associated with grapevine decline in China** VIRTUAL

Y. Y. ZHOU, X. H. LI, L. N. WU, H.M. ZHANG, W. ZHANG, J.Y. YAN

P.8 **Characterisation of the presence and distribution of grapevine trunk diseases in the vineyards of Quebec, Canada** VIRTUAL

C. PROVOST, P. CONSTANT, A. A. DURAND

P.9 **Identification and quantification of Grapevine trunk and black-foot diseases pathogens in the soil, using real-time PCR coupled with HRM**

S. TESTEMPASIS, E. STAVRIDOU, P. MADESIS, G. S. KARAOGLANIDIS

P.10 **Quantification of airborne inoculum of *Eutypa lata* and Botryosphaeriaceae spp. by real-time PCR in California**

J. CLERKIN, S. DUBROVSKY, A. L. FABRITIUS

P.11 **Effect of cover crops on the dispersal of *Phaeomoniella chlamydospora* inoculum in vineyards**

M. BERBEGAL, D. PINNA , E. GONZÁLEZ-DOMINGUEZ, G. HASANALIYEVA, T. CAFFI,V. ROSSI, J. ARMENGOL

P.12 **Study of environmental conditions influencing survival and reproductive structures development of *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora***

M. BERBEGAL, E. GONZÁLEZ-DOMÍNGUEZ, J. ARMENGOL

P.13 **Comparing disease incidence of grapevine trunk diseases at different sites in the Tokaj wine region, Hungary**

P. BALLING, T. KOVÁCS, A. KNEIP, P. MOLNÁR

P.14 **Spring shoot thinning wounds are susceptible to grapevine trunk disease pathogens** VIRTUAL

M. R. SOSNOWSKI, M. R. AYRES

P.15 **Investigating how *Lasiodiplodia brasiliensis* colonizes grapevine tissues**

E. A. RANGEL-MONTOYA, R. HERNÁNDEZ-MARTÍNEZ

P.16 **Endophytic mycobiome and anthocyanidins, two key features involved in grapevine leaves affected by 'tiger stripes'**

G. DEL FRARI, C. INGRÀ, A. GOBBI, M. R. AGGERBECK, T. NASCIMENTO, A. CABRAL, H. OLIVEIRA, A. FERRANDINO, L. HESTBJERG HANSEN, R. BOAVIDA FERREIRA

P.17 **High diversity of fungal grapevine trunk pathogens isolated from one-year canes including the first detection of *Neofabraea kienholzii* in Albariño cv grapevine in Spain** VIRTUAL

V. REDONDO-FERNÁNDEZ, A. ALONSO-NÚÑEZ, N. CAMBRA-GONZÁLEZ, L. AREAL-HERMIDA, C. SIEIRO

P.18 **BIOBESTicide project: Action of *Pythium oligandrum* on grapevine trunk diseases and its impact on microbial communities**

S. LOPEZ, A. CHATAIGNER, M. C. DUFOUR

P.19 **It will be possible to predict Esca symptoms manifestation based on the wood microbiome?**

VIRTUAL

B. GARCÍA-GARCÍA, M. M. ALGUACIL-GARCÍA, A. ACEDO, L. MARTÍN

P.20 **Effects of temperature on *in vitro* biocontrol of *Diplodia seriata* by psychrotolerant *Pseudomonas* strains**

A. LARACH, P. VEGA-CELEDÓN, P. SANHUEZA, N. RIQUELME, M. SEEGER, X. BESOAIN

P.21 **Phylogenetic host range in the Botryosphaeriaceae**

I. SILVA-VALDERRAMA, J. R. ÚRBEZ-TORRES, T. J. DAVIES

P.22 **Grapevine below-ground microbiome analysis identifies *Fusarium* spp. aggravating the severity of grapevine trunk disease (GTDs)**

VIRTUAL

Y. H. LI, X. H. LI, W. ZHANG, J. ZHANG, H. WANG, J. B. PENG, X. C. WANG, J. Y. YAN

TUESDAY JULY 12th

09:00 - 10:00 Session 5: S3 Host-Pathogen Interactions

Chairpersons: Kálmán Zoltán Váczy & Rufina Hernández-Martínez

09:00 - 09:15 **5.1** **VIRTUAL**

Susceptibility of pruning wounds to grapevine trunk disease pathogens in different Australian climates

M. R. SOSNOWSKI, M. R. AYRES, R. BILLONES-BAAIJENS, S. SAVOCCHIA

09:15 - 09:30 **5.2** **VIRTUAL**

Characterisation of the endomicrobiome of grapevine nursery plants in Australia

R. BILLONES-BAAIJENS, M. LIU, B. STODART, M. R. SOSNOWSKI, S. SAVOCCHIA

09:30 - 09:45 **5.3** **STUDENT**

Deciphering transcriptomic and metabolomic early host responses in grapevine wood to Esca pathogens *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*

A. ROMEO-OLIVÁN, J. CHERVIN, S. FOURNIER, V. PUECH-PAGES, C. BRETON, T. LAGRAVÈRE, J. DAYDÉ, B. DUMAS, G. MARTI, A. JACQUES

09:45 - 10:00 **5.4** **STUDENT**

Comparative transcriptomic and metabolomic responses of grape to biological control agent *Trichoderma atroviride* suggest early modifications before infection by Esca pathogens *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*

A. ROMEO-OLIVÁN, J. CHERVIN, S. FOURNIER, V. PUECH-PAGES, C. BRETON, T. LAGRAVÈRE, J. DAYDÉ, B. DUMAS, G. MARTI, A. JACQUES

10:00 - 10:30 MORNING COFFEE

10:30 - 12:00 Session 6: S3 Host-Pathogen Interactions

Chairpersons: Florence Fontaine & Pedro Reis

10:30 - 10:45 **6.1**

Deciphering the susceptibility level of Greek grapevine cultivars to grapevine trunk disease pathogens through measurements of defense-related genes expression

S. TESTEMPASIS, P. NTASIOU, T. TSOUVALAS, E. MPILA, G. S. KARAOGLANIDIS

10:45 - 11:00 **6.2**

Tracking the fungal pathobiome associated with young grapevine decline in California nurseries

C. TODD, J. F. GARCIA, A. JACQUES, D. CANTU, P. ROLSHAUSEN

11:00 - 11:15	**6.3**	**Esca-leaf symptoms: Histological alteration and its relation with the physiological and nutritional status** M. V. ALARCÓN, L. MÉNDEZ-GRANO DE ORO, A. FLORES-ROCO, A. DE SANTIAGO, J. RODRÍGUEZ-PRIETO, L. MARTÍN
11:15 - 11:30	**6.4**	**The physiology and drivers of Esca leaf symptom development in grapevine** G. BORTOLAMI, T. FREJAVILLE, J. POUZOULET, N. DELL'ACQUA, N. FERRER, L. GUERIN DUBRANA, P. LARIGNON, P. LECOMTE, G. GAMBETTA, C. DELMAS
11:30 - 11:45	**6.5**	**Influence of morphological traits of xylem vessels on the response to *Phaeomoniella chlamydospora* of table- and wine-grape and rootstocks** D. GERIN, N. CHIMIENTI, A. AGNUSDEI, R. M. DE MICCOLIS ANGELINI, P. ROTONDO, F. FARETRA, S. POLLASTRO
11:45 - 12:00	**6.6**	**Effects of tissue age on Botryosphaeria dieback caused by *Diplodia seriata* in cv. Cabernet Sauvignon plants** A. LARACH, E. SALGADO, P. SANHUEZA, A. SALINAS, F. CADIZ, M. SEEGER, X. BESOAIN
12:00 - 13:30		**LUNCH**
13:30 - 14:30		**Session 7: S3 Host-Pathogen Interactions**
		Chairpersons: Chloé Delmas & Gianfranco Romanazzi
13:30 - 13:45 **STUDENT**	**7.1**	**Wood degradation by *Fomitiporia mediterranea* M. Fischer: physiologic, proteomic and metabolomic approaches** M. SCHILLING, A. MAIA-GRONDARD, E. ROBERT, P. HUGUENEY, C. BERTSCH, S. FARINE, E. GELHAYE
13:45 - 14:00	**7.2**	**Occurrence of *Phaeomoniella chlamydospora* at different stages of grapevine propagation material, pathogen biomass fluctuation from the nursery to the field and association with the endophytic microbiome** C. TSOUKAS, P. ATHANASIADI, A. K. TZIMA, E. J. PAPLOMATAS
14:00 - 14:15	**7.3**	**Response of different grapevine cultivars to infection by *Lasiodiplodia theobromae* and *Lasiodiplodia mediterranea*** P. REIS, A. GASPAR, A. ALVES, F. FONTAINE, C. REGO
14:15 - 14:30 **STUDENT**	**7.4**	**Development of an analytical approach using magic-angle spinning NMR for the study of molecular markers of dieback in French vineyards** C. ROBERT, A. GRÉLARD, A. SAAD, M. GADRAT, P. REY, C. DELMAS, A. LOQUET
14:30 – 15:00		**AFTERNOON COFFEE**

15:00 - 17:45 Session 8: S4 Microbial Ecology

Chairpersons: Aleš Eichmeier & Emilie Bruez

15:00 - 15:15 **STUDENT**	**8.1**	**Drought stress results in a compartment-specific restructuring of the grapevine root-associated fungal microbiome** M. J. CARBONE, S. ALANIZ, P. MONDINO, M. GELABERT, A. EICHMEIER, D. TEKIELSKA, R. BUJANDA, D. GRAMAJE
15:15 - 15:30	**8.2**	**The fungus *Aureobasidium pullulans* may promotes the development of foliar symptoms on Esca-diseased grapevines** Z. KARÁCSONY, V. MONDELLO, A. SONGY, F. FONTAINE, K. Z. VÁCZY
15:30 - 15:45 **STUDENT**	**8.3**	**A study to characterise diversity of fungal endophytic environment of young nursery grapevine plants** M. AL SARRAJ, E. DEYETT, A. ROMEO OLIVAN, T. LAGRAVERE, P. ROLSHAUSEN, A. JACQUES
15:45 - 16:00 **STUDENT**	**8.4**	**Comparison of the grapevine-associated plant pathogenic fungal community among different microhabitats, among cultivars and between healthy and Esca-diseased plants** A. GEIGER, C. M. LEAL, Z. KARÁCSONY, R. GOLEN, K. Z. VÁCZY, J. GEML
16:00 - 16:15	**8.5**	**Predicting pathogens' virulence: linking host breadth and pathogenicity of the Botryosphaeriaceae fungal family in wine grapes (*Vitis vinifera)*** I. SILVA-VALDERRAMA, O. SILVA, J. BOULE, J. R. ÚRBEZ-TORRES, T. J. DAVIES
16:15 - 16:30 **STUDENT**	**8.6**	**Exploring the microbial terroir: communities of fungi associated with grapevine trunk diseases differ among terroirs and seasons** C. M. LEAL, A. GEIGER, K. Z. VÁCZY, J. GEML
16:30 - 16:45	**8.7**	**Drought influences fungal community structure and diversity inhabiting the grapevine vascular system and enhaces *Phaeomoniella chlamydospora* abundance** M. M. MALDONADO-GONZÁLEZ, M. J. CARBONE, A. EICHMEIER, T. KISS, R. BUJANDA, D. GRAMAJE
16:45 - 17:00 **VIRTUAL**	**8.8**	**AMF community diversity identification and their effects on grapevine growth parameters under black foot disease pressure** R. MOUKARZEL, H. J. RIDGWAY, J. LIU, A. GUERIN-LAGUETTE, E. E. JONES
17:00 - 17:15 **STUDENT** **VIRTUAL**	**8.9**	**Culturome versus DNA metabarcoding: Diversity of grapevine endophytic mycobiome in old and young vines of different health status in New Zealand** N. BESSELMA, H. J. RIDGWAY, D. C. MUNDY, B. R. VANGA, P. PANDA, E. E. JONES
17:15 - 17:30 **STUDENT** **VIRTUAL**	**8.10**	**Can the microbiome drive the suppression of grapevine trunk diseases?** D. ADEJORO, E. E. JONES, H. RIDGWAY, D. C. MUNDY, B. VANGA, S. BULMAN

17:30 - 17:45 **8.11** **Implications of abiotic and biotic stress on *Phaeomoniella chlamydospora* colonization in young 'Merlot' grapevines**
STUDENT
VIRTUAL
J. HRYCAN, P. BOWEN, T. FORGE, M. HART, J. R. ÚRBEZ-TORRES

17:45 - 19:00 Wine. Poster Viewing with Presenters

P.23 **Enhancing the regrowth of vines following remedial surgery**
VIRTUAL
M. R. SOSNOWSKI, M. R. AYRES

P.24 **Management of grapevine trunk disease with remedial surgery**
VIRTUAL
E. VAN ZIJLL DE JONG, H. TERNENT, S. ST GEORGE, R. KALLAS, M. R. SOSNOWSKI

P.25 **GTD prevention: A practical application of *Trichoderma* formulations under field conditions**
VIRTUAL
J. J. GUERA-NOGALES, L. MÉNDEZ-GRANO DE ORO, M. J. DORADO-RICO, L. MARTÍN

P.26 **Biological control of inoculum of *Diplodia seriata* in pruning debris of *Vits vinifera* in Chilean orchards**
E. DONOSO, L. ROMERO, W. HETTICH, D. BASCUÑAN, C. GARCIA, J. FIGARI

P.27 **Effect of the combined treatments with LC2017 and *Trichoderma atroviride* strain I-1237 on disease development and defense responses in vines infected by *Lasiodiplodia theobromae***
P. REIS, V. MONDELLO, I. DINIZ, A. ALVES, C. REGO, F. FONTAINE

P.28 **Will forestry waste be able to save the grapevines and help control GTDs?**
L. MERLEN, L. GALIMAND, C. TARNUS, C. GERARDIN, P. GERARDIN, M. GELLON

P.29 **Native isolates of *Trichoderma* spp. can protect pruning wounds against *Lasiodiplodia theobrome* in Argentinian vineyards, reducing the incidence of Hoja de Malvón disease**
VIRTUAL
V. LONGONE, A. PIERONI, G. ESCORIAZA

P.30 **Impact of different grapevine bench grafting methods on the xylem anatomy, hydraulic traits and wood necrosis associated with young declines of grafted vines**
VIRTUAL
E. BATTISTON, S. FALSINI, A. GIOVANNELLI, S. SCHIFF, C. TANI, R. PANAIIA, S. DI MARCO, L. MUGNAI

P.31 **Bio-products partially protect grapevine pruning wounds against infection of the trunk pathogen *Lasiodiplodia theobromae***
VIRTUAL
G. ESCORIAZA, V. LONGONE, I. FUNES PINTER, M. ULIARTE, A. NOLI

P.32 **Effect of hot-water treatment on grapevine viability and fungal trunk diseases pathogens diversity by RNA high-throughput amplicon sequencing**
M. ANDRÉS-SODUPE, A. EICHMEIER, D. GRAMAJE

P.33 **Sensitivity of fungal grapevine trunk pathogens to treatments with electrolyzed water *in vitro***

A. FRINKLER, M. BERBEGAL, F. BEN ATIA, J. V. ROS-LIS, G. GAUME, J. ARMENGOL

P.34 **The impact of sanitary status of scions regarding grapevine trunk diseases and various disinfectants on phenolic compounds in different parts of grapevine grafts of 'Cabernet Sauvignon'**

D. RUSJAN, D. KJUDER, A. ŠKVARČ, M. MIKULIC-PETKOVSEK

P.35 **Evaluation of *Talaromyces pinophilus* as an antagonist of the causal agents of Botryosphaeriaceae spp. in grapevine**

P. RODRÍGUEZ-HERRERA, F. GAÍNZA-CORTÉS

P.36 **Effects of the moss extracts on plant pathogenic fungus causing Phomopsis cane and leaf spot of grapevine**

N. LATINOVIC, M. SABOVLJEVIC, M. VUJICIC, A. SABOVLJEVIC, J. LATINOVIC

P.37 ***In vitro* evaluation of *Trichoderma* native strains as potential biological control agents against *Phaeoacremonium minimum***

G. CARRO-HUERGA, S. MAYO-PRIETO, A. RODRÍGUEZ-GONZÁLEZ, O. GONZÁLEZ-LÓPEZ, S. GUTIERREZ, P.A. CASQUERO

P.38 **Effectiveness of Mamull® (*Trichoderma* spp. and *Bionectria* spp.) in the control of trunk wood disease in table grape and blueberry**

VIRTUAL

L. A. ALVAREZ, E. DONOSO, C. TORRES

P.39 **Efficacy of Tachigaren® (hymexazol 360 g/L) SL to suppress branch lesions caused by *Lasiodiplodia theobromae* in grapevine plants**

VIRTUAL

L. A. ALVAREZ, G. ESPINO

VINAŘSKÝ FOND ČESKÁ REPUBLIKA

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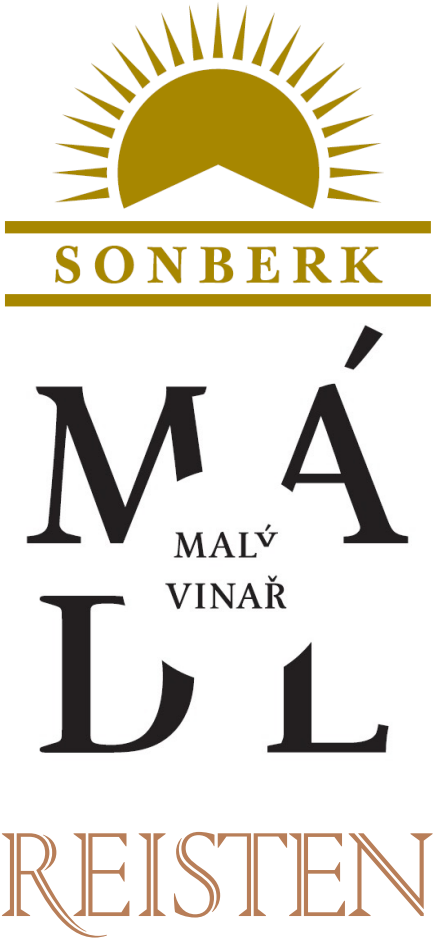
Projekt s podporou Vinařského fondu

WEDNESDAY JULY 13th

9:00 - 17:00	**Field Trip South Moravia** **Sponsored by SONBERK, MALÝ VINAŘ - MÁDL, REISTEN**



THURSDAY JULY 14th

08:30 - 10:00 Session 9: S5 Disease Management

Chairpersons: Francois Halleen & Sandra Alaniz

08:30 - 08:45 **9.1** **Evaluating treatments for the protection of grapevine pruning wounds from natural infection by trunk disease fungi**

R. BUJANDA, B. LÓPEZ-MANZANARES, S. OJEDA, O. ONEKA, L. GONZAGA SANTESTEBAN, J. PALACIOS, D. GRAMAJE

08:45 - 09:00 **9.2** **Hot water treatment (50 °C for 45 min) in grapevine nurseries: the dilemma of heat tolerant GTD pathogens**

F. HALLEEN, M. WEBBER, L. MOSTERT

09:00 - 09:15 **9.3** **The *in vitro* effects of selected phenolic compounds against *Diplodia seriata, Eutypa lata, Fomitiporia mediterranea* and *Neofusicoccum parvum***

STUDENT

K. ŠTŮSKOVÁ, F. FONTAINE, V. MONDELLO, E. HAKALOVÁ, J. WOHLMUTH, A. VAVŘINÍK, T. HELMOVÁ, Š. FRANKOVÁ, A. EICHMEIER

09:15 - 09:30 **9.4** **Minimal versus intensive: How the pruning intensity affects occurrence of grapevine leaf stripe disease and wood integrity in the trunk**

C. KRAUS, C. RAUCH, E. M. KALVELAGE, F. H. BEHRENS, D. D´AGUIAR, C. DUBOIS, M. FISHER

09:30 - 09:45 **9.5** **Effects of pruning on desiccation cone formation of three cultivars in France**

E. BRUEZ, C. CHOLET, T. MARTIGNON, M. GIUDICI, M. BOISSEAU, P. REY, L. GENY

09:45 - 10:00 **9.6** **Can spray coverage of wounds and Eutypa dieback control be improved by the addition of adjuvants to fungicide?**

VIRTUAL

M. R. AYRES, M. R. SOSNOWSKI

10:00 - 10:30 MORNING COFFEE

10:30 - 12:00 Session 10: S5 Disease Management

Chairpersons: José Ramón Úrbez-Torres & David Gramaje

10:30 - 10:45 **10.1** **Effects of biocontrol agents on *Fomitiporia mediterranea***

M. RIEDLE-BAUER, M. GORFER, M. MADERCIC

10:45 - 11:00	**10.2**	**Biological control of *Phaeomoniella chlamydospora* in young grapevines with *Bacillus velezensis* K165 and *Fusarium oxysporum* F2** F. I. GKIKAS, A. TAKO, D. GKIZI, C. LAGOGIANNI, E. A. MARKAKIS, S. E. TJAMOS
11:00 - 11:15 **STUDENT**	**10.3**	**Lignans extract from knotwood of Norway spruce as a possible novel bioprotectant agent against grapevine trunk diseases** M. ŠPETÍK, J. BALÍK, P. HÍC, E. HAKALOVÁ, K. ŠTŮSKOVÁ, L. FREJLICHOVÁ, J. TŘÍSKA, A. EICHMEIER
11:15 - 11:30	**10.4**	**Biological and chemical pruning wound protectants reduce infection of grapevine trunk diseases pathogens in California** R. BLUNDELL, A. ESKALEN
11:30 - 11:45	**10.5**	**Study of the impact of a copper-hydroxyapatite formulation on the vine physiology, microbiome, metabolome, for a potential use against grapevine trunk diseases** V. MONDELLO, O. FERNANDEZ, J. F. GUISE, C. LEMAÎTRE-GUILLIER, R. GOUGEON, A. ACEDO, P. SCHMITT-KOPPLIN, M. ADRIAN, C. PINTO, P. TROTEL-AZIZ, F. FONTAINE
11:45 - 12:00	**10.6**	***In vitro* evaluation of endophytic and rhizospheric bacteria as potential biocontrol agents of grapevine trunk diseases** M. I. BUSTAMANTE, K. ELFAR, A. ESKALEN
12:00 - 13:30		**LUNCH**
13:30 - 15:15		**Session 11: S5 Disease Management**
		Chairpersons: Vincenzo Mondello & Mercedes Maldonado González
13:30 - 13:45 **VIRTUAL**	**11.1**	**Biological control of Botryosphaeria dieback on grapevines** J. R. ÚRBEZ-TORRES, J. POLLARD-FLAMAND, J. BOULÉ, M. HART
13:45 - 14:00	**11.2**	**Combining a HA + Cu(II) site-targeted copper-based product with a pruning wound protection program to prevent infection with *Lasiodiplodia* spp. in grapevine** P. REIS, A. GASPAR, A. ALVES, F. FONTAINE, C. REGO
14:00 - 14:15 **VIRTUAL**	**11.3**	**Relationship between *Trichoderma* recovery from pruning wounds treated with biocontrol formulations and the control of *Diplodia seriata* in *Vitis vinifera* in Chilean orchards** E. DONOSO, L. ROMERO, W. HETTICH, D. BASCUÑAN, C. GARCIA, J. FIGARI
14:15 - 14:30 **STUDENT** **VIRTUAL**	**11.4**	**Hot water treatment as a tool to produce high-quality grapevine propagation material** D. SIMON, P. WINTERHAGEN, R. WALTER, T. WETZEL, A. KORTEKAMP, A. VON TIEDEMANN, J. EDER

14:30 - 14:45 **VIRTUAL**	**11.5**	**Removal of trunk disease pathogens in mature grapevines with remedial surgery** E. VAN ZIJLL DE JONG, H. TERNENT, S. ST GEORGE, R. KALLAS, M. R. SOSNOWSKI
14:45 - 15:00 **VIRTUAL**	**11.6**	**Trellis systems of rootstock mother vines affect the wood microbiome** E. BATTISTON, L. BORRUSO, S. FALSINI, C. PINTO, T. MIMMO, S. CESCO, S. DI MARCO, L. MUGNAI
15:00 - 15:15 **STUDENT** **VIRTUAL**	**11.7**	**Biological control agents for Botryosphaeria dieback of grapevine** C. S. DELGADO-RAMÍREZ, E. SEPÚLVEDA, C. VALENZUELA-SOLANO, R. HERNÁNDEZ-MARTÍNEZ
15:15 – 15:45		**AFTERNOON COFFEE**
15:45 - 16:45		**Session 12: Student Poster Flash Presentations**
		Chairperson: Laura Mugnai & Ximena Besoains
15:45 - 15:50	**P.40**	**Epidemiological survey of grapevine trunk diseases in the Eger Wine Region** A. CSÓTÓ, D. BARANYI, G. SZAKADÁT, E. SÁNDOR
15:50 - 15:55	**P.41**	**Potential GTDs antagonist microfungi isolated from grapevines** A. CSÓTÓ, M. FILE, A. N. PITI, B. ELLMANN, K. PÁL, G. SZAKADÁT, E. SÁNDOR
15:55 - 16:00	**P.42**	**Interactive effects of *Dactylonectria macrodidyma* inoculation on the rhizosphere and root microbiome of grapevine** M. J. CARBONE, A. EICHMEIER, T. KISS, D. TEKIELSKA, R. BUJANDA, B. LÓPEZ-MANZANARES, S. OJEDA, D. GRAMAJE
16:00 - 16:05	**P.43**	**Evaluation of *Trichoderma atroviride* SC1 and *Bacillus subtilis* PTA-271 combination against grapevine trunk diseases pathogens in nursery propagation process** C. LEAL, D. GRAMAJE, F. FONTAINE, P. TROTEL-AZIZ, J. ARMENGOL
16:05 - 16:10	**P.44**	**Incidence of newly described mycoviruses in *Diaporthe* sp.** M. KOCANOVA, L. BOTELLA, M. RIEDLE-BAUER, A. EICHMEIER
16:10 - 16:15	**P.45**	**Biological and physical protection of grapevine propagation material from trunk disease pathogens** E. ABARQUERO, M. P. MARTÍNEZ-DIZ, A. DÍAZ-FERNÁNDEZ, Y. BOUZAS, R. BUJANDA, D. GRAMAJE, E. DÍAZ-LOSADA
16:15 - 16:20	**P.46**	**Composition of phytopathogenic fungal communities in grapevine leaves differ among sampling months, but not between organic and conventional management** C. M. LEAL, A. GEIGER, J. GEML

16:20 - 16:25	**P.47**	**Bismuth Subsalicylate, a fungistatic compound and plant defenses stimulator, with potential for the treatment of grapevine trunk diseases** L. MERLEN, C. TARNUS, C. DELAITE, M. GELLON
16:25 - 16:30	**P.48**	**Trunk BioCode – A deep metagenomic study of Grapevine Trunk in Portuguese vineyards and biosensor adaptation** F. AZEVEDO-NOGUEIRA, A. GASPAR, C. REGO, H. M. R. GONÇALVES, A. M. FORTES, D. GRAMAJE, P. MARTINS-LOPES
16:30 - 16:35	**P.49**	***Sporocadaceae* species associated with grapevine trunk diseases in Cyprus** G. MAKRIS, M. CHRISTOFOROU, L. KANETIS
16:35 - 16:40	**P.50**	**Grapevine trunk diseases of cold-hardy varieties grown in Northern Midwest United States vineyards coincide with wounds and winter injury** D. H. DeKREY, A. E. KLODD, M. D. CLARK, R. A. BLANCHETTE
16:40 - 16:45 **VIRTUAL**	**P.51**	**The effect of dual inoculation (*Seimatosporium* species with/without GTD fungi) on lesion length (symptom expression) in Sauvignon Blanc vines** N. BESSELMA, H. J. RIDGWAY, E. E. JONES

19:00 – 23:00 **12th IWGTD BANQUET**

ABSTRACTS OF THE 12th IWGTD

ORAL PRESENTATIONS

1.1 Investigating the causal agents of Sudden Vine Collapse in California.

A. ESKALEN[1], E. HARDY[1], K. ELFAR[1], M. BUSTAMANTE[1], M. AL-RWAHNIH[1], C. STARR[2], S. BOLTON[3], N. MCROBERTS[1], M. BATTANY[4], L. BETTIGA[5], K. ARNOLD[6] and G. ZHUANG[7].

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Since 2011, grape growers in the San Joaquin Delta, Central Valley, and Coastal counties of California have reported Sudden Vine Collapse (SVC), in which patches of vines within the vineyard, especially the ones grafted on virus-sensitive rootstocks (Freedom, 039-16 and 101-14, among others), quickly die with no apparent cause. In some cases, dying patches are so large that can be seen via satellite images, with levels of loss that have caused growers to remove entire vineyards. In 2018, the disease reached an economic threshold of destruction that was affecting an increasing number of growers, therefore gaining greater attention. In this study, four vineyards exhibiting SVC in the San Joaquin Delta were sampled over July 2019. Samples were collected from the roots, rootstock, scion, cordon, spurs, and leaves of the selected vines. Samples from each tissue were plated and cultured on PDA amended with tetracycline. Isolated fungi were identified using molecular techniques. Additionally, leaf and rootstock samples were analyzed using high-throughput sequencing to characterize the microbial profiles, including viruses. Moreover, an iodine test was performed to evaluate the starch content and therefore estimate the extent of girdling at the graft union. Results showed that, on each vine with severe symptoms, both *Grapevine leafroll-associated virus 3* and a vitivirus, *Grapevine virus A* or *Grapevine Virus F*, were present. A myriad of Grapevine Trunk Disease (GTD) pathogens was isolated from both healthy-looking and symptomatic. These included *Botryosphaeriaceae*, *Fusarium* sp., and *Diaporthe* sp., among others. However, no single fungal pathogen was consistently found in affected

grapevines. Allegedly, the efforts of the rootstock to reject the scion following infection causes girdling at the graft union, preventing the flow of water and nutrients throughout the vine. Therefore, the inability of the plant to transport carbohydrates leads to starch depletion in the roots and a subsequent lack of feeder roots, further preventing the vine from uptaking nutrients from the soil. All these factors contribute to a quick collapse of the vine. In conclusion, we hypothesize that SVC is not caused by a single pathogen but is the result of a disease complex in which vines are predisposed to root stress due to co-infections by a leafroll virus (*GLRaV-3*), vitiviruses (*GVA, GVF*) and possibly others. Consequently, infected vines rapidly die by an additional infestation of fungal pathogens associated with grapevine trunk diseases.

1.2 Aspergillus Vine Canker: An overlooked canker disease of grapevine in California.

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Grapevine canker diseases are commonly associated with fungal pathogens of the *Botryosphaeriaceae*, *Diatrypaceae*, and *Diaporthaceae* families. These pathogens have been found and described in many cultivars worldwide. Symptoms include internal wood necrosis, stunted or poor shoot development after the budbreak, and dieback of cordons or the entire vine. Cankered tissues may also exhibit dark fruiting bodies (pycnidia and perithecia) on the surface, which are responsible for releasing the spores that will lead to further infections in the vineyard. A different wood canker disease was first detected in the San Joaquin Valley in 1989, affecting excessively vigorous young 'Red Globe' grapevines. Since then, the disease has been observed on different cultivars, including Chardonnay, Grenache, and Crimson Seedless. The pathogen was morphologically identified *Aspergillus niger,* a member of the group of black aspergilli, or *Aspergillus* section *Nigri*, and the disease was named as Aspergillus Vine Canker. From 2003 to 2010, Aspergillus vine canker was detected and monitored in Italy, affecting a number of table grape cultivars on different growing regions. Recently, through a collaboration with UC Cooperative Extension farm advisors, we identified Aspergillus vine canker on Grenache and Malbec cultivars in Fresno and Sonoma counties, respectively. Symptomatic vines tested negative for viral infections. Affected vines are easily distinguishable by their premature senescent leaves during the fall, while healthy vines are still green. Our lab has been focusing on the specific identification of the causal agent of Aspergillus vine canker in California using molecular tools, particularly by constructing phylogenetic trees using DNA sequences of the calmodulin- gene. Preliminary data suggest that our isolates correspond to *Aspergillus tubingensis*, a closely related species to the previously identified *A. niger.* We are currently studying the phylogenetic relationships between *Aspergillus* isolates from wood cankers and sour rot berries to understand accurately the etiology and epidemiology of both diseases.

1.3 Development of loop mediated isothermal amplification (LAMP) assays for rapid detection of Eutypa and Botryosphaeria dieback pathogens.

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Eutypa dieback (ED) and Botryosphaeria dieback (BD) pathogens are prevalent in Australian vineyards causing significant yield reduction and threatening the sustainability of vineyards. Current diagnostics for ED and BD rely primarily on fungal isolations and PCR-based techniques that are time-consuming, labour intensive or require expensive equipment, and highly skilled staff to perform the analysis. Thus, rapid and simple DNA-based methods designed for on-site diagnostics of infected grapevine plant materials allows testing for ED and BD pathogens in low resource environments. In this study, we developed real-time LAMP assays using the Genie II instrument to detect ED and BD pathogens in infected plant materials. Five species-specific and two genus-specific LAMP assays were developed for ED and BD pathogens, respectively. A low cost and simple DNA extraction protocol was also developed for LAMP and was found highly suitable for rapid DNA extraction from infected wood. All seven real-time LAMP assays were highly specific and suitable for detecting and discriminating different ED and BD pathogens using gBlocks, genomic DNA and crude DNA extracted from infected plant materials. All assays can detect as low as 1-2 pg of fungal DNA alone or in combination with plant DNA. The LAMP assays were at least two times more sensitive compared to conventional fungal isolation but were less sensitive compared to existing qPCR methods. The development of these LAMP assays was shown to be rapid and sensitive in detecting ED and BD pathogens from infected plant materials. LAMP assays offer cost effective, simple, and robust diagnostic tools for assessment of infected plant materials in the field or in low resource environments.

1.4 Species diversity of Diatrypaceous airborne spores in Australian vineyards.

R. BILLONES-BAAIJENS[1], M. LIU[1,2], M. R. AYRES[3], M. R. SOSNOWSKI[3,4] and S. SAVOCCHIA[1,2].

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Eutypa dieback (ED) is considered an important grapevine trunk disease in Australia, causing significant yield reduction, and threatening the sustainability of vineyards. *Eutypa lata* and other Diatrypaceous fungi produce ascospores that infect primarily through pruning wounds resulting in cankers, dieback and eventually death of vines. Understanding the prevalence and distribution of these Diatrypaceous airborne spores in vineyards will assist in elucidating their importance in disease spread and in developing subsequent disease management strategies. This study analysed more than 4800 DNA samples collected using Burkard spore traps from eight wine growing regions over eight years, using multi-faceted molecular tools to investigate the diversity and abundance of Diatrypaceous species in Australian vineyards. A multi-target quantitative PCR assay using SYBR green detected Diatrypaceous spores from 21% of the samples analysed with the spore numbers and frequency of detection varying between regions. Subsequent Sanger sequencing of amplified Diatrypaceous DNA identified seven Diatrypaceous species with *E. lata* being the most prevalent in South Australia, while *Eutypella citricola* and *Eu. microtheca* were frequently detected only in New South Wales. Two new species, *Diatrype stigma* and *Cryptosphaeria multicontinentalis* were also identified in high frequency with *D. stigma* being detected in several regions. The data generated from samples analysed to date showed high species diversity of Diatrypaceous spores that were trapped across different wine growing regions in Australia. Data continues to be generated from spore traps deployed across Australian wine regions, so this study will further elucidate the critical times of the year when ED spores are abundant in vineyards. This will provide localised data for each region that will assist growers in making decisions for optimal timing of pruning and wound protection in their vineyards.

1.5 Implementation of droplet digital PCR to determining the health status and abundance of grapevine trunk disease fungi in ready-to-plant nursery material imported into Canada.

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Fungi associated with Botryosphaeria dieback, black foot, and Petri disease are prevalent in the asymptomatic and symptomatic inner tissues of nursery material and in young vineyards. Canada is reliant on the import of ready-to-plant grapevines for industry growth, however, there are currently no studies investigating the health status of nursery material imported for use in Canada. Five nurseries, which import ready-to-plant grapevine material into Canada were selected to identify the most prevalent grapevine trunk disease (GTD) fungi present and their abundance. Fifteen plants each of 'Chardonnay', 'Merlot' and 'Pinot Noir' grafted on '3309C' rootstock were analyzed from four nurseries. The same number of self-rooted 'Chardonnay', 'Merlot' and 'Pinot Noir' plants were analyzed from one nursery. Tissue samples were collected from the roots, base, graft-union (in grafted plants), and scion from each plant. Primers were either adapted or newly developed to be used in droplet digital PCR (ddPCR) to determine the quantities of *Botryosphaeriaceae* spp., *Cadophora luteo-olivacea*, *Ilyonectria* spp., *Dactylonectria torrensensis*, *Phaeoacremonium minimum*, and *Phaeomoniella chlamydospora* from each plant part. Traditional re-isolations were conducted from each plant part tissue and isolated fungi were identified by means of morphological and molecular studies. Internal necrosis was measured at the base of each plant to further understand how the level of infection may correlate with symptoms' expression. Results revealed all plants used in this study were infected with at least one of the above mention GTDs fungi. Significant differences were found on pathogen concentration among plants within the same cultivar as well as between cultivars from the same nursery. Significant differences were observed on GTD fungi present among nurseries. GTDs fungi and their concentrations significantly varied among the different parts of the plant. Overall, the base and graft-union part were found to contain the highest quantity of GTD fungi.

1.6 Rapid detection of *Neofusicoccum parvum* using Loop mediated isothermal amplification (LAMP).

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The pathogen *Neofusicoccum parvum* belongs to the group of fungi associated with grapevine trunk diseases (GTD) which represents one of the most important economic problems for viticulture in Chile. Sanitary measures such as early detection of these pathogens represent the most efficient way to mitigate and control infections caused by this fungus. Currently, qPCR represents the most widely used tool due to its sensitivity; however, it represents a high economic cost at the time of intensifying and massifying these detection system to early detection practices. LAMP is a low-cost technique that amplifies DNA with high specificity, efficiency, and speed under isothermal conditions, being a very useful tool for the massification and intensification of grapevine trunk fungi detection in plants in greenhouse. In the present research, we developed a set of LAMP primers for the early detection of the pathogen *Neofusiccocum parvum* based on the locus MAT. The analysis of the sequencing products obtained from the MAT 1-2-1 gene of the MAT 1-2 idiomorph of the Chilean isolates allowed us to identify new distinctive regions of the species. In addition, results on visualization of the products obtained after LAMP amplification by colorimetry and electrophoresis, showed that we detected the pathogen *Neofusicoccum parvum* in only 35 min of reaction at 65 °C. This promising technique could be implemented in sanitary evaluations for the early detection of plant pathogens in high intensity production systems.

2.1 Botryosphaeria dieback is a major component of grapevine trunk disease complex in Oregon vineyards.

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Western states including California, Oregon, and Washington are the major wine grape-producing regions in the United States. While reports of grapevine trunk diseases (GTDs) are available from neighbouring states, there is limited information available on the extent of GTDs prevalence in Oregon vineyards. In this study, we characterized the types of GTDs in Oregon vineyards, and the effect of two distinct geographical regions on spore release by *Botryosphaeriaceae* spp. Northern Oregon has comparatively wetweather conditions with annual average rainfall of 1050 mm whereas, southern Oregon has dry weather conditions with annual average rainfall of 500 mm. Trunk tissue samples were collected from 29 vineyards in both regions during the fall of 2019 and 2020. Fungal species were identified through culture and PCR based methods. The identified GTD pathogens included *Botryosphaeriaceae* spp. and *Phaeoacremonium* spp. from 72 and 21 % of the surveyed vineyards respectively; *Phaeomoniella chlamydospora*, *Cryptovalsa ampelina*, *Truncatella angustata*, *Seimatosporium lichenicola*, *Hormonema viticola* from 7 % of the surveyed vineyards; and *Dactylonectria macrodidyma*, and *Pestaloptiopsis* sp. from 3 % of the surveyed vineyards. Pathogens were detected in both regions and in young and mature vines. The presence of GTD pathogens from the Esca disease complex was significantly affected by vineyard age (P=0.02) with pathogens being significantly more abundant in older vineyards compared to younger vineyards. *Botryosphaeriaceae* spp. were the most commonly detected species and their spore release were affected by the region. In northern Oregon, the spore detection occurred between December and February. In southern Oregon, the detection occurred between November and January. This study provides insight on common GTD pathogens in Oregon vineyards and their spore release during the critical months of vineyard pruning, which is important in implementing preventative disease management practices to prevent and mitigate the disease.

2.2 Carbohydrate-active enzymes and secondary metabolite production using two lignocellulosic biomass on *Neofusicoccum parvum* Bt-67, a grapevine trunk pathogen.

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Neofusicoccum parvum is one of the most aggressive *Botryosphaeriaceae* species associated with grapevine trunk diseases. This species may secrete enzymes capable of overcoming the plant barriers, leading to wood colonization. In addition to their roles in pathogenicity, there is an interest in taking advantage of its carbohydrate-active enzymes (CAZymes), related to plant cell wall degradation, for lignocellulose biorefining. Furthermore, *N. parvum* produces toxic secondary metabolites that may contribute to its virulence. In order to increase knowledge on the mechanisms underlying pathogenicity and virulence, as well as the exploration of its metabolism and CAZymes for lignocellulose biorefining, we evaluated *N. parvum* Bt-67 capacity in producing lignocellulolytic enzymes and phytotoxic secondary metabolites when grown *in vitro* on two biomasses: grapevine canes (GP) and wheat straw (WS). For this purpose, a multiphasic study combining enzymology, transcriptomic and metabolomic analyses was performed. Enzyme assays showed higher xylanase, xylosidase, arabinosidase, and glucosidase activities when the fungus was grown on WS in contrast with GP. Fourier Transform Infrared (FTIR) spectroscopy confirmed the lignocellulosic biomass degradation caused by the secreted enzymes. Transcriptomics indicated that the *N. parvum* Bt-67 gene expression in the presence of both biomass was similar. In total, 134 genes coding CAZymes were up-regulated, where 94 of them were expressed in both biomass growth conditions. Lytic polysaccharide monooxygenases (LPMOs), glucosidases, and endoglucanases were the most represented group of CAZymes and correlated with the enzymatic activities obtained. The secondary metabolite diversity, analyzed by HPLC-UV-Vis-MS, was variable depending on the carbon source. Preliminary results suggest that, contrary to enzymatic activities, secondary metabolites content was higher when *N. parvum* Bt-67 was grown with GP. Overall, these results provide insight into the influence of lignocellulosic biomass on virulence factor expression. Moreover, this study opens the possibility of optimizing the enzyme production from *N. parvum* with potential use for lignocellulose biorefining.

2.3 Exploration of a Non-Enzymatic Wood Degradation Pathway in *Fomitiporia mediterranea,* the Main White Rot Agent in European Esca-Affected Vineyards.

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Fomitiporia mediterranea (Fmed) is a basidiomycete identified as a white rot agent, historically associated with Esca in European vineyards. Although described as a white rot agent, its biomolecular wood degradation mechanisms are not yet fully understood. According to the white rot definition, it possesses an enzymatic pool (laccases, Class II peroxidases, carbohydrate-active enzymes) capable of attack and break down all components of lignocellulosic biomass (cellulose, hemicellulose and lignin), leaving grapevine wood as fibrous bleached residues. However, comparative genomics studies and other observations on wood cell microvoids size retrieved in the literature, allowed us to formulate the hypothesis that Fmed could adopt both non-enzymatic and enzymatic mechanisms to degrade grapevine wood. Our hypothesis is based on the CMF (Chelator Mediated Fenton) model, proposed in the late 1990s by American researchers for brown rot fungi. According to our results, under appropriate experimental conditions as close as possible to the physiological conditions of grapevine wood, Fmed demonstrates *in vitro* the ability to: *i*) produce low molecular weight (LMW) iron-chelating compounds, that can *ii*) reduce ferric iron to ferrous iron, and *iii*) redox cycle to produce hydroxyl radicals, thus satisfying all the conditions for a CMF-like non-enzymatic wood degradation mechanism. Moreover, carbohydrate oxidative experiments showed that reducing sugars are liberated when cellulose and hemicellulose are treated with the CMF reaction induced by Fmed LMW extracts. Finally, variations in the CMF reaction were observed among different Fmed strains. Further research is ongoing to study the non-enzymatic pathway effectiveness *in lignum* and *in planta*.

2.4 Analyzing the presence of trunk diseases fungi in heritage grapevines of Baja California.

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In Baja California, Mexico, vineyards with plants over 50 years old established without irrigation can be found. They derive from the first vines acquired by the Dominican missionaries around 1697. They can be called heritage vines. Mision cultivar stands out among them. It has very vigorous foliage, high resistance to drying out, and vigorous and long shoots. Currently, there are less than 38 hectares of heritage vines in Baja California. These plants are established in dry conditions, with rainfall of less than 250 mm per year and with little or no pesticides and fertilizer applications. Regarding the climate change scenarios that predict less rain with increases in temperature in the area, these vines are being revalued by some viticulturists. Therefore, it is important to know the pathogens present that could limit their establishment. The objective of this work was to analyze trunk disease fungi in heritage vines. For this, wood samples were collected in eight vineyards from plants with symptoms of dieback, dead arms, or cankers. Using PDA and water agar media, 78 fungal isolates were recovered, including 18 with morphological characteristics similar to trunk diseases fungi. Characterization was performed by microscopy and molecular identification of ITS and EF1-α fragments. Isolates belonged to *Cytospora*, *Diaporthe*, and *Diplodia* genera. *Diplodia seriata* was the most abundant species, followed by *Diplodia pinea, Diaporthe ampelina* and *Cytospora parasitica*. Other identified fungi were: *Alternaria* sp., *Aspergillus* sp., *Chaetomium* sp., T*richoderma asperellum* and *Sordaria fimicola*. Koch postulates are underway.

2.5 The most important fungal pathogens responsible of grapevine trunk disease (GTD) in south Italy.

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Grapevine cultivation in Italy has always been affected by biotic diseases affecting all parts and organs of vines including roots, trunks, cordons, leaves and berries. During the last 50 years, several studies carried out on grapevine diseases highlighted the presence of a conspicuous fungal flora responsible of decline of vineyards and decrease in yield. In particular, the studies carried out in Apulia region, in south Italy, during the last 30 years, reported *Phaeoacremonium* spp., *Phaeomoniella chlamydospora*, *Botryosphaeriaceae* spp., *Pleurostoma richardsiae*, some fungal agents of black foot (*Dactylonetrica*, *Ilyonectria*, *Thelonectria* species), and other fungi considered minor because less isolated such as *Seimatosporium*, *Truncatella*, *Cadophora* and *Colletotrichum* species to name a few. The isolation frequencies of above fungi, associated with different symptoms, external and internal, with organ kinds such as roots, trunks, branches, are variable due to the age and cultivar of vineyards used in surveys. In the present study fungal pathogens were isolated from wood with varying symptoms including asymptomatic samples. The majority, 45 - 50 %, of the wood tissue pieces were infected with mainly *Botryosphaeriaceae* species; 8-15 % of wood tissue pieces with *Phaeoacremonium* species; 3-10 % with *Phaeomoniella chlamydospora*; 8-10 % with black foot fungi, and the remaining 15 % with other fungi. The same fungal species were also isolated from asymptomatic wood tissue pieces although with lower isolation frequencies, allowing to consider that they could act as hemibiotrofic fungi during the first years of grapevines. The pathogenicity tests carried out during our studies confirmed the ability of all fungi to cause infections on grapevines with in vivo conditions. On the basis of these results, further studies are necessary in order to understand which conditions, environmental, geographic, cultivars etc. could switch the behaviour of above fungi from hemibiotrofic to pathogenetic.

2.6 4D imaging at the bedside of grapevines: towards non-destructive diagnosis of trunk diseases.

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Worldwide, vineyards sustainability is threatened by grapevine trunk diseases (GTD), which spread insidiously, irreversibly degrading internal trunk tissues and ultimately entailing vine death. Foliar symptoms can appear erratically, but the sanitary status of vines cannot be ascertained without injuring the plants. To tackle this challenge, a novel approach was developed based on medical multimodal 4D imaging and Artificial intelligence (AI)-based image processing that allowed a non-invasive GTD diagnosis. Each imaging modality contributed differently to tissue discrimination, and we identified quantitative structural and physiological markers characterizing wood degradation. The combined study of the anatomical distribution of degraded tissues and the foliar symptom history of plants collected in a vineyard in Champagne, France, demonstrated that white rot and intact tissue contents were key measurements. We finally proposed a model for an accurate GTD diagnosis. This work opens new routes for precision agriculture by permitting field monitoring of GTD and surveying plant health *in situ*.

3.1 Managing Esca in Bordeaux: An economic analysis of curative practices under appellation yield risk and uncertain disease severity.

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An increase in the incidence of GTDs over the last 10-15 years has been reported worldwide. In France, yield losses are estimated at 4.6 hl/ha/year due to Esca, the main GTD affecting grape vines in France. Despite the threat, many grapevine growers are reluctant to adopt field-tested preventive and curative practices. In our study, we combined an epidemiological model of Esca infection for representative vineyards in AOC Entre-Deux-Mers, AOC Pauillac, and AOC Cognac developed at INRAE with an economic decision-making model to analyze two curative practices (curettage and complantation). Within this combined nature-human model, we analyzed how risk preferences influence the grower's decision to implement a curative practice to reduce the incidence of Esca and achieve appellation yield requirements. We considered different decision criteria based on risk preferences and practice efficiency, and compared outcomes across practices, risk preferences, and efficiency to identify preferred strategies and the incremental value of high practice efficiency over low efficiency. Our simulations showed that the decision of the grapevine growers to implement a practice mostly favors curettage over complantation and doing nothing and is dependent on risk preference. We showed with the AOC Pauillac that adopting curettage generates a maximum cumulative loss over 40 years of 239,257 €/ha instead of 514,615 €/ha with the decision of inaction (when practice efficiency is high). Also, the results show that a high practice efficiency generates an additional gain of 67,147 €/ha over 40 years. These results suggest non-economic factors may be discouraging growers from adopting these curative practices or a lack of knowledge about the economic gains may exist. Providing growers with evidence of the economic benefit could lead to greater adoption, and a more profit and sustainable wine industry in France. In addition, investments in practice efficiency can have substantial benefits, especially in high-valued wine grape producing regions.

3.2 Variation of mycorrhizal colonization in plants with canopy symptoms of Esca disease.

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Esca disease is one of the most important diseases of grapevine worldwide. The disease is associated with a list of fungal pathogens, among which *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum* and *Fomitiporia mediterranea* are the most frequent. The onset of disease can be affected by several factors, including the cultivar, the rootstock, weather conditions, the trellising system and phytosanitary management. However, the effects of stress may have a role in disease onset, which has yet to be fully clarified. Intensive investigations were carried out on the microorganisms affecting the canopy, in particular on trunk and branches, while relatively poor information is available on the colonization of the root system by both pathogens and putatively beneficial microorganisms. Our investigation aimed to use molecular tools to identify the microorganisms associated with the roots of symptomatic plants, compared to apparently symptomless vines, in three vineyards of the Marche region of central-eastern Italy. The arbuscular mycorrhizal fungi (AMF) colonization intensity showed higher values in grapevines with symptoms of Esca (ranging from 24.6 % to 61.3 %) than in neighboring asymptomatic plants (ranging from 17.4 % to 57.6 %). Specific primer pairs for native *Funneliformis mosseae* and *Rhizophagus irregularis* AMF species allowed us to gain a higher number of DNA copies (67 vs 47 and 292 vs 201, respectively in 20 µl) of both fungal species in Esca symptomatic vines. Therefore, our investigation suggests a possible relationship between Esca and native AMF in grapevine.

3.3 Diversity and pathogenicity of fungi causing GTDs in Chilean patrimonial vineyards.

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Trunk diseases are a major problem in Chilean commercial vineyards, reducing their productivity, quality and longevity. However, in ancient patrimonial vineyards, mostly from the cultivars País, Moscatel, Carignan, País, Torontel and Moscatel, which are being recently rescued to make historical wines, the problem is beginning to be studied. Preliminary identifications were performed for 235 fungal isolates from affected plants collected in Cauquenes and Itata valley. The objective was to determine the identity of the most frequently isolated species and the pathogenicity of representative isolates on grapevine. Isolates were macro and microscopically studied on PDA after 14 days of incubation at 25 °C. To confirm the identity of the isolates, DNA was extracted from pure cultures on PDA and genes were amplified depending on the morphological identification. Plants were produced rooting 1-year old healthy canes cv. Petit Syrah in tap water amended with 500 ppm of indole butyric acid. Pathogenicity was assessed inoculating fresh cuts with 0.5 cm diameter mycelial plugs of actively growing colonies on PDA of the species *Arambarria destruens*, *Seimatosporium vitifusiforme*, *Diplodia seriata*, *D. mutila* and *Neofusicoccum parvum*. Plants (n=7) were incubated at 25 +/- 3 °C for 60 days in flowing tap water. After the incubation period, bark was removed, and the necrotic lesions were measured and compared (Tukey <0.05). The results showed differences in virulence levels among species and isolates from the same species. *Botryosphaeriace* species *N. parvum, D. seriata* and *D. mutila* were the most virulent, followed by *S. vitifusiforme* and *A. destruens*.

4.1 Establishment of a cell model to study molecular interactions between grapevine and Esca-associated pathogens.

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Esca is a complex and poorly understood trunk disease which causes important economic losses in vineyards. Genetic solution or biostimulation are the main approaches being considered to achieve vineyard protection. Thus, the characterization of molecular events occurring throughout the first steps of the interactions between grapevine cells and Esca-associated pathogens is a prerequisite, but impossible to perform directly in the vine trunk. We have developed different cell strategies: calli and stomata (specialized structures consisting of two guard cells surrounding a pore) to study these interactions. The guard cell, a model for the characterization of cell signaling, perceives stimuli and respond by inducing the opening/closing of stomata, a parameter that can be quantified. Assays on epidermal peels reveals that guard cells from *Arabidopsis thaliana* and *Vitis vinifera* can detect and respond to Esca-associated pathogens. Additionally, using pharmacological and genetic approaches, we explored the involvement of ethylene signaling in the cell response to pathogens. Overall results showed that, guard cell and stomatal responses are efficient and promising tools to establish a cell pathosystem model for characterize and explore molecular events that occur during grapevine and Esca -associated pathogens.

4.2 Diversity of *Neofusicoccum parvum* for the production of the phytotoxic metabolites (-)-terremutin and (R)-mellein.

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Plant pathogens have evolved various strategies to enter hosts and cause diseases. Particularly *Neofusicoccum parvum*, an aggressive member of the Botryosphaeria dieback consortium, can secrete the phytotoxins (-)-terremutin and (R)-mellein during grapevine colonization. Although an exogenous supply of purified phytotoxins to plantlets was shown to weaken grapevine immunity, their contribution to Botryosphaeria dieback symptoms in lignified plants still remains unknown. Two N*eofusicoccum parvum* isolates and a UV mutant were thus characterized for their phytotoxin production *in vitro*, their pathogenicity on grapevine, and their genome sequenced. Isolate Np-Bt67 produced high level of (-)-terremutin, but almost no (R)-mellein, and it was the most aggressive on grapevine, triggering apoplexy. Similar symptoms were not induced by purified (-)-terremutin. Isolate Bourgogne S-116 (Np-B) produced 3-fold less (-)-terremutin and high amounts of (R)-mellein, but it was less aggressive on grapevine than Np-Bt67. The UV9 mutant obtained from Np-B (NpB-UV9) no longer produced (-)-terremutin but overproduced (R)-mellein by 2.5-fold, and it was as pathogenic as its parent. NpB-UV9 differed from its parent by simple mutations in two genes (transcription factor UCR-NP2_6692, regulatory protein UCR-NP2_9007), not located neither near (R)-mellein, nor (-)-terremutin biosynthetic genes, but likely involved in the control of (-)-terremutin biosynthesis. Grapevine immunity was disturbed upon challenge with these pathogens or purified phytotoxins, leading to an upregulation of SA-dependent defenses, while (-)-terremutin interfered with host JA/ET-dependent defenses. Our results suggest that neither (-)-terremutin nor (R)-mellein alone is essential for the pathogenicity of *N. parvum* on grapevine, since a (-)-terremutin non-producing mutant isolate which overproduced (R)-mellein *in vitro* was as pathogenic as the parent isolate. However, these phytotoxins could play a quantitative role in the infection process.

4.3 The beneficial effects of *Bacillus subtilis* PTA-271 and *Trichoderma atroviride* SC1 against Botryosphaeria-dieback pathogen *Neofusicoccum parvum* may vary with grapevine cultivar.

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Grapevine trunk diseases (GTDs) threaten viticulture worldwide. Biocontrol strategies may serve as alternatives to better cope with environmental concerns over pesticides. A combination of biological control agents (BCAs) may be more effective than one BCA alone. In this study, we evaluated the combination of *Bacillus subtilis* (Bs) PTA-271 and *Trichoderma atroviride* (Ta) SC1 for the protection of *Vitis vinifera* 'Chardonnay' and 'Tempranillo' rootlings against *Neofusicoccum parvum* Bt67, an aggressive species that causes Botryosphaeria dieback (BD). Indirect effects on the host of each BCA alone and in combination were characterized *in planta*, as well as their direct effects on the pathogen *in vitro*. Results suggest that: (1) the cultivar contributes to the beneficial effects of Bs PTA-271 and Ta SC1 against *N. parvum*, and that (2) the *in vitro* BCA mutual antagonism switches to the strongest fungistatic effect toward *N. parvum* in a three-way confrontation test. We also report for the first time the beneficial potential of a combination of BCAs against *N. parvum*, especially in Tempranillo. Our findings highlight a common feature for both cultivars: salicylic acid (SA)-dependent defences were low in plants protected by the BCA, in contrast with symptomatic plants. We thus suggest that the high level of expression of SA-dependent defences in Tempranillo is associated with its susceptibility to *N. parvum*. Nonetheless, the cultivar-specific responses to the BCAs require further investigation.

5.1 Susceptibility of pruning wounds to grapevine trunk disease pathogens in different Australian climates.

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Grapevine trunk diseases (GTD), caused by fungal pathogens that infect pruning wounds, pose a major threat to vineyard sustainability. Reports from different regions around the world indicate that there is variation in the duration of susceptibility of pruning wounds to infection at different times during the pruning season. A series of wound susceptibility trials were conducted from 2013 to 2019 in three climatically different regions of Australia; McLaren Vale (MV) SA (warm-dry), Adelaide Hills (AH) SA (warm-wet) and Big Rivers (BR) NSW (hot-dry) using cvs Shiraz (MV and AH) and Cabernet Sauvignon (BR). One-year-old canes were pruned in early (June), mid (July) and late (August) winter, followed by inoculations at weekly intervals for up to 16 weeks with the GTD pathogens *Eutypa lata* (Eutypa dieback) and *Diplodia seriata* (Botryosphaeria dieback). Wounds were highly susceptible to infection by both pathogens immediately following pruning, after which the susceptibility decreased rapidly over the following 14 days. From 21 days post-pruning, susceptibility was generally negligible, similar to non-inoculated controls, apart from *D. seriata* in BR, where susceptibility remained higher, including in non-inoculated controls. For *E. lata*, wounds were less susceptible to infection when pruned in late winter in AH, but susceptibility was similar across the pruning times in MV. Wounds were generally more susceptible to infection by *D. seriata* in BR than in AH. These results indicate that, in AH, delaying pruning to later in the dormant season could reduce the risk of *E. lata* infection. The greater susceptibility to *D. seriata* in BR is likely due to its prevalence in the hot-dry region, and the higher inoculum doses used, compared to that of the warm-wet region of AH, where *E. lata* is prevalent. These results also highlight the importance of wound protection for the 2-week period of greatest susceptibility following pruning.

5.2 Characterisation of the endomicrobiome of grapevine nursery plants in Australia.

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The plant microbiome is the community of microorganisms that may form symbiotic, commensal, or pathogenic relationships with their host. A well-balanced and highly diverse microbiome plays an important role in increasing plant fitness by facilitating adaptation to changing environmental conditions. In this study, the endophytic microorganisms present in grapevine nursery plants were characterised, with a particular focus on grapevine trunk disease pathogens, to understand the dynamics associated with the health and fitness of planting materials and young vines in vineyards. Total DNA was extracted from inner tissues and roots of dormant grafted and own-rooted vines collected from four grapevine nurseries in Australia. All DNA samples were analysed utilising high-throughput amplicon sequencing targeting the 16S rRNA gene and ITS sequences. Preliminary results demonstrate microbial communities varied greatly between nurseries, grape varieties, and tissue-type. One nursery had higher overall microbial populations compared to the other three nurseries. Ramsey had the highest microbial population among rootstocks, while microbial populations were similar for Shiraz and Chardonnay. Microbial populations were also higher in the roots than in the above ground tissues. For the bacteria, the most predominant families were *Pseudomonadaceae* (30 %) and *Enterobacteriaceae* (22 %) some of which are considered harmless or symbionts, although some species are also known to be enteric microorganisms. The most abundant fungal taxa are considered common environmental species and beneficials, including *Trichoderma* spp. and mycorrhizal fungi. Of the grapevine trunk disease (GTD) pathogens, the genera *Phaeoacremonium, Cadophora* and *Eutypella* were most abundant followed by *Ilyonectria*, *Phaeomoniella*, *Fomitiporia* and *Cryptovalsa*. Further investigations are required to understand the importance of these GTD pathogens in grapevine nursery plants and their potential impact in vineyard establishment and longevity in Australia.

5.3 Deciphering transcriptomic and metabolomic early host responses in grapevine wood to Esca pathogens *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*.

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The early host responses to two Esca pathogens, *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*, were compared, based on transcriptomic and metabolomic analyses. The aim of the study was to characterise the host responses in the grapevine wood to either pathogen alone. Transcriptomic analysis identified specific sets of differentially expressed genes associated with each pathogen. Functional analysis of these genes revealed differences mainly in “Signaling”, “Hormonal signaling” and “Biotic stress response”. Metabolomic analysis highlighted a group of flavonoids and stilbenoids that were overproduced in inoculated plants, compared to non-inoculated plants. Further, metabolomic analysis identified specific metabolites associated with each pathogen. For instance, *P. minimum* inoculation was associated with the accumulation of a lipophilic flavonoid cluster. Thus, our findings showed different transcriptomic and metabolomic responses, depending on the pathogen. Altogether, these observations suggest that the grapevine may differently ‘perceive’ and thus respond with a different set of defences to wood colonization by *P. minimum* and *P. chlamydospora*.

5.4 Comparative transcriptomic and metabolomic responses of grape to biological control agent *Trichoderma atroviride* suggest early modifications before infection by Esca pathogens *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum.*

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The biocontrol agent (BCA) Vintec® is a commercial strain of *Trichoderma atroviride* labeled for management of Esca in grape. Several *Trichoderma* species and strains have been shown to stimulate plant-defense mechanisms against different phytopathogens. In this work, we evaluated whether the pre-inoculation with Vintec® modifies the early molecular responses in the grapevine wood to later infection by Esca pathogens *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*. Global transcriptional analysis identified clusters of genes differently regulated in the presence of Vintec®, compared to non-treated plants. Phenylpropanoid metabolism and stilbene biosynthesis-related genes were significantly represented among the genes differently expressed in the presence of Vintec®. A global metabolomic analysis identified clusters of plant compounds synthesized in response to both Vintec® and the pathogens. These compounds belong mainly to the stilbene class. Five relevant 'biomarkers' were chosen for *in vitro* evaluation of their antifungal activity on *P. chlamydospora*. The results suggest that these compounds may play a role in limiting the *in planta* development of the pathogens. Altogether, our results suggest that the efficacy of Vintec® may be associated with an early or an enhanced biochemical response to limit colonization by the pathogens.

6.1 Deciphering the susceptibility level of Greek grapevine cultivars to grapevine trunk disease pathogens through measurements of defense-related genes expression.

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Grapevine Trunk Diseases (GTDs), caused by several fungal pathogens, represent a major threat to viticultural production globally. Management of GTDs is influenced by several biotic and abiotic factors such as pathogen, geographic region, climate, cultivar, cultural practices etc. In this study, we evaluated the susceptibility of the 13 most important Greek grape wine cultivars to three GTD pathogens (*Phaemoniella chlamydospora, Phaeoacremonium aleophilum* and *Diplodia seriata*). Plants of each cultivar (n=30) were artificially inoculated and incubated in a greenhouse for 9 months. The disease severity on each cultivar was evaluated by measuring the lesion of necrotic wood tissue using a 5-scale disease index. The results revealed four highly tolerant (Limnio, Xinomavro, Robola, Kotsifali) and four highly susceptible (Vidiano, Roditis, Savatiano, Malagouzia) cultivars to the infection by the three pathogens tested. To investigate possible mechanisms of resistance to GTDs, a transcriptomic analysis was conducted on one tolerant (cv. Limnio) and one susceptible (cv. Vidiano) cultivar by measuring the expression level of 12 defense-related genes 12, 24, 48, 72 and 168 hours post-inoculation (hpi). A significant early gene accumulation (12hpi) was observed at cv. “Limnio” compared to cv. “Vidiano”, in which most of the genes were overexpressed in later time points (72 and 168 hpi). Interestingly, in the most tolerant cv. “Limnio”, several pathogenesis-related proteins were triggered (PR6, PR10, PR10.1, and chitinase). At the same time, significant accumulation was observed in genes related to biotic stress response (Glutathione S-transferase and lipoxygenase) and genes encoding enzymes involved in phytoalexins synthesis, such as stilbene synthase and phenylalanine ammonia-lyase. To the best of our knowledge, this is the first study deciphering the susceptibility of Greek grape wine cultivars to GTDs and enhance our understanding of the molecular basis of grapevine tolerance to this disease complex.

6.2 Tracking the Fungal Pathobiome Associated with Young Grapevine Decline in California Nurseries.

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Young grapevine decline can impact longevity and productivity of new field plantings, leading to decreased economic returns. Understanding the critical steps during the plant propagation phase that are conducive to the spread of fungal pathogens will allow nurserymen to develop cost effective management practices to limit disease incidence in nursery stocks. We have been conducting a survey of grapevine cuttings throughout the grapevine production process to determine how different cultural practices shape the fungal pathobiome. Identical Chardonnay and Cabernet Sauvignon clones grafted on 1103P rootstocks were collected across several nurseries at different steps of the propagation pipeline (mother field, callusing, green vines, dormant vines) for two consecutive years. Endophytic fungal community composition was assessed with culture dependent and independent (HTS) methods in three different trunk compartments comprising the root-rootstock, below the graft union, and above the graft union sections. We first profiled culturable fungi using standard isolation techniques from wood samples coupled with ITS PCR and Sanger sequencing. Second, we identified fungal community composition using metabarcoding primers designed *in silico* to target Grapevine Trunk Associated Ascomycetes (GTAA). Using these methods, many known fungal pathogens of grapevine were found including *Phaeoacremonium*, *Phaeomoniella*, *Cadophora*, *Neofusicoccum* and *Diaporthe*. A comparison of these methods confirms that the HTS approach is a more sensitive tool for pathogen detection than culturable methods. Our methods show that the differences in disease associated fungal community composition were driven by nurseries and at the different steps of the production pipeline. Our results highlight several possible infection routes during the propagation.

6.3 Esca-leaf symptoms: Histological alteration and its relation with the physiological and nutritional status.

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Manifestation of the Esca chronic form in leaves follows a pattern known as “tiger stripes”, also reported as Grapevine Leaf Stripe Diseases (GLSD). Esca is a plurennial disease, but symptoms are not regular throughout consecutive years, as the same vine may show GLSD symptoms one year but be asymptomatic the following. Esca-leaf manifestation was monitored from 2018 to 2021 in a vineyard of cv. Tempranillo located in Extremadura (Spain). This work aimed to compare grapevine leaves under four conditions: (A) green leaves from asymptomatic vines, (S) symptomatic leaves entirely affected by the disease, (SA) green leaves from vines partially affected by Esca, and (SS) symptomatic leaves from vines partially affected by Esca. Histological, physiological, chemical, and nutritional characterization of leaves was performed at the time of GLSD manifestation in 2021. Morphometric measurements showed that GLSD reduce the leaf area. The leaf damage was significantly superior on S and SS. At the histological level, leaf cross-sections showed less thickness and area in S and SS than A. Similarly, a decrease in the upper and lower epidermis and a reduction in the thickness of the palisade and spongy parenchyma were observed. Using a Dualex® leaf clip sensor, a significant decrease in chlorophyll and NBI index were found on S, while the anthocyanins index increased. No changes were found in the flavonoid content. Physiological activity measured as net photosynthesis, stomatal conductance, transpiration, and water use efficiency tends to be low in S leaves. No significant deficiencies in nutrient compositions were found among the conditions.

6.4 The physiology and drivers of Esca leaf symptom development in grapevine.

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The development of Esca leaf symptoms is an erratic and misunderstood phenomenon, which varies among grape varieties and growing regions in the world. Esca development is likely driven by multiple factors, such as pathogen populations, vine physiology, and the climatic and cultural conditions. Grapevine hydraulic functioning is at the core of the interaction between biotic and abiotic factors during vascular pathogenesis. The xylem tissue is colonized by a myriad of microbes, including the Esca pathogens. The ascent of sap is impacted by soil water availability and climatic conditions. Our research focused on exploring (*i*) the impact of Esca leaf symptom development on xylem hydraulic integrity and (*ii*) the interaction between drought, climatic conditions, and leaf symptom incidence under controlled conditions and in the field. We demonstrated that leaf symptom development was associated with hydraulic failure caused specifically by xylem occlusion and subsequent loss of hydraulic conductivity from the trunk to the leaf lamina. Xylem occlusion by tyloses was specific to Esca leaf symptoms, compared to other types of leaf premature or autumnal senescence, and varied among grape varieties. Using mature potted plants uprooted from the vineyard and naturally infected by Esca pathogens, we demonstrated that a long drought (-1MPa of predawn water potential over 3 months) inhibited Esca leaf symptom development, suggesting that vine water status plays a key role in Esca pathogenesis. We compared water relations and carbon dynamics during the combined effects of Esca and drought stress. Finally, using bi-monthly leaf symptom monitoring in 50 vineyards in southern France (over several years) and statistical modeling, we demonstrated that soil humidity, evapotranspiration, and temperature were the key drivers of Esca incidence and phenology. These findings provide new insights on the role of plant physiology and microbial communities, and their interaction with climate, as key drivers in Esca pathogenesis.

6.5 Influence of morphological traits of xylem vessels on the response to *Phaeomoniella chlamydospora* of table- and wine-grape and rootstocks.

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Phaeomoniella chlamydospora causes heavy economic losses on grapevine under field and nursery conditions. Xylem structural features might be a key determinant of sensitivity or resistance of the host plant. Here the relationship between *P. chlamydospora* infection and xylem vessels features of ten table grape and seventeen wine grape varieties or clones, as well as three rootstocks, was investigated. The observations were based on image analysis of cross sections and fungal isolation of cuttings artificially inoculated with a conidial suspension (106 conidia mL^{-1}) of the benomyl resistant-marked strain PCHCIA43.2 of *P. chlamydospora* at 30-90 days after inoculation (DAI). The average vessels diameters of table grape cultivars ranged from 58.8 ('Allison') to 70.5 µm ('Red Globe'); 'Allison', 'Sable' and 'Timco' showed higher vessel densities than 'Italia', 'Sugar Crisp' and 'Sugraone' in the diameter classes 151-180 µm and >180 µm. Vessel diameters of wine-grape varieties were in the range from 44.8 ('Nero di Troia') to 72.3 µm ('Merlot') and their densities ranged from 20.3 ('Nero di Troia') to 29.7 vessels/mm² ('Sangiovese'). In rootstock, vessel diameters were 50.8, 54.0 and 60.9 µm for 34 E.M., 140 Ruggieri and 1103 Paulsen, in the order, and their density was always lower than 25.5 vessels/mm^2. Regarding the bioassay *in planta*, at 90 DAI PCHCIA43.2 was isolated from 93.3 % of 'Timco' cuttings and 13.3 % of 'Sugar Crisp' and 'Sugraone'. These data resulted positively correlated with the frequency of large vessels. With few exceptions, on wine-grape varieties, the percentage of re-isolation was related to the frequency of large vessels or vessel density. The pathogen was isolated from 33 - 51 % of rootstock cuttings and infection data were more related to vessel densities than their size. These results highlight the relationship between the susceptibility to *P. chlamydospora* and the size and density of xylem vessels which are worthwhile of further research.

6.6 Effects of tissue age on Botryosphaeria dieback caused by *Diplodia seriata* in cv. Cabernet Sauvignon plants.

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Symptoms associated with Botryosphaeria dieback (BD) can occur in young or mature vines (>7 years in age). However, in vineyards, it has been observed that the prevalence and severity of the disease increase considerably with the age of the grapevines. In Chile, *Diplodia seriata* is the most prevalent species in vines cv. Cabernet Sauvignon. However, pathogenicity tests have been carried out in 1- to 2-year-old shoots, and no information is available about the susceptibility of mature tissues to pathogen infection. Therefore, two strains of *D. seriata* (50 µl of 1.104 conidia.ml^{-1}) were inoculated in wounds done on tissues of 1, 2, and 10 years of age in Cabernet Sauvignon plants under vineyard conditions. Lesions were compared with previous inoculations made in pieces of canes. Our results showed that *D. seriata* was significantly more aggressive in 10-year-old tissue (doubling its aggressiveness), and that grapevine inoculations were representative of damage observed in the field. This aspect must be considered when evaluating the importance of each pathogenic species causing BD, especially considering that the aggressiveness of the disease increased when the grapevines were more than seven years old.

7.1 Wood degradation by *Fomitiporia mediterranea* M. Fischer: physiologic, proteomic and metabolomic approaches.

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Fomitiporia mediterranea (Fmed) is considered as the main fungal species found in grapevine wood rot, also called "amadou", one of the most typical symptoms of the grapevine trunk disease Esca. This fungus is functionally classified as a white-rot, able to degrade all wood structure polymers, i.e. hemicelluloses, cellulose, and lignin, the most recalcitrant component. Specific enzymes are secreted by fungi to degrade those components, namely carbohydrate active enzymes for hemicelluloses and cellulose, which can be highly specific to the nature of polysaccharides, and peroxidases, which enable white-rot to degrade lignin, with specificities to lignin composition as well. Furthermore, besides polymers, a highly diverse set of metabolites often associated with antifungal activities is found in wood, this set differing among the considered wood species. Wood decayers possess the ability to detoxify these specific extractives and this ability could reflect the adaptation of these fungi to their specific environment. The aim of this study is to better understand the molecular mechanisms used by Fmed to degrade wood structure, and in particular its potential adaptation to grapevine wood. To do so, Fmed was cultivated on sawdust from different wood species: grapevine, beech, and spruce. Carbon mineralization rate, mass loss and contents in wood structure polymers, targeted metabolites (extractives) and secreted proteins were measured. We used Trametes versicolor, an ubiquitous white-rot model organism, for comparison. Whereas no significant degradation on spruce was observed, a higher mass loss was measured on Fmed grapevine culture compared to beech culture. Moreover, on both substrates, a simultaneous degradation pattern was demonstrated, and proteomic analysis revealed only few differences in secreted enzymes responsible of wood degradation. These results represent a first step in the understanding of a potential adaptation of Fmed to its ecological niche, and place emphasis on the need for further research.

7.2 Occurrence of *Phaeomoniella chlamydospora* at different stages of grapevine propagation material, pathogen biomass fluctuation from the nursery to the field and association with the endophytic microbiome.

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Grapevine trunk diseases (GTDs) pose a huge threat to grapevine cultivation worldwide. *Phaeomoniella chlamydospora* (*Pch*), associated with young grapevine decline syndrome, has been widely reported in vine nurseries and young vines. The aim of this study was to investigate the occurrence and the fluctuation of *Pa. chlamydospora* biomass at three (3) stages of propagation material and to correlate pathogen biomass with the endophytic microbiome. Dormant cuttings (1) from two Greek varieties and two rootstocks and different rootstock-scion combinations of grafted unrooted (2) and rooted vines planted for six months in the field (3), were sampled. TaqMan qPCR assays were carried out to quantify the pathogen biomass within the collected samples. The mean biomass of *Pch* for all dormant cuttings (1) was 3,94 pg.100 ng of plant DNA (or 3,94 *10-5 g *Pch* DNA.g plant DNA). In addition, the mean *Pch* biomass for all rootstock-scion combinations was 1,21 pg.100 ng plant DNA for grafted unrooted (2) and 62,14 pg.100 ng of plant DNA for rooted vines (3), indicating a 50-fold increase of *Pch* biomass from the nursery to the field. To investigate a possible correlation between the biomass of *Pa. chlamydospora* within the different types of propagation material and the endophytic microbiome, DNA samples in which the lowest and highest biomass values were detected, were pooled for Next Generation Sequencing analysis. Considering the above but also according to the literature, *Pa. chlamydospora* is detected in dormant cuttings deriving from nursery mother vines. The huge increase of pathogen biomass from the grafted unrooted vines to the grafted rooted vines shows that propagation process plays a crucial role in the dissemination and development of Petri disease. Knowledge on the endophytic microbiome during the propagation process will improve the understanding of the mutual interactions and contribute to the management of this important disease.

7.3 Response of different grapevine cultivars to infection by *Lasiodiplodia theobromae* and *Lasiodiplodia mediterranea*.

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Botryosphaeria dieback is a grapevine trunk disease that affects all viticulture regions of the world. Species of the genus *Lasiodiplodia* have been reported as pathogenic towards grapevine in several growing regions and have also been previously reported from Portuguese vineyards. Species in this genus, particularly *Lasiodiplodia theobromae*, have been reported to be more aggressive than other *Botryosphaeriaceae* species associated with Botryosphaeria dieback. The aim of this study was to assess the response of the most representative cultivars planted in Portugual, Touriga Nacional, Touriga Franca, Alvarinho, Aragonez (=Tempranillo) and Cabernet Sauvignon, by performing artificial inoculations with *Lasiodiplodia* spp. collected in different geographic locations worldwide. Two experiments, each repeated twice, were performed using: (1) two-year-old grapevines kept under controlled greenhouse conditions inoculated with six isolates of *L. theobromae* and one isolate of *L. mediterranea*; and (2) seven-year-old field-grown grapevines inoculated with two isolates of *L. theobromae*. Response of the cultivars was assessed by evaluating the lesion length caused by the isolates under study, five months after inoculation. The results showed that all isolates were able to infect the annual shoots since they were always re-isolated and produced internal wood discoloration. Significant differences were found for all isolate/cultivar combinations. For both experiments, Touriga Nacional showed the largest lesions while Aragonez recorded the smallest lesions amongst the cultivars inoculated with *Lasiodiplodia* spp. Portuguese isolates were more aggressive than those from Peru, which demonstrated to be mildly aggressive. These results give a first insight on the response of selected Portuguese cultivars to *Lasiodiplodia* species, present in Portugal, but not commonly associated with Botryosphaeria dieback. This contributes to improving the knowledge of the impacts that Botryosphaeria dieback causal agents have on crucial national cultivars, which may help winegrowers to manage current cultural practices, and also to optimize decision making when planning the establishment of new vineyards.

7.4 Development of an analytical approach using magic-angle spinning NMR for the study of molecular markers of dieback in French vineyards.

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Grapevine trunk diseases (GTDs) such as Esca or Black Dead Arm have disastrous consequences for viticulture. The economic cost of the replacement of vines is very costly. To date, neither chemical snor biological treatment has been able to eradicate GTDs since the prohibition in Europe of sodium arsenite, nor diagnostic methods for detecting GTDs in their early steps. Dieback of vine wood tissues is associated with necrosis processes that affect the chemical structure and the molecular architecture of wood. Here we present a new analytical approach to investigate at the atomic scale how wood tissues are affected by the dieback. We employed magic-angle spinning nuclear magnetic resonance (MAS NMR) to characterize the main polymer components of vine wood extracted at different dieback stages. We compared apparently healthy grapevine wood tissues and diseased wood tissues to detect and identify possible molecular markers of dieback at the atomic level. Our approach allows for the identification of molecular markers such as the cellulose cristallinity, degradation of specific carbohydrate linkages and apparition of acidic degradation products that were used to investigate wood dieback of french vineyards. Our approach will open an avenue to investigate other wood tissues and vineyards impacted by GTDs.

8.1 Drought stress results in a compartment-specific restructuring of the grapevine root-associated fungal microbiome.

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Physiological and molecular responses of grapevine to water stress have been extensively studied, however, little is known about the effect of drought on the grapevine-associated belowground fungal microbiome. The plant root supports complex microbial communities that can influence nutrition, plant growth and health. In this study, we examined the drought-induced shifts in fungal composition in the root endosphere, the rhizosphere and bulk soil by ITS high-throughput amplicon sequencing (HTAS). We imposed three irrigation regimes (100 %, 50 % and 25 % of the field capacity) to one-year old grapevine rootstock plants cv. SO4 when plants had developed 2-3 roots. Root endosphere, rhizosphere and bulk soil samples were collected 6- and 12-months post-plantation. Results showed that severe water deficit (25 %) modified the overall fungal composition of all three compartments, with the root endosphere compartment showing the greatest divergence from well-watered control (100 %). We identified a significant enrichment in several fungal genera such as the arbuscular mycorrhizal fungus *Funneliformis* within the roots at severe water deficit regime. The overall response of the fungal microbiota associated with black-foot disease (*Dactylonectria* and "*Cylindrocarpon*" genera) and the potential biocontrol agent *Trichoderma* to drought stress was consistent across compartments, being their relative abundances significantly higher at 50 - 100 % than at 25 % irrigation regime. Our results reveal that drought stress, in addition to its well-characterized effects on plant physiology, also results in restructuring of grapevine root microbial communities and suggest the possibility that members of the altered grapevine microbiota might contribute to plant survival under extreme environmental conditions.

8.2 The fungus *Aureobasidium pullulans* may promote the development of foliar symptoms on Esca-diseased grapevines.

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Esca disease is a member of grapevine trunk diseases, and it is one of the most devastating fungal infections of the grapevine. Besides the main pathogens *Phaeomoniella chlamydospora* (*Pch*), *Phaeoacremonium minimum* (*Phm*) and *Fomitiporia mediterranea*, several fungal species are associated with this syndrome. While the complex nature of Esca is well known, only the interactions between the main pathogens were studied in the context of pathogenesis and little is known about the possible role of non-pathogenic fungi. The abundance of *Aureobasidium pullulans* (*Apu*) in the healthy wood of Esca-symptomatic cv. Cabernet sauvignon grapevines were assessed by both isolation-based methods and qPCR measurements. The results revealed a positive correlation between the abundance of *Apu* and the severity of foliar symptoms (relative area of necrosis). The *in vitro* investigation of the interactions between *Apu* and *Pch* or *Phm* showed that *Phm* has a strong inhibitory effect on *Apu*, while there was a mutual antibiosis between *Pch* and *Apu* without toxic effects. Further studies of the *Apu-Pch* interactions suggested the mutual induction of sporulation and inhibition of growth which the effects are mediated by secreted molecules. Artificial infections on grapevine shoot sections with 3 *Pch* and 3 *Apu* isolates were carried out individually and in combination. Results showed that *Apu* can enhance the development of foliar symptoms on *Pch*-infected plants in a strain-dependent manner. Phytotoxicity tests with the water extracts of leaves severely damaged by *Apu+Pch* inoculations suggest that the increased symptom severity is the result of the accumulation of toxic metabolites in the co-inoculated shoots. Protein and polysaccharide secretion of the examined *Pch* and *Apu* isolates was also investigated and revealed some differences, which may explain the positive effect of some *Apu-Pch* co-infections on the development of foliar symptoms of Esca disease.

8.3 A study to characterise diversity of fungal endophytic environment of young nursery grapevine plants.

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In sharp upsurge for three decades, ESCA threatens grape production from all viticulture regions. It is caused by a complex of fungal pathogens that attacks the perennial organs of the vine. Disease symptoms vary according to the age of the plant, the severity of the infection, and the pedoclimatic context. The overarching goal of our research project is to understand the microbiome assemblage of vines from nurseries and if it could influence the pathophysiology of the Esca pathosystem. Our working hypothesis postulates that the variabilities of the vine microbiome could affect the early host defense responses against Esca causal agents (*P. minimum* and *P. chlamydospora*). The first step was to determine if there are differences in microbiome profiles of rooted grafts from different grapevine nurseries. In 2020, we initiated characterizations of the microbiomes of grafted vines from two continental regions in the US (California) and France (South-West). Plants were the same genetic materials, Cabernet-Sauvignon grafted on Richter 110, and from 2 nurseries within each region. The results presented are those obtained from North American plants. The results from the French plants are still being analyzed. The analysis already shows significant differences between the two nurseries both in terms of alpha diversity and beta diversity, thus validating the possibility of rapidly prospecting the influence of this microbiome on the responses of wood to pathogenic agents associated with Esca. The diversity of the observed microbiomes will allow us to investigate the effect of these variabilities on disease outcome.

8.4 Comparison of the grapevine-associated plant pathogenic fungal community among different microhabitats, cultivars and between healthy and Esca-diseased plants.

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Grapevine trunk diseases (GTDs) are among the major threats to the wine industry, causing yield loss and dieback of grapevines. While the increasing damage caused by GTDs in recent decades have spurred several studies on grapevine-associated pathogenic fungi, key questions about the emergence and severity of GTDs remain unanswered, such as whether differences in richness, abundance, and composition of plant pathogenic fungi exist among below- and aboveground microhabitats, among cultivars, and among asymptomatic and symptomatic Esca-affected grapevines. We addressed these questions using DNA metabarcoding of soil, bark, and perennial wood samples from asymptomatic and symptomatic grapevines of four different cultivars, two global (Chardonnay and Cabernet sauvignon) and two local (Leányka and Kékfrankos). We observed larger compositional differences in plant pathogenic fungi within grapevine plants than among them. This is driven by the dominance of GTD-associated fungi in perennial wood and to a lesser extent in bark and the dominance of non-GTD pathogens in soil, as well as by the lack of significant differences among cultivars and among asymptomatic and Esca symptomatic grapevines. These results suggest that fungi generally associated with Esca disease belong to the core grapevine microbiome and likely are commensal endophytes and/or latent saprotrophs, some of which can act as opportunistic pathogens on stressed plants. Furthermore, the role of environmental factors may be particularly important for the development of Esca disease and studies are needed to investigate the abiotic conditions on fungal compositional dynamics in Esca-affected plants.

8.5 Predicting pathogens' virulence: linking host breadth and pathogenicity of the Botryosphaeriaceae fungal family in wine grapes (*Vitis vinifera*).

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Plant diseases can have devastating effects on crops and food sources, elevating world hunger and malnutrition. Fungal pathogens are responsible for 30 % of emerging plant diseases and have caused epidemics with catastrophic consequences for ecosystems and people. Previous work has linked host breadth and pathogen emergence, illustrating important connections with the phylogeny of the plant hosts. However, the importance of the pathogen phylogeny in shaping pathogen-host association has been under-explored, yet it may be a useful tool for describing pathogens likely to shift to novel hosts and predicting the potential for disease emergence following host jumps. Here, we describe the phylogenetic signal in host use by *Botryosphaeriaceae*, a globally distributed fungal family that infects most woody perennial plants, and explored the link between host breadth, phylogenetic relatedness, and virulence. First, we reconstructed the phylogeny of *Botryosphaeriaceae* spp. infecting grapevines (*Vitis vinifera*) and examined whether closely related pathogens infect similar host species. Second, we quantified virulence of the *Botryosphaeriaceae* known to infect *V. vinifera* using a high-throughput detached cane assay. We trained a machine learning algorithm to differentiate between asymptomatic and necrotic tissue, and quantified lesion size as a proxy for virulence. We then modelled the relationship between host breadth and lesion size. Preliminary results show large differences in host breadth within the *Botryosphaeriaceae*, and a positive relation between the phylogenetic host breadth and pathogen virulence in *V. vinifera.* This work provides a first step towards predicting virulence of a known pathogen on a novel host following a host jump. We suggest our approach could be useful for the coordinated global monitoring of high-risk species within *Botryosphaeriaceae*.

8.6 Exploring the microbial terroir: communities of fungi associated with grapevine trunk diseases differ among terroirs and seasons.

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In viticulture and oenology, the terroir concept is widely used to explain some of the differences in sensory and chemical characteristics of grapes and wines. The concept itself partly is based on known or presumed spatial differences in edaphic and mesoclimatic factors. These environmental differences also likely affect plant-associated microbes, with possible implications for plant health as well as crop yield and quality. In this study, we compared the compositional dynamics of fungi in woody tissue of grapevine, with emphasis on fungi associated with grapevine trunk diseases (GTDs). We sampled living woody tissue from grapevine plants of the same cultivar Furmint - (local white variety), in late winter and late summer, in three different terroirs in Tokaj, Hungary. DNA sequence data was generated by Illumina NovaSeq at BaseClear (Leiden, the Netherlands) with fungi-specific primers targeting the rDNA internal transcribed spacer (ITS) region. Of the fifteen different GTD-associated fungal genera found, *Phaeomoniella*, *Diplodia* and *Phoma* showed the highest richness, with some variation among terroirs. In addition, we found significant compositional differences among terroirs and sampling months, with terroir explaining 11.5 % and season 6 % of the variance in the community composition of GTD-associated fungi. These observed differences may be due to the inherent influence of different edaphic and mesoclimatic conditions that comes with different terroirs and seasonality. They affect the mycobiome composition and diversity possibly by secondary effect since living conditions in trunk woody tissues are expected to be relatively stable. We provide here novel information on the compositional dynamics of GTDs in wood tissues among terroirs and different seasons with implications for plant health studies.

8.7 Drought influences fungal community structure and diversity inhabiting the grapevine vascular system and enhances *Phaeomoniella chlamydospora* abundance.

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Grapevine productivity in the Mediterranean regions could be gravely affected by global warming, resulting in an increase of competitiveness by water resources. Recent studies showed that water deficit can alter the root-associated microbiota of grapevines, in particular those organisms able to alleviate the effects of abiotic and biotic stress factors. In this study, we investigated how drought influences fungal community structure and diversity inhabiting the grapevine vascular system, with special attention to the Petri and Esca disease pathogen *Phaeomoniella chlamydospora*. We subjected one-year-old grapevine rootlings grown under greenhouse conditions to three water regimes (WRs): 100 %, 50 % and 25 % of field capacity. Wood samples were taken before planting (t0), and one year (t1) and two years (t2) after planting from the bottom, medium and apical parts of the rootstock by using a non-destructive sampling method. Fungal composition and *P. chlamydospora* abundance were assessed by ITS high-throughput amplicon sequencing (HTAS) and droplet-digital PCR (ddPCR), respectively. Drought significantly altered the overall fungal compositions in the vascular system, being diversity significantly higher at 100 % WR than at the other WRs in t1 and t2. Several fungal taxa associated with grapevine trunk diseases (GTDs) were predominant and determined the dissimilarities among WRs, i.e., the species *P. chlamydospora* at 25 % WR in t1 and t2, the genus *Cadophora* at 50 % WR in t1, and the genera *Cadophora* and *Ilyonectria* at 100 % WR in t2. Both HTAS and ddPCR methods showed an increase in the *P. chlamydospora* OTUs and abundance at 25 % WR. Correlation analyses showed positive (*Cadophora*/*Ilyonectria*) and negative (*Cadophora*/*Phaeoacremonium*) interactions among several genera associated with GTDs. Water deficit reduced the complexity of the co-occurrence networks among taxa, resulting in higher interactions with more dense and compact networks at 100 % WR.

8.8 AMF community diversity identification and their effects on grapevine growth parameters under black foot disease pressure.

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Arbuscular mycorrhizal fungi (AMF) have potentially significant applications for sustainable agricultural ecosystems. AMF have been also shown to reduce infection and mitigate the effect of black foot disease on grapevine rootstocks. However, limited information is available regarding AMF-grapevine interactions worldwide and especially in New Zealand. Moreover, most studies to date have researched the effect of one, two or a combination of only a small number of AMF species on fungal pathogens associated with grapevines. Therefore, this work aimed to (*i*) characterise the AMF community diversity associated with different commercial grapevine rootstocks sampled from New Zealand vineyards, (*ii*) investigate the beneficial effect of AMF community on grapevine growth parameters, and (*iii*) evaluate how young grapevine rootstocks inoculated with their 'home' and 'away' whole AMF communities would respond to challenges with a black foot pathogen species mixture. The AMF communities identified from these rootstocks were assigned to *Ambispora* spp., *Claroideoglomus* spp., *Funneliformis* spp. and *Glomus* spp. The community analyses demonstrated that rootstock significantly influenced the AMF community composition in all sites. The findings of the second part of experiment showed that the AMF communities had a significant direct effect by increasing plant biomass and nutrient uptake and indirectly by influencing the chlorophyll content in grapevine leaves through the increase of specific nutrients such as K, Mn, and Zn. The outcome of the third part of experiment revealed that high disease incidence and severity did not reduce growth in vines with AMF inoculation compared to vines inoculated with the pathogen only. It also showed that the high level of disease present in rootstocks limited the effect of the AMF community with only little evidence that AMF treatments lowered disease incidence and severity in vines. Further research is required to understand the mechanistic effect of AMF colonisation on plant growth parameter especially under high disease pressure.

8.9 Culturome versus DNA metabarcoding: Diversity of grapevine endophytic mycobiome in old and young vines of different health status in New Zealand.

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The grapevine harbours a diverse community of fungi in the woody trunk tissue, termed the “endophytic mycota”. These communities can have a profound effect on the vine’s physiology, health, growth, and ability to adapt to stress. Some of these include pathogenic fungi as the causal agents of grapevine trunk disease (GTD), with many considered latent pathogens. For GTD, understanding the factors affecting latency is still limited. This study aimed to compare the fungal endophyte community in young and old Sauvignon blanc vines, both symptomatic and asymptomatic for GTD, using culture-dependent and culture-independent approaches. Nine vineyards were sampled, with 60 mature vines (>10 years old) and 30 young vines (<9 years old) sampled. Each age group consisted of equal numbers of apparently healthy and symptomatic vines. Trunk cores were taken from each vine using a sterilised 4-mm drill bit after removing the bark with a knife. Fungal communities were characterized by isolation and metabarcoding of the ITS1 region. For the culturome, a collection of 2116 endophytic fungi were recovered, representing 42 fungal genera. Trunk microbiota was dominated by species of the genera *Alternaria*, *Aureobasidium*, *Diplodia*, *Epicoccum*, *Phaeomoniella*, *Eutypa*, *Botrytis*, *Cladosporium*, and *Diaporthe*. Differences in the taxa recovered into culture were observed between vines of different ages, and symptomology. In the metabarcoding approach, 1892 OTUs were obtained. The same fungal genera were identified as the most abundant using metabarcoding. Alpha diversity analysis revealed that greater diversity was detected in old compared to young vines and in asymptomatic compared to symptomatic trunks. Beta diversity analysis demonstrated significant differentiation in the fungal communities structure for both age and health status. This study has produced new baseline information on Sauvignon blanc endophytic mycota and further work will determine the impact of these microbial communities on the latency of GTDs.

8.10 Can the microbiome drive the suppression of grapevine trunk diseases?

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Grapevine trunk diseases (GTDs), caused by several fungal species, are among the most destructive grapevine diseases in New Zealand and other grape-growing countries. The control of the diseases is problematic, and there is currently no approved fungicide for their eradication. This has necessitated seeking alternative strategies, including a sustainable biological control approach, to manage the diseases. Therefore, this study aimed to identify taxa in the grapevine microbiome that contribute to plant health. In some New Zealand vineyards, observations have revealed vines that remain healthy within a background of trunk diseases. These grapevines were termed 'disease-escape' to represent their apparent health under heavy disease pressure. Recent research on the grapevine microbiome has shown that microorganisms from these 'disease-escape' plants could contribute to disease suppression. Putative disease escape vines were identified in vineyards in two grape-growing regions in New Zealand: Hawke's Bay and Canterbury. The vines were selected based on their presence in a diseased area, maturity, and absence of trunk disease symptoms. Trunk core samples were taken from the disease-escape vines and neighbouring symptomatic vines. Subsequently, the samples' total fungal and bacterial communities were identified and compared using culture-independent DNA metabarcoding and culture-dependent approaches. After analysing the metabarcoding and culturing results, microbial taxa that were differentially more abundant in disease-escape grapevines and the ones that correlated negatively with GTD pathogens were identified. The next stage of the study is to design a synthetic community using members of the taxa of interest from the disease-escape grapevines. This SynCom will be introduced into young grapevines and monitored for their ability to suppress the development and severity of GTDs. The research results will provide information on the roles (if any) that the grapevine trunk's microbiome plays in suppressing GTDs.

8.11 Implications of abiotic and biotic stress on *Phaeomoniella chlamydospora* colonization in young 'Merlot' grapevines.

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Growing evidence has led to the hypothesis that some grapevine trunk disease (GTD) fungi may behave as latent pathogens, transitioning from an endophytic to a pathogenic lifestyle triggered by plant stress. Two greenhouse experiments were conducted over two growing seasons to determine the impact of drought stress and ring nematode (*Mesocriconema xenoplax*) infestation on *Phaeomoniella chlamydospora* fungal growth and disease development in young 'Merlot' grapevines. Research into the potential role of arbuscular mycorrhizal (AM) fungi as stress reducers an biocontrols has also grown in recent years. The AM fungus *Rhizophagus intraradices* was inoculated into the soil to investigate its potential in mitigating plant stress and thus mitigate its impact on fungal growth. *Phaeomoniella chlamydospora* was vacuum inoculated into the base of dormant 'Merlot' canes at a high (25,000 spores), medium (5,000 spores), and low (1000 spores) inoculum to investigate whether a threshold required for disease to occur would be reached sooner in highly infected plants. Fungal quantity was determined at time of inoculation, time of planting, and upon conclusion of the experiment with the use of droplet digital PCR to determine the effect of stress on fungal growth. Pruning weights were collected throughout the experiment and internal necrosis was measured at the base of each grapevine upon conclusion of the experiment to monitor symptom expression. Preliminary results showed no phenotypical differences (dry root weight and pruning weights) between stressed and non-stressed plants no matter their infection status. Percent necrosis at the base of the trunk was higher in water stressed grapevines but no difference was observed in nematode infested plants. ddPCR analysis revealed increased fungal growth in water stressed plants, but not in nematode infested plants, indicating water stress may play a role in Petri disease development in young grapevines.

9.1 Evaluating treatments for the protection of grapevine pruning wounds from natural infection by trunk disease fungi.

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Infection of grapevines by grapevine trunk disease (GTD) fungal pathogens primarily occurs through annual pruning wounds made during the dormant season. This study aimed to evaluate and compare the efficacy of various liquid formulation fungicide (pyraclostrobin + boscalid) and paste treatments as well as biological control agents (*Trichoderma atroviride* SC1, *T. atroviride* I-1237, and *T. asperellum* ICC012 + *T. gamsii* ICC080), for their potential to prevent natural infection of grapevine pruning wounds by trunk disease fungi in two field trials over two growing seasons. Vineyards were located in Samaniego, Northern Spain (19-years-old; cv. Tempranillo) and Madiran, Southern France (24-years-old; cv. Cabernet Franc). Wound treatments were applied immediately after pruning in February. Untreated controls were mock treated with sterile distilled water. One year after pruning, canes were harvested from vines and brought to the laboratory for assessment of *Trichoderma* spp. and fungal trunk pathogens. More than 1,000 fungal isolates associated with five GTDs (Esca, Botryophaeria, Diaporthe and Eutypa diebacks, and Cytospora canker) were collected from the two vineyards each growing season. The efficacy of each product varied according to the GTD fungi and the grape-growing region, although, in some cases, the low incidence of some GTDs in the untreated control did not allow determining significant differences between treatments. In general, *T. atroviride* I-1237 was the most effective treatment followed by pyraclostrobin + boscalid. *Trichoderma* recovery percentages ranged from 16.7 to 93.3 % in Samaniego, and from 32.5 to 94.2 % in Madiran. The experiment will be undertaken for a third season during 2022-2023.

9.2 Hot water treatment (50 °C for 45 min) in grapevine nurseries: the dilemma of heat tolerant GTD pathogens.

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The benefits of hot water treatment (HWT; 50 °C for 30 min) in grapevine nurseries is well known, although it is also known that it does not completely eradicate all infections. A HWT protocol of 50 °C for 45 min has been recommended for the control of Aster Yellows in South Africa. The aim of this study was to determine the effect of HWT (50 °C for 45 min) on GTD pathogens in South African nurseries, firstly *in vitro*, followed by artificially inoculating rootstock cuttings of Ramsey, Richter 110, US 8-7, Paulsen 1103 and 143B Mgt. Pathogens included *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum*, *Pm. parasiticum*, *Cadophora luteo-olivacea*, *Pleurostoma richardsiae* (Petri disease), *Campylocarpon fasciculare*, *Camp. pseudofasciculare*, *Ilionectria liriodendri*, *Dactylonectria macrodidyma* (Black foot disease), *Neofusicoccum australe*, *N. parvum* (Botryosphaeria canker and dieback) and *Diaporthe ampelina* (Phomopsis dieback). *In vitro* results concluded that HWT was able to cause complete inhibition of conidial germination and mycelial growth of all pathogens associated with Black foot disease, Bot. canker and dieback and Phomopsis dieback. Pathogens associated with Petri disease were more heat tolerant, with *Pl. richardsiae* being the most tolerant species and *Pa. chlamydospora* the most sensitive, followed by *Ca. luteo-olivacea*. The effect of HWT temperatures greater than 50 °C was also investigated. *Pleurostoma richardsiae* showed the highest tolerance with temperatures of up to 60 °C not able to achieve complete control. In the in vivo experiments, HWT was highly effective in eradicating *Pa. chlamydospora* and *Ca. luteo-olivacea* and significantly reduced *Pm. minimum* and *Pm. parasiticum* incidence and severity, based on isolation studies. The effect of HWT on *Pl. richardsiae* was less consistent. The incidence of *Pl. richardsiae* was not significantly reduced, however, the severity of the infections was significantly reduced, although inconsistently. Even though HWT may not eradicate all infections, it is highly recommended for use in an integrated approach to control GTDs.

9.3 The *in vitro* effects of selected phenolic compounds against *Diplodia seriata*, *Eutypa lata*, *Fomitiporia mediterranea* and *Neofusicoccum parvum*.

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Grapevine trunk pathogens (GTPs) cause serious damage and have a significant economic impact on the cultivation of grapevines worldwide. There is currently no direct and fairly effective protection against GTPs in nursery and established vineyards. The inhibitory effects of eugenol, epigallocatechin-3-o-gallate (EGCG) and thymol against the GTDs pathogens *Diplodia seriata*, *Eutypa lata*, *Fomitiporia mediterranea* and *Neofusicoccum parvum* were thus monitored by *in vitro* test. The most significant inhibition of fungal growth was observed for eugenol, which showed the lowest value of half-maximum effective concentration (EC_{50}). For eugenol at a concentration of 1.5 µL mL^{-1}, a complete inhibition of growth was observed in both *F. mediterranea* and *N. parvum* with *E. lata* and *D. seriata* showing also high inhibition values, 99 % and 98 %, respectively. For eugenol, EC_{50} values of 0.94, 0.95, 0.96, and 1.00 µL mL^{-1} were obtained for *F. mediterranea*, *E. lata*, *D. seriata*, and *N. parvum*, respectively. Thymol at the highest concentration used (45 µL mL^{-1}), showed statistically significant inhibitory activity against all four pathogens. *D. seriata* showed 90 % inhibition, *N. parvum* 88 %, *F. mediterranea* 74 % and *E. lata* 67 %. The relative thymol EC_{50} values of 25.37, 25.45, 29.02 and 34.28 µL mL^{-1} were observed with for *N. parvum, D. seriata*, *F. mediterranea* and *Eutypa lata*, respectively. To compare, EGCG did not show any statistically significant inhibitory effects on selected GTPs. The next step will be to evaluate the potential use of eugenol to control GTPs by *in planta* test.

9.4 Minimal versus intensive: How the pruning intensity affects occurrence of grapevine leaf stripe disease and wood integrity in the trunk.

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Previous works in managing Grapevine trunk diseases (GTDs) indicate that non- or minimal-pruning schemes under certain circumstances can significantly reduce the risk of Esca. Nevertheless, knowledge of the mechanisms behind these observations is limited. Therefore, it was the aim of this study to investigate in more detail the effect of pruning intensity on the occurrence of grapevine leaf stripe disease and grapevine trunk integrity. Two German vineyards ('Dornfelder' and 'Müller-Thurgau') partially trained with intensive- and minimal-pruning schemes were chosen for this survey due to the accessibility of multi-annual Esca monitoring data (five years and six years, respectively). Incidence of external symptoms and the proportion of white rot and necrosis in the trunk of Esca positive and negative vines was analysed and compared between the two pruning intensities. The results revealed that only in the 'Dornfelder' vineyard the incidence of external Esca symptoms was significantly reduced over a period of five years (2017–2021) by minimal pruning and this up to 73.7 % compared to intensive pruning. In both vineyards, trunks of intensive-pruned vines not only had more pruning wounds on the trunk (by 86.0 % and 72.9 %, respectively) than minimal-pruned vines, but also exhibited a larger (by 19.3 % and 14.7 %, respectively) circumference of the trunk head. Only in the 'Dornfelder' vineyard, the proportion of necrosis was higher for intensive-pruned vines (23.0 %) than for minimal-pruned vines (11.5 %).

9.5 Effects of pruning on desiccation cone formation of three cultivars in France.

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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). A panoply of methods, including pruning, are currently being used in attempts to prolong grapevine life. In our forthcoming article, we consider the different effects of high or short pruning in grapevine. High pruning leaves a chicot and preserves the diaphragm, unlike short pruning, which damages the diaphragm. Our experiment, which focused on necrosis formation, examined the correlation between diameter spur and necrotic length, and also measured the evolution of desiccation cones according to pruning type. The grapevines were pruned in February and, 4 or 8 months later, five vines per modality (short or high pruning) were sampled. There was no correlation between spur diameter and necrotic length for Cabernet sauvignon, Sauvignon blanc and Ugni blanc. However, there was a correlation between necrotic length and quality of pruning wound length for Cabernet sauvignon and Ugni blanc. Necrotic length also varied with the vintage, particularly so as regards Sauvignon blanc. Overall, high pruning was effective in stopping desiccation cone development in the chicot. Keeping the diaphragm safe allows the sap flow path to function fully.

9.6 Can spray coverage of wounds and Eutypa dieback control be improved by the addition of adjuvants to fungicide?

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Infection of grapevine pruning wounds by the pathogen *Eutypa lata*, which causes Eutypa dieback, can be efficiently and effectively controlled by the spray application of fungicides. The level of disease control is correlated to wound coverage, however, as tractor-driven sprayers are designed to target leaves, they require adjustment to achieve adequate coverage of pruning wounds on dormant vines. A vineyard trial was established in winter 2017, and repeated in winter 2018, in McLaren Vale, South Australia, to evaluate whether the addition of spray adjuvants (di-1-p-menthene and trisiloxane ethoxylate) to fungicide treatments (tebuconazole and fluazinam) could improve wound coverage and protection against infection by *E. lata*. Optimal spray output to achieve adequate coverage of pruning wounds is at least 600 L/ha, but in order to ascertain any benefits from the addition of adjuvants, in this trial, a recycle sprayer applied treatments at a low output rate of 200 L/ha, prior to inoculation of wounds with *E. lata*. A novel technique for evaluating wound coverage was developed, using fluorescent pigment added to treatments, and was compared with the use of water-sensitive papers (WSPs). Digital images of fluorescent pigment on wounds, captured under UV light, and from WSPs, were assessed using Image J image analysis software and showed little effect of the addition of adjuvants on coverage. However, when compared directly, WSPs indicated twice the coverage than the fluorescent pigment deposited directly on pruning wounds, from the same treatment. Treated canes were removed 11 months after inoculation and assessed for pathogen recovery. Overall, there was little difference in recovery between treatments, indicating that the adjuvants did not improve efficacy of fungicides. Disease control was minimal with the application of the fungicides, reiterating the importance of applying the recommended minimum of 600 L/ha to achieve sufficient wound coverage.

10.1 Effects of biocontrol agents on *Fomitiporia mediterranea*.

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White rot inducers such as *Fomitiporia mediterranea* (Fmed) and other *Fomitiporia* species are considered, among other fungal species, main pathogens associated with the ESCA syndrome. In the current study, the effect of *Trichoderma simmonsii* 804 and 1056, *T. citrinoviridae* 232, *Bacillus subtilis* 224, *B. subtilis* 230, *B. amyloliquefaciens/velezensis* 624, *B. amyloliquefaciens/velezensis* 2277, *B. amyloliquefaciens/velezensis* 2143 and *Pseudomonas koreensis* (all isolated from grapevines) and *T. atroviridae* (Vintec, Belchim) was studied on growth of Fmed in dual culture assays and using a wood disc model. For the dual culture assay mycelial disks (1 cm) of Fmed cultures were placed in the centre of MEA plates. Similar disks excised from BCA cultures, were quartered and placed at the edge of the plates. After 14 days the diameter of the Fmed culture was measured. The wood disk models included either fresh or autoclaved halved cross sections of grapevine trunks (from dormant 10-15 y old asymptomatic vines cv. 'Rotburger' ('Zweigelt') approximately 4 mm thickness) on water agar. Two inoculation timings were established. Either inoculation with Fmed was carried out 7 days before treatment with the biological control agents (BCAs) or 7 days thereafter. For pathogen inoculation, halved mycelial disks were placed in the middle of the wood pieces, for BCA treatment wood pieces were briefly immersed in inoculation suspensions (bacterial isolates: OD600 0.2-0.3 in PBS; *Trichoderma* sp. 108 cfu/ml in tap water). Growth of Fmed on the wood disks was visually evaluated after 3-5 weeks. In dual culture all BCAs significantly reduced growth of Fmed as compared to the control, the *Trichoderma* species being most effective. Fmed successfully colonized the mock inoculated wood disks. A significant effect of all *Trichoderma* species on pathogen growth was observed both in case of fresh and autoclaved wood for both inoculation timings. Inhibitory effects of bacterial BCAs were also recorded.

10.2 Biological control of *Phaeomoniella chlamydospora* in young grapevines with *Bacillus velezensis* K165 and *Fusarium oxysporum* F2.

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The emergence of grapevine trunk diseases (GTDs) as the major problems for viticulture in the last two decades all over the worlds puts in danger the sustainability of the crop. *Phaeomoniella chlamydospora* (Pch), a tracheomycotic fungus is among the most predominant GTD-associated species and a major player in Petri disease causing decline of young grapevines. There are no available strategies to reduce infections causing Petri disease except prevention in the nursery, therefore there is an urgent need for alternative and eco-friendly approaches to control the disease. In the present study, we evaluated the effectiveness of *Bacillus velezensis* K165 (formerly known as *Paenibacillus alvei* K165) and *Fusarium oxysporum* F2 against *Pch* in young grapevines. In dual culture bioassays, in a growth medium simulating the xylem environment, F2 decreased *Pch* growth and sporulation, whereas K165 did not have any effect on *Pch*. In rooted grapevine cuttings K165 was applied through root drenching while F2 was applied by stem injection. K165 significantly reduced wood discoloration, the typical symptom of *Pch* infection, whereas the application of F2 by stem-puncture did not. Both K165 and F2 reduced the endophytic relative DNA amount of *Pch* by 90 % and 82 %, respectively, compared to controls as revealed by qPCR analysis. Also, both K165 and F2-treated grapevines harbored higher lignin levels compared to non-inoculated control. Therefore, we assume that F2 and K165 have the potential to be used as biocontrol agents against *Pch* in grapevines.

10.3 Lignans extract from knotwood of Norway spruce as a possible novel bioprotectant agent against grapevine trunk diseases.

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Grapevine trunk diseases (GTDs) pose a major threat to the wine industry causing significant economical loses worldwide. The nonexistence of effective treatment declares an urgent need to find a solution to the current situation. In the present study, we used an extract from the knotwood of spruce trees as an antifungal agent against GTDs. In the *in vitro* trial, we focused on the antifungal effect of the extract against particular strains of the most common GTD pathogens. Complete inhibition of growth was observed against *Cadophora luteo-olivacea*, *Dactylonectria torresensis*, and *Phaeoacremonium minimum*. Partial inhibition was observed against *Diaporthe ampelina* (62.5 %), *Diaporthe bohemiae* (58.4 %), *Diplodia seriata* (30 %) and *Eutypa lata* (79 %) using 1 $mg.mL^{-1}$ extract. Subsequently, the *in vitro* trial was followed by an *in planta* experiment. Commercial grafts of grapevine were treated with the extract and then planted. The total genomic DNA of grapevines was extracted 10 days and 180 days after the treatment. The fungal microbial diversities of the treated/untreated plants were compared using high-throughput amplicon sequencing. Treated plants showed 76.9 % lower relative abundance of the genus *Diaporthe* and 70 % lower relative abundance of the genus *Phaeoacremonium* 10 days after the treatment. A similar scenario was observed for the genus *Cadophora* 180 days after treatment, where treated grapevines showed 76 % lower relative abundance of this genus compared with untreated grapevines.

10.4 Biological and chemical pruning wound protectants reduce infection of grapevine trunk diseases pathogens in California.

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Grapevine trunk diseases (GTDs) are currently considered some of the most important challenges for viticulture, curtailing vineyard longevity and productivity in nearly every raisin, table, and wine grape production region in California and worldwide. Pruning wounds provide the main entry point for fungal pathogens responsible for these diseases; pathogens enter the wounds following precipitation events. The aim of this study was to evaluate the efficacy of selected chemical and experimental biological fungicides for the protection of pruning wounds against two of the most common and virulent fungal pathogens causing GTDs: *Eutypa lata* and *Neofusicoccum parvum*. This study was conducted on Chenin blanc at the UC Davis Department of Plant Pathology Field Station for six months. Results showed that several chemical and biological fungicides, notably the chemical fungicide Fluopyram/trifloxystrobin, the biofungicide *Trichoderma atroviride*, and a combination *Trichoderma asperellum*, *T. gamsii* and a blend of crab and lobster shell powder provided significant protection against at least two of the canker pathogens used in this study. However, the majority of products tested did not provide simultaneous control of both *E. lata* and *N. parvum* pathogens, highlighting the continuing challenge of controlling GTDs.

10.5 Study of the impact of a copper-hydroxyapatite formulation on the vine physiology, microbiome, metabolome, for a potential use against grapevine trunk diseases.

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The concerns on human and environment health are driving industry to formulate more innovative and eco-sustainable plant protection products (PPPs). Copper (Cu) is a PPP active ingredient subjected to regulation, especially in Europe where it is considered as “candidate for substitution”. Indeed, Cu is recognized as an effective fungicide, used for more than 130 years against mildew and the only authorized product in organic viticulture. Previous studies using a new Cu-based formulation, namely the HA+Cu(II), using Cu in low concentration (3.5 %) that is transported to plants by hydroxyapatite, showed its efficiency against *Plasmopara viticola* and *Phaeoacremonium minimum* under greenhouse conditions, correlated with the induction of some plant defense responses. To evaluate the use of this formulation for GTD control, we studied the impacts of HA+Cu(II) on plant physiology in vines cv Chardonnay and Cabernet sauvignon, healthy or infected by *Diplodia seriata* and *Neofusicoccum parvum* in greenhouse. We further characterized the effects of HA+Cu(II) against GTDs in vineyard infected by Esca (cv Chardonnay from Champagne region, France). This last study aimed to determine the impact of HA+Cu(II) on: (*i*) the incidence of Esca, (*ii*) the vine microbiome, (*iii*) the vine physiology and (*iv*) enological parameters of the juice. In greenhouse, the HA+Cu(II) application induced several defense genes without adversely affecting neither the plant growth nor photosynthetic activity, with induction levels comparable to those of a commercial product. The fungistatic effect on the two *Botryosphaeriaceae* reported *in vitro* was confirmed *in planta*. In field, we observed a decreasing trend in the incidence of Esca cumulate over years and neither deleterious effects on the vine microbiota, vine physiology nor on the enological properties of the juice. Some similarity of HA+Cu(II) treatments to those of sodium arsenite were observed. Our results therefore fully support the potential of HA+Cu(II) as a promising PPP towards GTDs.

10.6 *In vitro* evaluation of endophytic and rhizospheric bacteria as potential biocontrol agents of grapevine trunk diseases.

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Management of grapevine trunk diseases (GTDs) constitutes an ongoing challenge for viticulture and therefore there is a need for effective and long term sustainable strategies. A total of 20 commercial vineyards throughout California were sampled over the summer of 2019 for isolating endophytic and rhizospheric bacteria from different parts of grapevines including cordon, trunk, and root. A collection of 1,344 bacterial isolates was obtained and tested *in vitro* against *Neofusicoccum parvum* and *Diplodia seriata*, from which a subset of 172 isolates exerted mycelial growth inhibition levels over 40 %. The majority of isolates of this subset corresponded to an undescribed species of *Bacillus* (n=154), closely related to *B. velezensis*, whereas the remaining belong to a range of species of *Pseudomonas* (12 isolates), *Serratia* (2 isolates) and other genera excluded from this study. Representative isolates of *Bacillus* sp., *Pseudomonas chlororaphis* and *Serratia plymuthica* were challenged in dual antagonism assays against *N. parvum, D. seriata, Lasiodiplodia theobromae, Eutypa lata*, *Diaporthe ampelina, Phaeoacremonium minimum, Fomitiporia polymorpha* and *Ilyonectria liriodendri*. Mycelial inhibition levels were consistent across bacterial species, being slightly higher against slow growing fungi than against *Botryosphaeriaceae*. Moreover, the volatile and agar-diffusible metabolites produced by these bacteria were tested against the mycelium growth of *N. parvum* and *E. lata, Bacillus* sp. isolates strongly inhibited the growth of both pathogens through their diffusible metabolites at all tested concentrations (1, 15, and 30 % v/v) but not by their volatile compounds. The isolates of *P. chlororaphis* and *S. plymuthica*, however, caused lower inhibition levels against both pathogens, yet a combination of both volatile and diffusible metabolites seem to be involved in the antifungal activity. Currently, these isolates are being tested in field experiments to evaluate their effectiveness against trunk disease pathogens.

11.1 Biological control of Botryosphaeria dieback on grapevines.

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Botryosphaeria dieback (BD) is a grapevine trunk disease (GTD) causing important yield losses and limiting the lifespan of vineyards. *Botryosphaeriaceae* spp. causing BD infect grapevines through pruning wounds. Therefore, pruning wound protection is currently the most effective and available management strategy. A demand for alternatives to chemical products and more sustainable control methods to manage GTDs has significantly increased in the last years worldwide. With no control products currently registered in Canada against GTDs, the main objectives of this research were to identified local biological control agents (BCAs) in the *Trichoderma* genus and evaluate their potential biocontrol activity against BD fungi *Diplodia seriata* and *Neofusicoccum parvum*. A total of 29 *Trichoderma* isolates were obtained from vineyards in British Columbia (BC). Morphological studies along with phylogenetic analyses of the ITS1-5.8S-ITS2 gene and TEF-1α partial gene identified seven species, including *T. asperelloides, T. atroviride, T. harzianum, T. koningii, T. tomentosum*, and two novel species, *T. canadense* and *T. viticola*. *In vitro* dual culture antagonistic assays showed several isolates to inhibit fungal pathogen mycelial growth by up to 75 %. *In planta* detached cane assays under controlled greenhouse conditions identified *T. asperelloides, T. atroviride* and *T. canadense* isolates from BC to provide 70 % to 100 % pruning wound protection against *D. seriata* and *N. parvum* for up to 21 days after treatment. Field trials conducted in cv. Merlot vines in 2019 and 2020 showed mixed-species inoculum of *T. asperelloides, T. atroviride* and *T. canadense* to provide high biocontrol activity against BD fungi for up to 60 days after treatment. Field results also showed *Trichoderma* spp. from BC to provide similar or better pruning wound protection when compared against commercial chemical and biocontrol products. This study provides needed data towards the development and registration of the first control products against GTDs in Canada.

11.2 Combining a HA + Cu(II) site-targeted copper-based product with a pruning wound protection program to prevent infection with *Lasiodiplodia* spp. in grapevine.

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The genus *Lasiodiplodia* have been reported from several grape growing regions and is considered as one of the fastest wood colonizers, causing Botryosphaeria dieback. These fungi can infect grapevines through any type of wounds, but pruning wounds are currently considered as the principal site of infection. Therefore, it is of the utmost importance to find an effective integrated pest management that includes cultural practices, organic products, BCAs, responsible use of chemical pesticides, and management strategies that may combine both chemical and biological controls. The aim of this study was to *i*) evaluate the efficacy of Esquive®, a biocontrol agent, as grapevine pruning wound protection applied single or in a combined protection strategy with a new site-targeted copper-based treatment (LC2017), and *ii*) compare their efficacy with a chemical protection provided by the commercially available product, Tessior®. For two seasons, protectants were applied onto pruning wounds, while LC2017 was applied throughout the season according to manufacturer's instructions. Pruning wounds of two different cultivars were inoculated with three isolates of *Lasiodiplodia* spp. Efficacy of the wound protectants varied between both years of the assay. However, according to the cultivar studied they were able to control the pathogen to some extent. The application of LC2017 did not show clear evidence of improving the control obtained by the sole application of the other products tested. Nevertheless, LC2017 showed a fungistatic effect against *Lasiodiplodia* spp., *in vitro*, and has previously shown an elicitor effect against grapevine trunk diseases. Therefore, this combination of two protection strategies may constitute a promising long-term approach to mitigate the impact of Botryosphaeria dieback.

11.3 Relationship between *Trichoderma* recovery from pruning wounds treated with biocontrol formulations and the control of *Diplodia seriata* in *Vitis vinifera* in Chilean orchards.

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The use of sprayed *Trichoderma* based products have raised doubts about the relationship between the recovery of *Trichoderma* from treated cuts and control efficacy. This work sought to establish a correlation between the recovery of *Trichoderma* and the decrease in damage by wood fungi in field conditions of several vineyards. Two experiments were established in San Felipe (Aconcagua Region) and Talca (Maule Region), using grape cvs. Red Globe and Cabernet Sauvignon, respectively. Twenty-four hours after pruning 100 g/hl of the commercial product Mamull (Bio Insumos Nativa SPA) was sprayed using conventional equipment in five 0.5 ha replicates, alternated with controls without treatment, under a randomized block design. At 180 days after application, samples (n=50) were evaluated for *Trichoderma* and pathogen recovery (natural infection), also incidence and severity of lesions. At 15 days after field application, *Trichoderma* was recovered from samples (n=50) by isolating on malt extract agar, and a sub sample of detached canes (n=20) were inoculated in the laboratory with *Diplodia seriata* conidia suspension and incubated in glass flask for 30 days, for evaluation of symptoms. In the field, a greater ($P<0.05$) incidence of symptoms (dieback (mm of lesion) X pathogens recovery (0, 1)) (7.5 %) was observed in controls compared with 0.2 % when treated with *Trichoderma*. The average recovery of *Trichoderma* from the treated canes (52.3 %), was significantly higher ($P < 0.05$) than the control canes (11.3 %). Recovery of *D. seriata* was significantly higher ($P<0.05$) in the control canes (16.4 %) compared to those treated with *Trichoderma* (5.4 %). The detached canes inoculated in the laboratory had longer ($P<0.01$) lesions in the control (1.8 cm) compared with those having Trichoderma treatment (0.2 mm). Low correlation was found between the presence of *Trichoderma* and field damage ($R= 0.45$; $P<0.05$). It is concluded that the recovery of *Trichoderma* from treated cuts is less reliable than the damage measurements of the samples treated in the laboratory, the biocontrol formulation show a significant control of wood disease, also natural infection in field, as with artificial inoculation 15 days after spray.

11.4 Hot water treatment as a tool to produce high-quality grapevine propagation material.

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Hot water treatment (HWT) of dormant woody plant material has been shown to reduce infections by Grapevine Trunk Disease (GTD) pathogens. In this study, HWT on individual developmental stages of three pathogens [*Phaeomoniella chlamydospora* (*Pch*), *Phaeoacremonium minimum* (*Pmi*), *Botryosphaeriaceae* (*Bot*) species] was assessed at different combinations of temperature and exposure time. Conidia and mycelial suspension of *Pch*, *Pmi* and *Bot* were first subjected to HWT between 30 and 45 min at 40 to 55 °C and then incubated on malt extract agar in a moist chamber to assess colony formation. In addition, field experiments were carried out to evaluate the effect of HWT on the targeted fungal pathogens and on the commercially available biological control agent (BCA) [*Trichoderma atroviride* strain SC1 (TASC1) (Vintec®, Belchim Crop Protection Deutschland GmbH). Inoculated scion cuttings were grafted with healthy rootstocks following HWT (50 °C, 45 minutes) under practical conditions, planted in nurseries and analysed at distinct time points for pathogen development. Our results *in vitro* indicated that *Pmi* showed reduced sensitivity to HWT at the ungerminated spore stage, whereas *Pch* and *Bot* conidia were more sensitive to HWT. Isolations from the inoculated cuttings confirmed that infestations caused by *Pmi* and *Pch* were reduced, whereas *Bot* was completely eliminated through HWT. Also, the results showed no negative impact of HWT on the commercial BCA product. Therefore its antagonistic ability against the fungal trunk pathogens should not be affected.

11.5 Removal of trunk disease pathogens in mature grapevines with remedial surgery.

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Remedial surgery has been used to improve the productivity and extend the life of vines infected by Botryosphaeria dieback and Eutypa dieback. Efficacy is dependent on the removal of infected wood and growth of new shoots to re-establish a trunk. Over the last 3 years, remedial surgery has been carried out in mature (>18 years) commercial vineyard blocks of cvs Sauvignon Blanc, Cabernet Sauvignon and Merlot. Each year, the trunks were removed the same distance above the graft union (200 mm for cv Sauvignon Blanc and 150 mm for the other varieties). The incidence of dieback in the canopy was >90 % in cvs Sauvignon Blanc and Cabernet Sauvignon and increased over this time from 64 % to 91 % in cv Merlot. Internal trunk staining symptoms associated with dieback were frequently detected at the top of the trunk below the head in cv Sauvignon Blanc (98 %) and cv Merlot (84 %) and at the remedial cut site (55 % and 59 %, respectively). These symptoms were not as frequent in the trunks of cv Cabernet Sauvignon (62 % top and 10 % cut site). Staining symptoms were also observed from spur and watershoot wounds on the trunk. Over three growing seasons, the distance of staining from the remedial cut site decreased significantly in all three varieties and in cvs Sauvignon Blanc and Merlot, there was an increase in the incidence of staining at the remedial cut site. *Botryosphaeriaceae* species were frequently detected in the trunks in advance of the staining in all three cvs. Trunks were shown to be infected with multiple species of *Botryosphaeriaceae*. When present *Eutypa lata*, was usually found together with these pathogens. *Botryosphaeriaceae* species, and occasionally *E. lata*, occurred over distances >200 mm in advance of the staining. Growers are now advised to intervene earlier and cut as low as practical to improve the efficacy of remedial surgery.

11.6 Trellis systems of rootstock mother vines affect the wood microbiome.

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The management of rootstock mother vines represents the first stage of bench-grafted vines production along the grapevine nursery process. With few exceptions, in source mother blocks, rootstocks shoots are usually sprawled on the ground, an inexpensive method that may favour infections by trunk disease pathogens, compromising the quality of the propagation material. Trellis systems might be applied to rootstock mother vines to improve the functional leaf area and its exposure to sunlight. Different trellis system, therefore, influences the canopy microclimate: this and the lack of contact with the soil occurring by trellising rootstock mother vines may positively shape the dynamics of the epiphytic and endophytic microbial communities. By applying a DNA metabarcoding approach, this study investigated the impact of two trellising methods (a vertical trellis system and a transpiring fabric applied to protect sprawled rootstocks from the soil) compared to the traditional sprawled rootstocks, on the resident fungal and bacterial communities, with a specific focus on wood pathogens, of two rootstock cultivars: Kober 5 BB and 110 Richter. Bacterial and fungal beta-diversity, including epiphytic and endophytic communities, resulted to be affected by the temporal distribution of the rootstock. Such diversity was more evident when comparing a trellised rootstock (cv. Kober 5 BB) with the same plant material sprawled on the ground. The results show that the sprawling shoots, compared to vertical-positioned shoots, are more exposed to soilborne microorganisms and pathogens due to the contact with the inoculum in the soil and the higher temperatures and humidity.

11.7 Biological control agents for Botryosphaeria dieback of grapevine.

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Botryosphaeriacea fungi cause Botryosphaeria dieback; this disease is responsible for considerable damage to grapevines worldwide. In Mexico, one of the highly virulent causal agents is *Lasiodiplodia brasiliensis*. There are no effective control strategies available for this disease; therefore, integrated management includes eliminating diseased plants, fungicides, wounds protection, and biological control agents. This work aimed to identify and evaluate microorganisms with biocontrol activity against Botryosphaeria dieback fungi to provide growers with environmentally friendly strategies for disease control. One hundred thirty-seven endophytic bacteria were isolated from old grapevines, and 37 *Trichoderma* fungi were recovered from our lab collection. All isolates were evaluated *in vitro* to select those with antagonistic activity against *L. brasiliensis*. From the results, eleven *Bacillus* and four *Trichoderma* isolates were selected to assess characteristics associated with plant growth promotion and their ability to produce volatile and non-volatile compounds with antifungal activity. In the end, thirteen isolates were selected to perform greenhouse assays. These were applied directly to the soil as a preventive treatment while *L. brasiliensis* was inoculated into a hole made in the grapevine plants. Only the plants inoculated with *Bacillus subtilis* BEVP26 showed significantly smaller lesions than the control. Finally, nine of the previously tested isolates were applied preventively in a pruning wound made on branches of grapevines established in a commercial vineyard, one hour later *L. brasiliensis* was applied at the top. The plants inoculated with eight of the isolates showed smaller lesions compared to plants inoculated only with *L. brasiliensis*. These potential biological control agents were molecularly identified as belonging to *B. subtilis*, *B. velezensis*, *T. asperellum*, and *T. longibrachiatum*.

POSTER PRESENTATIONS

P.1 Diversity of *Dactylonectria* and *Ilyonectria* species causing black foot disease in grapevine nursery stock in Uruguay.

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Black foot caused by "*Cylindrocarpon*"-like asexual morph fungi is one of the main diseases affecting young grapevines worldwide. Several studies have shown that grapevines are primarily infected by black foot in the nursery process, highlighting the role of infected nursery plants in the spread of the disease. In a previous study carried out in Uruguay, typical symptoms caused by black foot disease were observed in grapevine planting material. Symptoms consisted in brown to dark streaks that developed from the base of the rootstocks, wood necrosis at the base of the trunk, sunken necrotic roots lesions and reduced root biomass. To know the diversity of "*Cylindrocarpon*"-like fungal species associated with black foot disease, grapevines ready to be sold to growers with black foot symptoms of different cultivars grafted onto Gravesac, 1103P, SO4, 101-14 and 3309C rootstocks, were evaluated from 2017 to 2019. A total of 77 "*Cylindrocarpon*"-like strains were isolated and identified by DNA sequence analysis of the partial histone H3 gene. The BLAST search was conducted against type specimens in GenBank and analysed phylogenetically by the Maximum Likelihood method. Five species belonging to the genus *Dactylonectria* (D.), and three of the genus *Ilyonectria* (I.) were found. The most prevalent black-foot species was *D. macrodidyma* (n = 32), followed by *D. novozelandica* (n = 15), *D. torresensis* (n = 10), *I. liriodendri* (n = 9), *D. pauciseptata* (n = 5), *D. valentina* (n = 1), *I. robusta* (n = 1) and *Ilyonectria* sp. (n = 4). The present study improves our knowledge on the etiology of black foot disease affecting Uruguayan nursery grapevines.

P.2 *Aspergillus* spp. causing Aspergillus vine canker on grapevine in Mexico.

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The genus *Aspergillus* comprises a diverse group of species with high economic and social impact. Aspergillus vine canker is relatively rare; the fungus infects vigorous new shoots and canes when the vine is being trained, entering through a wound. Recently, grapevines with symptoms similar to Aspergillus vine canker have been observed in Mexico, thus our aim was to study these diseased plants. Woody samples of symptomatic plants were obtained from vineyards established in Sonora, Baja California, and Guanajuato. Samples were sterilized with alcohol and fire and placed onto Potato Dextrose Agar medium. Seventeen isolates were obtained that showed similar morphology to the genus *Aspergillus*. The colony and microscopic characteristics were observed in Czapek Yeast Extract Agar and Malt Extract Agar. Phylogenetic analysis performed using calmodulin (CMD) and β-tubulin (BenA) genes revealed three species of *Aspergillus*. Nine isolates were identified as *A. niger*, seven as *A. tubingensis,* and one as *A. welwitschiae*. Pathogenicity studies were carried out using grapevine plants cv. Merlot. Eleven isolates were inoculated in the woody tissue through a wound and on leaves. *Aspergillus niger* A10BCMX, *A. niger* A8SMX, and *A. tubingensis* A13SMX were the most virulent, causing lesions of up to 2 cm in length after 50 days. On the inner side of a wound, inside the cambium, powdery black conidia were found. On the leaves, necrotic lesions, mycelia, and black conidia formed. This is the first report of *Aspergillus* species associated with Aspergillus vine canker in vineyards of Mexico.

P.3 Molecular methods in detection and quantification of *Diplodia seriata* and *Phaeomoniella chlamydospora* in vine plants.

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Grapevine trunk diseases (GTDs) are one of the most phytosanitary problems affecting vineyards, currently reporting 133 fungi species, divided into 9 families associated with them. In Chile, *Phaeomoniella chlamydospora*, *Diplodia seriata* and *Inocutis* sp. are the phytopathogenic fungi most frequently isolated from adult plants with GTDs. The diagnosis of these fungi by classical methods is a laborious process that takes a long time to be processed. In addition, the low growth rate in semi-selective media in relation to other microorganisms can generate false negatives due to overgrowth of these fungi, underestimating incidence levels. Consequently, methods based on molecular detection can be a complementary and effective tool for these wood fungi, being faster and more precise in their results. In the current study, 54 grapevine plants cv. Cabernet Sauvignon clone #337 on rootstock 110-14 was inoculated with *P. chlamydospora* or *D. seriata*. 10 months after inoculation it was detected and quantified both phytopathogenic fungi by qPCR in four zone, 5 and 15 cm over and below from inoculated zone. In our result, it was detected the growing of both fungi in the distal zone of 27 plants analysed for *P. chlamydospora* and 24 of 27 plant infected for *D. seriata*. The above validates the complementary use of this molecular tool along the classical methods in the diagnosis and detection of *P. chlamydospora* and *D. seriata* and enables their detection in asymptomatic wood as in the propagation material.

P.4 Investigating the role of *Fusarium* spp. in young vine decline in California.

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From 2018 to 2021, young vineyards of wine and table grape have been observed showing decline symptoms in several counties including Fresno, Kern, Monterey, Napa, San Joaquin, Sonoma, Tulare, Yolo, and Yuba, in California. Symptomatology was diverse and characterized by poor or no growth during the season, dieback, sap exudation and discoloration of the vascular tissue around the graft union. Absence of feeder roots and graft failure has also been observed. In 10 vineyards, the estimated decline prevalence ranged from 5 to 50 %. Isolations were performed from the margin of the vascular discoloration of affected vines by placing wood sections (1×1 mm) onto acidified potato dextrose agar (APDA) and incubated for 7 days at 25 °C in the dark. Although different fungal pathogens were obtained, such as *Botryosphaeriaceae*, *Phaeoacremonium* and *Cylindrocarpon*-like species, colonies of *Fusarium* were present in all the isolates plant samples. Pure cultures were obtained from single hyphal tips and further identified using a phylogenetic approach. After DNA extraction, the translation elongation factor 1-alpha (tef1) and the RNA polymerase II second largest subunit (rpb2) partial gene regions were amplified and sequenced using the primers EF1/EF2, 5F2/7cR and 7cF/11aR, respectively. Consensus nucleotide sequences were used to search the closest species in the NCBI database using BLAST. Phylogenetic trees revealed the occurrence of 13 species, members of the *F. fujikuroi*, *F. oxysporum*, *F. solani*, *F. sambucinum* and *F. incarnatum-equiseti* species complexes. The most frequent species (47.4 %) was *F. annulatum* (*F. fujikuroi* species complex) and pathogenicity was confirmed completing Koch's postulates in one-year-old 'Chardonnay' vines (Bustamante et al. 2022). Currently, the 12 remaining species are being tested for pathogenicity assays in both 'Chardonnay' and '1103P' vines.

P.5 *Diaporthe* spp. associated with dieback in Baja California vineyards.

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Phomopsis dieback is a grapevine trunk disease caused by several species of the *Diaporthe* genus. The main symptoms in affected plants are leaf spots, growth retardation, smaller bunches, fruit rot, and plant dieback. Some of the species associated with grapevines are *D. eres*, *D. viticola* and *Diaporthe ampelina* being the late one of the most virulent species. In Mexico, the cultivation of vines is of great socio-economic importance, mainly in Sonora and Baja California. There are reports of species associated with Botryosphaeria dieback and Eutypa dieback but, so far, there are no reports of *Diaporthe* species in the country's wine regions. Therefore, the objective of this work was to isolate and characterize fungi of the genus *Diaporthe* associated with grapevines. Isolates with morphology features similar to *Diporthe* spp. were obtained from grapevine plants with dieback symptoms in different vineyards of Baja California. The identification of isolates was made by morphology characterization and molecular analysis using the ITS and EF1-α markers. Through phylogenetic analysis allowed the identification of strains belong to the species of *D. ampelina, D. eres*, and *D. foeniculina*. Pathogenicity tests are being carried out. Although these species have been identified in vineyards worldwide, to our knowledge these are the first reports on grapevines in Mexico.

P.6 Hymenochaetaceae fungus *Arambarria destruens* associated with Grapevine Trunk Diseases in Chilean patrimonial vineyards.

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Grapevine is one of the most important fruit crops in Chile with 141,000 ha for wine production. Grapevine Trunk Diseases (GTDs) are well studied in commercial cultivars such as Cabernet Sauvignon, Sauvignon blanc, Merlot and Chardonnay, but there is lack of information regarding these diseases in Patrimonial Vineyards, mostly from País, Moscatel, Cinsault and Carignan cultivars. One of the most frequent symptoms observed in Patrimonial Vineyards were trunks showing yellowish-spongy-wood cankers. The objective of this work was to identify the fungal species associated with these symptoms. A survey was conducted from 2019 to 2021 on Patrimonial Vineyards, in the south of Chile. Fungi were isolated in quarter strength potato dextrose agar (PDA) amended with antibiotics and subsequently purified on PDA. Yellow cottony colonies with irregular margins and dark areas were consistently isolated from the cankers (18 %) and were preliminarily identified as Basidoimycetes based on microscopic structures like basidia and basidiospores. Mycelia was collected from the edge of 20 pure cultures growing on PDA. Genomic DNA was extracted and used to amplify the internal transcribed spacer (ITS) region and the ribosomal large subunit fragments (LSU). ITS and LSU consensus sequences were compared to reported ones and combined to perform a multigenic analysis. The alignments were individually edited, and then concatenated, and phylogenetic trees were performed using Maximum Parsimony and Maximum Likelihood algorithms. Based on the phylogenetic analyses, the Basidiomycetes species associated with the previously described symptoms was identified as the *Hymenochaetaceae* fungus *Arambarria destruens*.

P.7 Fungal species associated with grapevine decline in China.

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Grapevine is one of the most important fruit crops in China, with 765,018 ha of cultivation area what makes ranking the third in the world. In order to characterize fungal species within the roots associated with grapevine decline, field surveys were conducted in Beijing, Hebei, Ningxia and Yunnan Provinces of China in 2021. Root samples were collected from symptomatic plants showed stunted growth, small leaves with yellow lesions, uneven size fruits, and necrotic vascular or xylem, as well as from neighbouring symptomless plants. Tissue isolation was conducted and isolates were identified based on morphological characterization and multi-gene phylogenetic analysis. 93 isolates were obtained from symptomatic samples, among which *Clonostachys* accounted for the highest proportion (40.9 %), followed by *Neocosmospora* (25.8 %), *Fusarium* (16.1 %) and *Dactylonectria* (5.3 %). *Rhizoctonia*, *Botrytis* and *Meyerozyma* were also isolated. As for asymptomatic samples, 119 isolates were obtained, the four most frequent genera were the same as symptomatic group, while and the ratio of *Fusarium* was the highest (27.7 %). More genera were isolated including *Robillarda*, *Cylindrocladiella*, *Lasiodiplodia*, *Lophiostoma*, *Sacrocladium*, *Acrocalymma*, *Sporothrix*, *Stagonospora* and *Acremonium*. According to the phylogenetic result, isolates belonging to *Fusarium* were clustered with 3 species, *F. oxysporum*, *F. commune* and *F. clavum*. *Neocosmospora* was identified as *N. solani*, *N. falciformis* and *N. pisi*. *Clonostachys* isolates were all identified as *C. rosea*. In conclusion, main fungi isolated in this study was *Nectriaceae* including *Neocosmospora* spp., *Fusarium* spp., *Dactylonectria* spp. and *Cylindrocladiella* spp., among which 5 species were first reported on grapevine. Pathogenicity of the species will be further assessed, and the relationship between fungal species of symptomatic and symptomless plants will be explored.

P.8 Characterization of the presence and distribution of grapevine trunk diseases in the vineyards of Quebec, Canada.

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Grapevine trunk diseases (GTD) are very damaging for the sustainability of the vineyard heritage in all major wine regions of the world. GTD can affect young plantations as well as aging vineyards. In both cases, the risk of contamination by these diseases is increasing in Quebec vineyards, as many of them are more than 20 years old, and many producers plan to expand. However, the presence and distribution of GTD is unknown in Quebec. The objective of this project was to characterize the distribution of GTD in Quebec vineyards according to different criteria, such as region, grape varieties and age of the vineyards. Five GTD were targeted: Esca (Petri disease) (*Phaeomoniella chlamydospora, Phaeoacremonium minimum*), Eutypa dieback (*Eutypa lata*), Botryosphaeria dieback (*Botryosphaeriaceae* spp.), Excoriosis (*Diaporthe ampelina*), Black foot (*Cylindrocarpon*-like asexual morphs.). Sampling was done in several vineyards and qPCR analysis were performed to detect GTD. Results showed the presence of these diseases in Quebec vineyards, mainly Botryosphaeria dieback. The knowledge gained from this project will allow us to establish a portrait of GTDs in Quebec vineyards, allowing us to study the epidemiology of these diseases and evaluate cultural practices to limit their spread.

P.9 Identification and quantification of Grapevine trunk and black-foot diseases pathogens in the soil, using real-time PCR coupled with HRM.

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Identification of plant pathogens and inoculum quantification in soil samples using conventional methods is rather labor-intensive and time-consuming. Therefore, the development of rapid, and simple to perform PCR-based identification methods that use pathogen-specific primers is necessary. Herein, a real-time quantitative PCR approach coupled with high-resolution melting (HRM) analysis was developed with one primer set to identify and distinguish several fungal species associated with grapevine trunk and black-foot diseases. In detail, the developed method targeted several *Cylindrocarpon*-like asexual morphs belonging to the genera *Ilyonectria* or *Dactylonectria* and the fungal species *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Diplodia seriata*. The technique's reliability was first assessed on DNA extracted from pure fungal cultures. The melting curve analysis of the amplicons allowed for the distinction of all target species with confidence levels > 99 %. For each targeted genera/species HRM curve profiles were generated. The identification of the target pathogenic species in the fortified soil samples was achieved in a range confidence between 60 - 75 %. The quantification of the detected pathogen DNA in the soil material was assessed with quantitative PCR and the sensitivity was evaluated using standard curve. The standard curve was constructed using serial dilution of plasmid calibrator containing the produced amplicon of qPCR. High PCR efficiency was reached (nearly 100 %), and results were extremely reproducible throughout the time of storage and calibration ranges for a specific plasmid calibrator. The reaction was linear over a large dynamic range (R2 > 0.99) of 5 log10 concentrations tested. The method was validated in soil samples obtained from commercial grapevine nurseries and the concentration of the detected DNA was found to range from 2 to 105 copies. This study provides the development of a new molecular tool to detect and quantify several GTD or Black foot pathogens in soil samples of grapevine nurseries and may contribute to the optimization of vegetative material production in grapevine nurseries.

P.10 Quantification of airborne inoculum of *Eutypa lata* and *Botryosphaeriaceae* spp. by real-time PCR in California.

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Annually pruned vineyards and orchards suffer significant losses each year due to diseases caused by *Eutypa* and *Botryosphaeria* fungi. Airborne spores of these fungi gain access to the vine through pruning wounds, from which they grow into the wood, causing cankers, dieback and declined growth. In California, the diseases caused by *Eutypa lata* and *Botryosphaeriaceae* spp. are commonly initiated during winter, when the seasonal rains trigger the spore release of these fungi. The objective of this study was to find out if the Rotorod- type spore traps can be used for capturing spores of *Eutypa lata* and *Botryosphaeriaceae* spp. in the vineyard air. Real-time PCR was used to detect and quantify numbers of the spores in the spore traps on weekly basis from December till April 2020-2022. Results from this study were comparable with those obtained either by using glass slides or volumetric spore traps, suggesting that *Eutypa* and *Botryosphaeriaceae* spp. spore release correlates with the rain events in California. However, differences were found between the release of the spores of these two groups of fungi. While *Botryosphaeriaceae* spores were present in the traps throughout the winter, *Eutypa* spores were detected more sporadically. Data from the current study also suggested that in some locations, the spores were released without the presence of rain, possibly due to high humidity. Differences were also found in spore release patterns between old and newly established vineyards. In younger vineyards, lower counts of *Botryosphaeriaceae* and *Eutypa* spores were detected compared to the older, established vineyards. The study also suggested that the disease pressure could differ significantly by the location of the vineyard and the spore trap. The release of the spores did not always correlate with the weather data, suggesting that the predictions of the spore release cannot be done based on the weather forecasts only.

P.11 Effect of cover crops on the dispersal of *Phaeomoniella chlamydospora*.

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The effect of different cover cropping on the dispersal of *Phaeomoniella chlamydospora* inoculum was evaluated in an experimental plot located in Piacenza (Italy) during two consecutive growing seasons. We hypothesized that the use of cover crops may act as a physical barrier to reduce *P. chlamydospora* airborne and rain-splash inoculum dispersal. Spore traps (microscope slides) were placed in plots with natural grass, bare soil and four or one cover crops grown in the first and second season, respectively. Grapevine cuttings artificially inoculated with *P. chlamydospora* were placed on the ground in the middle of every two subplots per treatment; in each subplot two spore traps were located near the inoculum source and 40 cm above it. The traps were replaced weekly from December 2018 to June 2019 and from December 2019 to May 2020. Quantitative PCR using specific primers for *P. chlamydospora* was used to determine DNA concentration in the spore trap samples and the effect of treatments on the inoculum dispersal. Rainfall and average temperature data were obtained from a weather station placed in the experimental site. Soil coverage was also evaluated according to cover cropping development. The dispersal of *P. chlamydospora* was associated with rainfall events in both seasons. Results from the first season showed no reduction in *P. chlamydospora* DNA detected in grass and cover crop plots relative to the bare soil. This result is most likely explained by nonuniform cover and plants development due to dry weather conditions. Second season results showed lower DNA concentrations in natural grass and cover crop plots, especially during weeks with higher levels of detection, which accounted for most of the spore dispersal. Analysis on the traps placed 40 cm above the inoculum confirmed a reduction in spore dispersal in grass and cover crop plots relative to the bare soil plots.

P.12 Study of environmental conditions influencing survival and reproductive structures development of *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*.

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In this study, we set up an *in vitro* experiment to determine the effect of temperature on the development of perithecia of *Phaeoacremonium minimum* and pycnidia of *Phaeomoniella chlamydospora* on grapevine cuttings. Pieces of 1-year-old grapevine cuttings of 110 Richter (110 R) rootstock were inoculated with four isolates of *Pm. minimum* (representing complementary mating groups) and two isolates of *Pa. chlamydospora* and were incubated at 5, 10, 15, 20, 25 and 30 °C under continuous white light. After 6 weeks, the cuttings were examined under a stereoscope to evaluate mature reproductive structures formation. Both species were able to produce abundant fruiting bodies at temperatures ranging from 15 to 25 °C, but *Pm. minimum* produced more perithecia at 25 °C and *Pa. chlamydospora* produced more pycnidia at 20 °C. At 30 °C, only very few reproductive structures were observed. Additionally, a field experiment was conducted in two vineyards located in Villar del Arzobispo (Valencia, Spain) and Villena (Alicante, Spain), in which one-year-old grapevine cuttings of 110 R rootstock inoculated with the above-mentioned isolates, were deposited into perforated aluminium trays covered with a plastic grid, being exposed to environmental conditions from December 2019 until June 2020. Cuttings were randomly collected every 15 days and were examined under a stereoscope to determine the presence/absence of fruiting structures. Moreover, fungal isolations were performed to verify the survival of the inoculated fungi. No fruiting bodies were observed during the experiment, but both fungal species were systematically recovered from the cuttings. A GLM analysis showed differences between species and localities in the species survival along the time. Differences observed between *in vitro* and field experiments suggest that the development of reproductive structures is unfrequent in vineyards.

P.13 Comparing disease incidence of grapevine trunk diseases at different sites in the Tokaj wine region, Hungary.

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Grapevine Trunk Diseases (GTD) have a great impact on grape-producing countries worldwide. The presence of a GTD pathogen in the vine does not usually result in the immediate appearance of disease symptoms. Moreover, there is still limited information on the importance of environmental factors on disease incidence. This research, conducted in Hungary, aimed to estimate the occurrence of GTD in the Tokaj wine region, and to gain knowledge about which biotic and abiotic factors affect disease incidence. Four vineyards within 15 km, with different topology, soil types, varieties, and ages were studied, and monitored between 2016 and 2019. Parallel to this study, in the same period, 50 random sites across the Tokaj wine region were selected in order to monitor the GTD symptoms in different vineyards. Results of this study indicated that the topology, slope characteristics, and soil type were the most important abiotic factors affecting the incidence of GTD symptoms. The biotic factor with the greatest effect was the age of the vineyards, with disease incidence increasing with age. Neither disease incidence nor any biotic or abiotic factors could be correlated with the infection incidence, but there were significant differences among the sites. The slope characteristics of the sites was also a factor influencing symptoms incidence. August was the best month to pick the infected vines in August, as the symptoms were observed more frequently. The importance of removing the dead parts from the sites was also an important factor in the disease incidence. Our results suggest that infected cuttings can act as the primary infection source of the healthy vines.

P.14 Spring shoot thinning wounds are susceptible to grapevine trunk disease pathogens.

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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 undertaken during the spring to improve airflow for reduced foliar disease, increase leaf exposure to sunlight for increased photosynthesis, maintain crop yield and quality, and to reduce the number of pruning wounds in the following winter. 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. A preliminary trial was established on Shiraz vines grown in pots in a shade house. In November 2020, green shoots were either cut with secateurs 1 cm above the second or third node leaving a smooth pruning wound, or the whole green shoot was torn off at the joint between lignified cane and the base of the shoot, leaving a rough socket wound. Wounds were artificially inoculated with 200 spores of *Eutypa lata* (ED) or *Diplodia seriata* (BD). Spurs were removed 9 months later and assessed for presence or absence of the pathogens. Pathogens were recovered from 62 and 69 % (*E. lata*) and 96 and 91 % (*D. seriata*) of pruning and socket wounds, respectively. No pathogens were isolated from uninoculated controls. With these results, and the reports of detection of ED and BD pathogen spores throughout spring and summer, it is now important to determine the risk of infection in the vineyard under natural conditions following shoot thinning activities.

P.15 Investigating how *Lasiodiplodia brasiliensis* colonizes grapevine tissues.

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Lasiodiplodia brasiliensis is one of the most virulent species among *Botryosphaeriaceae*. It was recently reported in Sonora and Baja California, Mexico. Little is known about how *Botryosphaeriaceae* interact with grapevines during the infection process. This work aimed to evaluate the colonization of *L. brasiliensis* in the grapevine using histological techniques. One-year-old rooted cuttings of cv. Cabernet Sauvignon were inoculated by mechanical wounding and maintained in greenhouse conditions for 2-months. Subsequently, transverse and longitudinal sections of 70 µm thickness were obtained and stained with 0.1 % Toluidine B for phenolic compounds; 5 % iodine and 10 % potassium iodide for starch; 0.001 % Sudan black IV for suberin deposits; 0.1 % phloroglucinol-HCl and Mäule stain for lignin and 0.02 % Calcofluor M2R White and 0.5 % and Congo Red for cellulose and hemicellulose. Hyphae were observed using the Fontana-Masson stain. Observations were done in a Nikon Eclipse E200 microscope with a camera AxioCam HRc and epifluorescence microscopy on an Axio-Vert200 microscope supplied with a HBO100 100W Mercury Lamp with ebq100 power. Cellulose and suberin were observed using a DAPI filter (excitation at 330–380 nm, emission at 420 nm), and a TEXAS RED filter (excitation at 542–595 nm, emission at 644 nm). Infected plants lacked starch in ray's parenchyma; and cellulose, hemicellulose, and lignin in the lesions. Phenolic compounds and suberin were observed in the cork and vascular cambium, vascular bundles, and pith. The fungus colonized the vascular cambium, vascular bundles, occlusions, and pith. Melanized hyphae were observed mainly in the pith. According to this, *L. brasiliensis* is able to overcome the defense mechanisms of the plant and modify its cell wall, mainly degrading hemicellulose and using starch as a carbon source; over time, it degrades lignin and suberin, colonizing the rays and inducing the formation of the typical Botryosphaeria canker.

P.16 Endophytic mycobiome and anthocyanidins, two key features involved in grapevine leaves affected by 'tiger stripes'.

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A well-known symptom manifest in grapevines affected by grapevine leaf stripe disease (GLSD) and Esca proper is the 'tiger stripes' pattern on leaves. In red grape varieties, affected leaves display interveinal necrotic lesions, followed by a red portion of lamina, chlorotic tissue, and green tissue, the latter being closely associated to the main veins. Yet, two more coloring patterns are occasionally found in symptomatic leaves. One follows the sequence: necrotic-purple-green tissues, and some authors correlate it to the black dead arm syndrome (BDA); the other follows the sequence: necrotic-green tissues, and it is found in pre-apoplectic shoots. Despite these appreciable differences, it is still unclear the identity of the triggering factor(s) that lead to different coloring patterns, the anthocyanidin profile of symptomatic leaves, and the involvement of the endophytic mycobiome. In this study, we investigated the endophytic mycobiome composition of grapevine leaves manifesting GLSD and BDA-associated symptoms, as well as their anthocyanidin profile. We studied cultivars Cabernet Sauvingon and Touriga Nacional, sampling symptomatic and asymptomatic leaves in July 2020 and July 2021, in a vineyard in Lisbon. To unveil the mycobiome profile, we used DNA metabarcoding (Illumina® NGS), targeting the ITS1 region, employing primer set ITS1F2-ITS2. The anthocyanidin profile was analyzed using high-performance liquid chromatography. The leaves mycobiome analysis revealed 125 taxa, detected at relative abundances greater than 0.1 %. Among the most represented, we found Oomycete *Plasmopara viticola*; Ascomycetes *Cladosporium* spp., *Aureobasidium* spp., *Stemphylium* spp.; and Basidiomycetes *Sporobolomyces* spp. and *Filobasidium* spp. We detected significant differences in alpha and beta diversity when examining sampling year, cultivar, and symptom type, as well as taxa over- and under-representation. Similarly, the anthocyanidin profile, dominated by peonidin and cyanidin, differed quantitatively and qualitatively when examining cultivar and symptom type. Overall, this data suggests strong links among symptom coloring pattern, plant response and mycobiome profile.

P.17 High diversity of fungal grapevine trunk pathogens isolated from one-year canes including the first detection of *Neofabraea kienholzii* in Albariño cv grapevine in Spain.

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Albariño is a grapevine cultivar from NW Spain and North of Portugal which produces an excellent white wine with an increasing exportation rate worldwide. During the last years, reducing productivity was observed in a mature Albariño vineyard located near the Miño river between Galicia and Portugal. In 2021, 90 grapevine plants from three different plots were labelled and monitored. After pruning in January 2022, one-year canes were carefully analyzed looking for grapevine trunk disease (GTDs) symptoms. A total of 450 pure cultures and about 70 morphotypes were obtained. One representative isolate for each morphotype was selected. Genomic DNA was extracted, and the Internal Transcribed Spacer (ITS) region was amplified and sequenced by Sanger dideoxy sequencing technology. Blast searches classified our samples into at least 18 species in 9 different genera: *Botryosphaeria*, *Cadophora*, *Diaporthe*, *Diplodia*, *Neofusicoccum, Neopestalotiopsis, Pestalotiopsis, Phaeoacremonium* and *Phaeomoniella* involved in GTDs complex. Furthermore, in this survey, an isolate of *Neofabraea* was detected which could be involved as well in GTDs. Further work is still in progress order to accurate this identification. On the other hand, about 20 species of endophytes with potential activity against GTDs were found including *Alternaria*, *Arthrinium*, *Aureobasidium*, *Clonostachys*, *Cladosporium*, *Epicoccum* and *Trichoderma*. Next-generation sequencing gives us comprehensive information about microbial biodiversity and dynamics whereas morphological traditional methods let us to cultivate microorganisms to be able to perform subsequent experiments to evaluate the potential antagonisms of these endophytes. This study also states a high cultivable mycobiota associated to grapevine annual shoots obtained by sampling method that is not destructive for the trunk.

P.18 BIOBESTicide project: Action of *Pythium oligandrum* on grapevine trunk diseases and its impact on microbial communities.

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Grapevine trunk diseases (GTDs) have become a major concern in viticulture (15% yield loss). Since the 2001 ban on the use of sodium arsenate, the development of alternative control methods, such as biocontrol, has become increasingly important. Among the promising microorganisms, the oomycete *Pythium oligandrum* is known to improve plant health by increasing their natural defenses and reducing GTDs by at least 40%. The BIOBESTicide project (BIO-Based pESTicides production for sustainable agriculture management plan) aims to commercialise the production of a biopesticide solution to fight GTDs. The efficiency of a *P. oligandrum* based formulated product will be evaluated from the nursery to vineyards and the environmental impact of the product will be assessed to ensure its safety. One of the objectives supported by INRAE is to get a better understanding of the role of plant microbiota in plant health, and more generally, the effectiveness of biocontrol treatments. This objective is divided in two parts: 1) assessing the impact of the biopesticide on microbial communities of the vine through a microbial community diversity approach, and 2) understanding the factors contributing to the success or failure of the biopesticide. Thus, an experiment on 240 grafted vines (Merlot grafted onto rootstock SO4) in semi-controlled conditions was carried out in a greenhouse. Vines were treated with a *P. oligandrum* formulation or left untreated and were inoculated with fungal pathogens involved in GTDs: either *Neofusicoccum parvum* or *Phaeomoniella chlamydospora*. At different times during three-months growth plants were destructively harvested and different plant compartments (leaves, wood and rhizosphere) sampled. Potential changes in the microbial communities associated with GTD pathogen or *P. oligandrum* inoculation was evaluated using an Illumina high-throughput sequencing approach. The results obtained will extend the approval dossier which will be submitted in all European countries to ensure the environmental safety of the product used.

P.19 It will be possible to predict Esca symptoms manifestation based on the wood microbiome?

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Esca is the most destructive grapevine disease worldwide. A large range of fungi have been described as causal agents of Esca because of its isolation from degraded wood in leaf-esca symptomatic vines. The aim of this work was to compare both culture-dependent and non-culture dependent methods to identify and quantify present fungal community. Twenty four wood samples from six vines (3 leaf-esca symptomatic + 3 asymptomatic) were analysed in three different ways: *i*) Culture dependent microbiological analysis; *ii*) Non culture dependent microbiome analysis using Illumina Technology and *iii*) Quantification of pathogens *Phaeoacremonium minimum* (Pm) and *Phaeomoniella chlamydospora* (Pch) by TaqMan q-PCR. To determine the most informative point for detection of pathogens within a vine, four different sample points were compared: above the graft (A), at the strain cross (B) and arms (C, D). Fungus such as Pm, Pch, *Diplodia seriata*, *Diaporthe ampelina* and Basidiomycota species associated to Esca were found using both cultured and non-cultured dependent methodologies, as it was for other genera like *Penicillium*, *Acremonium*, *Alternaria* and *Epicoccum*. The number of OTUs found in culture (11) represented only 0,73 % of OTUs found using Illumina (1512). There was a significant effect of the methodology in the α-diversity indexes. The sampling point exerted a no significant effect on the wood microbiome. The pathogen Pch was detected in 22, 9 and 4 samples using Illumina, TaqMan q-PCR and culture dependent methods, respectively. The pathogen Pm was detected in 6, 5 and 10 samples respectively, showing a higher representation using culture dependent analysis. A non-metric multidimensinal scaling (NMDS) analysis of β-diversity showed discrimination of fungal communities coming from Esca affected vines (with manifestation of “tiger stripe” / apoplexy in canopy). Some species have been selected as indicators of apoplexy. Finally, when modelling the relationship between network properties and the disease phenotype, samples without Esca symptoms tend to have a lower proportion of co-exclusions on average.

P.20 Effects of temperature on *in vitro* biocontrol of *Diplodia seriata* by psychrotolerant *Pseudomonas* strains.

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Timely preventive control for Botryosphaeria dieback is essential for disease management; biocontrol agents have this protective effect. Conversely, from an epidemiological point of view, it is important to consider that the infectious process can occur at different temperatures. Therefore, knowledge of the biocontrol capabilities of microorganisms at different temperatures allows us to improve the selection of biocontrol agents. The *in vitro* biocontrol capabilities of four psychrotolerant *Pseudomonas* strains (CpR2b, GcR15a, TmR1b, TmR8) on the mycelial development of three *Diplodia seriata* isolates (2120, 2142 and 2183) were evaluated using the agar diffusion method at low (8 °C), medium (20 °C), and high temperatures (35 °C). At 7, 14, and 21 days after the confrontation, the internal growth radius of *D. seriata* was measured. Our results showed that the three *D. seriata* strains grew at 8 °C, 20 °C, and 35 °C. Interestingly, two strains of *Pseudomonas* showed a consistent inhibition of the mycelial growth of all three *D. seriata* isolates at 8 °C and 20 °C. However, at 35 °C a higher variation in biocontrol efficacy was observed.

P.21 Phylogenetic host range in the Botryosphaeriaceae.

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Botryosphaeriaceae is a diverse fungal family composed of endophytes, pathogens, and saprobes, and is ubiquitous across all terrestrial plant biomes. Anthropogenic movement of some fungal taxa, for example, on infected host material, is a major concern as the family includes species known to be some of the most aggressive pathogens in endophyte communities. In grapevines (*Vitis vinifera* L.), Botryosphaeria dieback is one of the most devastating grapevine trunk diseases, generating significant economic losses worldwide. Prevention of novel infections remains the most effective management tool, but it is difficult to identify highly virulent isolates before they emerge. Phylogenetics are a useful tool for describing pathogens likely to shift to novel hosts and the potential for disease emergence following host jumps. However, the taxonomy of *Botryosphaeriaceae* is still in flux, and we have little phylogenetic information on the species infecting grapevines. Here, we reconstruct the phylogeny of *Botryosphaeriaceae* species infecting *V. vinifera*, using molecular sequence data, and explore the link between host breadth and phylogenetic relatedness. We first show the distribution of *Botryosphaeriaceae* hosts across the megaphylogeny of vascular plants. We then describe the large variation in host breadth among *Botryosphaeriaceae* pathogens, revealing the complex associations linking host and fungal phylogenies. This work provides a first step towards predicting emergence of a known pathogen on a novel host. We suggest our approach will be useful for coordinated global monitoring of high-risk species within *Botryosphaeriaceae*.

P.22 Grapevine below-ground microbiome analysis identifies *Fusarium* spp. aggravating the severity of grapevine trunk disease (GTDs).

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Grapevine trunk diseases (GTDs) are disease complex which are considered as major threat for viticulture. The microbiota colonizing the belowground part of plants can form complex associations with plants and play important roles in promoting plant productivity and health in natural environments. The primary aims of this study were to investigate below-ground fungal communities in symptomatic or asymptomatic grapevines. For this purpose, the spatial dynamics of the fungal communities associated with three soil-plant compartments (bulk soils, rhizospheres and roots) were characterized by ITS high-throughput amplicon sequencing across two years. The diversity and composition of fungal communities were largely affected by soil-plant compartments ($p < 0.001$, 12.04 % of variation explained) and sampling year ($p < 0.001$, 8.83 %), whereas GTD symptomatology exhibited a weaker but still significant association with these characteristics ($p < 0.001$, 1.29 %). The effects of the latter were particularly prominent in root and rhizosphere community comparisons. A few confirmed GTD-associated pathogens were detected in the communities, but their relative abundances were not correlated (or negatively correlated) to symptomatology. Further analysis revealed that *Fusarium* spp. were enriched in symptomatic roots and rhizospheres compared to asymptomatic counterparts, suggesting that their abundances were positively correlated with symptomatic vines. Inoculation tests revealed that *Fusarium* isolates, similar to *Dactylonectria macrodidyma* which is a pathogen associate with black foot disease, caused dark brown necrotic spots on grapevine stems in addition to root rot that blackened lateral roots. The disease indices were higher in the co-inoculation treatment than due to single inoculation with a *Fusarium* isolate or *Dactylonectria macrodidyma*, suggesting that *Fusarium* spp. can exacerbate disease severity when inoculated with other known GTD-associated pathogens. These results demonstrate the effects of fungal microbiota of roots and rhizospheres on GTDs severity, while providing new insights into GTDs and potential control practices.

P.23 Enhancing the regrowth of vines following remedial surgery.

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Grapevine trunk diseases (GTDs) reduce yield, and cause vine decline and death. Remedial surgery has long been used to rejuvenate grapevines and has more recently been demonstrated to control GTDs in Australia. Current recommendations are to cut out all visibly affected wood, and train watershoots to replace the vine. However, there are reports of as little as 42 - 55 % of 30-year-old trunks producing shoots following surgery in Shiraz and Cabernet Sauvignon. A trial was established in four rows of a 22-year-old Cabernet Sauvignon vineyard in the Clare Valley, South Australia with 100 % incidence of GTD, to evaluate methods reported anecdotally to induce watershoots on trunks following remedial surgery. Treatments included hitting with a hammer, making an X-shaped cut with a tomahawk, rubbing bark with a wire brush, application of the plant growth regulator cyanamide (Dormex) and grafting with a chip bud, and surgery was performed in winter or spring for comparison. Over each of the following 3 years, a further four rows were subjected to remedial surgery, and stumps without a watershoot were chip bud grafted. In the final year, watershoots were retained from the previous year. Results indicated that natural watershoot production was 83-85 %, with no difference between the season vines were cut, and there was no influence of most treatments applied in the first year, except for grafting which increased shoot production by 11 %. In subsequent years, natural watershoot production varied from 70-78 %, and grafting trunks without watershoots increased shoot growth by 6-19 %. In the final year, retention of watershoots from the previous season resulted in 87-88 % shoot growth, similar to that of grafting in previous years. In conclusion, regrowth of vines subjected to remedial surgery for control of trunk diseases can be enhanced by grafting or retaining shoots from the previous year.

P.24 Management of grapevine trunk disease with remedial surgery.

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Remedial surgery is increasingly being used by growers in New Zealand to improve yields and extend the lifespan of vines infected with grapevine trunk diseases. Infected vines are “renewed” by removing the diseased trunk above the graft union and growing a new shoot to replace the old trunk. Remedial surgery has been carried out in winter and spring over the last 3 years in mature (>18 years) commercial vineyard blocks of cvs Sauvignon Blanc, Cabernet Sauvignon and Merlot to study over time the disease and efficacy of remedial surgery, and to determine the optimal time to carry out this practice. Dieback was widespread in the canopy of cvs Sauvignon Blanc and Cabernet Sauvignon (> 90 %), and only in cv Merlot, did the incidence of dieback significantly increase from 64 % to 91 % over the last three growing seasons. There was an increase over this time, in the severity of dieback in cv Cabernet Sauvignon but not in cvs Sauvignon Blanc and Merlot. Vine recoveries after remedial surgery were high in cv Merlot. In cvs Sauvignon Blanc and Cabernet Sauvignon, some of the vines with more severe disease symptoms did not recover from remedial surgery. For all three varieties, there were no differences in recoveries between vines cut in winter or spring. The vines were out of production for the first growing season following remedial surgery but by the third year there were no significant differences in yield between these vines and the untreated control vines in cvs Cabernet Sauvignon and Merlot. In cv Sauvignon Blanc, a change in pruning strategies affected the yield in the reworked vines. There have been no obvious signs of trunk disease in the reworked vines to date.

P.25 GTD prevention: A practical application of *Trichoderma* formulations under field conditions.

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Grapevine Trunk Diseases (GTDs) are a major concern for grapevine growers all over the world. There are no curative fungicides for GTDs. Protection of pruning wounds have been demonstrated to be a successful strategy avoiding wood infection by pathogens. The use of biological control agents (BCA) to protect pruning wounds has been recently authorized for the Spanish Ministry of Agriculture to manage GTDs. In this work three biological products based on different strains of *Trichoderma* fungus were evaluated under two commercial vineyards. In the first assay three products were compared under the same field conditions in a vineyard cv Tempranillo with a 3 % of Esca symptoms manifestation and training in a head system. In the second assay, one product were evaluated in three different years in a vineyard cv. Macabeo with no foliar GTD symptoms and transformed from double cordon to double Guyot system. Evaluation of the treatments were made by microbiological analysis of wood samples (n=20) to isolate both fungal pathogens involved on GTDs and *Trichoderma* spp. Two times were evaluated: 30 and 90 days after application. Our results demonstrated that *Trichoderma* was well implanted in pruning wounds with values ranged 50 - 100 % and little differences between all three formulations. The Mean Percentage of Infection (MPI) was calculated resulting in low recovery of pathogens *Phaeomoniella chlamydospora* and *Botryosphaeria* species. This was calculated by the (number of GTD infected samples /number of total samples) · 100. The efficacy of the treatments were recorded by the mean percent disease control (MPDC) calculated on the basis of MPI of the control (no treated) as (100 · (1 - (MPI treatment/ MPI control). Our results suggest that *Trichoderma* could be more easily installed in head training system and that the efficacy is highly correlated to both the implementation of the BCA but also the disease pressure.

P.26 Biological control of inoculum of *Diplodia seriata* in pruning debris of *Vitis vinifera* in Chilean orchards.

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The greater availability and understanding of the use of biological control agents, with mechanisms other than those of chemical fungicides, has opened new opportunities for pathogen control, thus being able to new strategies based on the application of biocides taking into consideration the periods of susceptibility of tissues and the residual time length of these products. The incorporation of biological agents in control strategies, with the ability to control resistant and reproductive structures as sclerotia or picnidium, allow control the pathogens in new places and times, adding a different tool at the control strategies. For the above cited reasons, a field trial was carried out, where applications of the commercial formulation of *Trichoderma* spp. and *Bionectria ochreoleca*, Mamull® (Bio Insumos Nativa SPA, Chile), were carried out, on pruning remains of Cabernet Sauvignon vines with the presence of pycnidia of *Diplodia seriata*. Selected cane samples (N = 30), were observed under a magnifying glass, determining pycnidia number per cm², marking and coding 5 sectors of 1 × 3 cm. Later these samples were placed in the field, where they were sprayed with Mamull, 100 gr hL^{-1} by an herbicide bar 300 L ha^{-1}. At 30 and 360 days, measurements of the level of parasitism of the pycnidia and viability of conidia release were taken, by addition of sterile water. Both treatments showed a significant effect ($P < 0.05$, LSD) in the reduction of viable pycnidia, which increased from 25.3 % at 30 days to 72.3 % at 180 days, also showing a significantly higher level of parasitism ($P < 0.01$ LSD) than the control (12.3 %), at 30 days (34.5 %) and even higher at 180 days (59 %). This trial shows a promising additional application of biocontrol agents to wood pathogens control strategies.

P.27 Effect of the combined treatments with LC2017 and *Trichoderma atroviride* strain I-1237 on disease development and defense responses in vines infected by *Lasiodiplodia theobromae*.

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Grapevine trunk diseases (GTDs) constitute one of the major problems for viticulture worldwide, being Botryosphaeria dieback considered one of the most important of these diseases. The increasing restrictions on effective chemical fungicides to mitigate GTDs have led to more in-depth research on biocontrol agents (BCAs) and new and improved fungicides with more efficient and innovative methods for pathogen control. However, their integration as part of integrated disease management has only now started to generate interest. In this work, we aimed to (*i*) evaluate the effect of the combination of Esquive® (a *Trichoderma*-based product) and LC2017 (a low-copper-based product), in the control of *Lasiodiplodia theobromae*, on grapevines cvs. Cabernet Sauvignon and Touriga Nacional in greenhouse and, (*ii*) investigating their elicitor effect on plant defense responses, through the analysis of the expression of a set of genes from grapevine inoculated. The pathogen was always re-isolated from the infected tissues and able to cause wood discoloration. Touriga Nacional exhibited longer lesions than Cabernet Sauvignon and the application of both products did not appear to reduce lesion length when compared to LC2017 applied alone. The elicitor effect of LC2017 on grapevine defense was confirmed by gene expression analysis, and no significant differences were found between plants treated with LC2017 and with both products. Moreover, a specific response related to the cultivar was verified, but this apparently unique interaction between product, cultivar, and pathogen remains to be further investigated.

P.28 Will forestry waste be able to save the grapevines and help control GTDs?

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Grapevine trunk diseases are a serious challenge for viticulture. They cause significant economic losses on a national and international scale. The three main diseases - Esca, Botryosphaeria and Eutypa diebacks - are currently without reliable and effective control solutions available to the wine industry. The only existing one, sodium arsenite, has been banned since 2001 in France and 2003 in Europe. Numerous alternatives are being studied, including substances of natural origin. The wood of tree knots represents an important source of extractives that meet these criteria, since some of these compounds are being studied in human health and cancer research. Knots are a co-product of the wood and paper industries as they can be removed from certain processes for which they are prejudicial. Using wood extractives is a source of bio-based substances in the context of bioeconomy constituting also a way to valorise wood co-products through circular economy. Our project consists of evaluating the antifungal potential of an extract of Douglas knots (*Pseudotsuga menziesii*) against two pathogenic fungi involved in GTDs: *Neofusicoccum parvum* and *Fomitiporia mediterranea*. *In vitro* tests confirmed a fungal growth inhibiting effect with an EC_{50} of the product evaluated between 0.5 and 1.0 mg/mL for both pathogens. Douglas knots extract could thus be a good candidate for the treatment of GTDs because of their ability to inhibit the growth of certain pathogens. Knots extracts showed no lethality effect on grapevine callus of *V. vinifera* cv Gewurztraminer and we observed a protective effect of these extracts on detached canes when applied after inoculation of the pathogens. Additional *in planta* trials, in the greenhouse and then in the vineyard, will allow us to evaluate the potential of these natural substances in the context of the preventive control of GTDs.

P.29 Native isolates of *Trichoderma* spp. can protect pruning wounds against *Lasiodiplodia theobrome* in Argentinian vineyards, reducing the incidence of Hoja de Malvón disease.

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One of the principal way of sustainable managing of grapevine trunk diseases is to prevent opportunistic pathogen to entry through the pruning woods. The application of biocontrol organisms on pruning wounds could be a possibility to reduce infection from pathogens that cause the common disease in Argentina: called “Hoja de Malvón” (HdM). This name refers to the fact that a diseased grapevine leaf resembles a geranium leaf. The present study was carried out to determine the protective effect of native *Trichoderma* spp. isolates with the highest *in vitro* inhibitory potential against *Lasiodiplodia theobromae* (Lt) on pruning wounds. Lt is one of the most prevalent fungi associated with HdM at different pruning times. The vineyard trial was carried out in a completely randomized design with a factorial arrangement: 3 pruning time (early: June, medium: July and late: August) × 5 treatments (4 native *Trichoderma* spp. and a control). After pruning, the wounds were sprayed with each biocontrol agent (BA): ACB 3, ACB 4, ACB 20 and ACB 25 and 24h later were inoculated with 50 µL (105 conidia mL^{-1}) of the pathogen. Sixty canes per treatment were collected after 28 days and prepared for reisolation. Effectiveness was calculated as the mean percent disease control (MPDC). Data analysis revealed a significant ($P<0.03$) pruning time × treatment interactions for MPDC. All BA were reisolated from pruning wounds even 28 days after spraying. The best treatments were: in early pruning time ACB 3 (81 %), ACB 25 (69 %) and in medium pruning time ACB 3 (62 %). Also in June and July pruning time ACB 4 (59 %), ACB 20 (48 %), ACB 25 (43 %) reduced significantly the entry of the pathogen through the pruning wounds. Finally, MPDC of all BA in late pruning fell down below 40 %. Native isolates of *Trichoderma* spp. provided effective control against Lt mainly in early and medium pruning time, in the regional climatic conditions.

P.30 Impact of different grapevine bench grafting methods on the xylem anatomy, hydraulic traits and wood necrosis associated with young declines of grafted vines.

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Grapevine grafting is an essential practice in viticulture and over the years, various bench grafting techniques have been developed to mechanize the nursery process and to increase the yield in number of viable cuttings. Bench grafting is a fundamental nursery practice that can potentially affect the quality of propagation material also in young decline associated with grapevine trunk diseases and has recently been reported to influence leaf symptoms development associated with diseases of the Esca complex. The study aimed to investigate how three bench grafting methods (i.e. *i*) omega graft as mechanical technique, *ii*) whip and tongue graft as manual technique and *iii*) full cleft graft as semi-mechanical technique) can influence these phenomena. Specifically, the different methods were compared for their effect on the anatomical development of the grafting point and the functionality of the xylem, also considering two factors: cultivar (Cabernet Sauvignon, Glera and Teroldego) and scion/rootstock diameter (thin and large). Light microscopy observations on the anatomical evolution were correlated with the grafting methods and the investigated varieties. Significant differences between cultivars and/or grafting types were also detected in necrotic areas on the grafted tissues. Statistical analysis of the grapevine vessels suggested differences in xylem parameters between cultivars, while grafting type had no significant effects. On the other hand, the graft type significantly affected the intrinsic growth rate. The results confirm the potential incidence of lesions and dysfunctionalities correlated with the grafting method applied, which can potentially induce grafted vine declines in vineyards due to the necrotic area detected on the grafted tissues.

P.31 Bio-products partially protect grapevine pruning wounds against infection of the trunk pathogen *Lasiodiplodia theobromae*.

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One of the challenges related with grapevine trunk diseases in Argentina, called “Hoja de Malvón” (HdM), is the implementation of efficient, safe and organic control strategies. Bio-products derived from organic wastes that exploit the presence of beneficial microorganisms could be an alternative option. In the present study, aerated (AT), non-aerated compost teas (NAT) and bio-slurry (B) were evaluated to protect pruning wounds against *Lasiodiplodia theobromae*, one of the most prevalent fungi associated with HdM. In addition, sodium bicarbonate (SB), thiophanate methyl (TM) and an untreated control were included in the field trials. The compost was made from exhausted grape marc, goat manure, leaves from garden raking and alfalfa, which was used to obtain AT and NAT. B was brewed from fresh material in an anaerobic process. In total, twelve treatment combinations (two pruning times, July and August, and five products and control) were applied on grapevines cv. Malbec in a completely randomized design. After pruning, the wounds were sprayed and 24 h later inoculated with 50 µL (105 conidia mL^{-1}) of a conidia suspension of *L. theobromae*. Sixty canes per treatment were removed after 28 days and prepared for re-isolation of the pathogen. Control effectiveness was calculated as the mean percent disease control (MPDC). TM yield in more than 75 % of control in July and August. Although the effectiveness of AT (33 %), NAT (23 %) and B (24 %) were lower compared to TM, but they were higher than SB (12 %). All products showed a higher effectiveness in July than in August, where MPDC did not exceed 20 %. These findings suggest that beneficial microorganisms need adequate conditions, more time and repeated applications to become established on pruning wounds and before being challenged by the pathogen. This study is the first approach of reuse of agro-industrial residues as a sustainable control alternative against *L. theobroamae.*

P.32 Effect of hot-water treatment on grapevine viability and fungal trunk diseases pathogens diversity by RNA high-throughput amplicon sequencing.

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The effect of hot-water treatment (HWT) on fungal communities in grapevine is so far largely derived from studies using culture dependent methods. In this study, we investigated changes in the potentially active fungal communities of internal grapevine wood after two HWT protocols (50 ºC-45 min and 53 ºC-30 min) by RNA high throughput amplicon sequencing (HTAS) in several cultivar/rootstock combinations during two years. Fungal diversity was only reduced by both HWT protocols during the second year of study. The effect of HWT on grapevine trunk disease (GTD) pathogen abundances varied according to the fungal genera and the HWT protocol. In some cases, HWT increased the abundance of GTD fungi (i.e. *Diaporthe*) but in other cases, GTD abundances decreased after HWT (i.e. *Dothiorella*). In order to evaluate the viability of planting material, treated plants were planted in a vineyard immediately after HWT, and one, two or three months after treatments. Grapevine viability was high one month after treatment, but it decreased as the time from HWT to the establishment of the vineyard increased. Plants established 3 months after treatments had less than 90 % viability on 30 % of combinations in the first year, and more than 50 % of combinations were affected in the second year.

P.33 Sensitivity of fungal grapevine trunk pathogens to treatments with electrolyzed water *in vitro*.

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In this study, the effect of *in vitro* treatments with different electrolyzed water (EW) based products on the mycelial growth of grapevine fungal trunk pathogens was evaluated. The species studied were: *Botryosphaeria dothidea* (*Bd*), *Dactylonectria torresensis* (*Dt*), *Eutypa lata* (*El*), *Ilyonectria liriodendri* (*Il*), *Lasiodiplodia theobromae* (*lt*), *Neofusicoccum parvum* (*Np*), *Phaeoacremonium minimum* (*Pmin*) and *Phaeomoniella chlamydospora* (*Pch*). Agar discs with mycelium of each of these fungi were treated by immersion in each of the products. The treatments lasted 30 s, and 1, 5, 15 and 30 min. Mycelium survival (%) of each fungal species at the different products-time of treatment combinations was determined. The effect on conidia germination was also evaluated for *Cadophora luteo-olivacea* (*Clo*), *Dt*, *Il*, *Pm* and *Pch*. Conidial suspensions of each fungus adjusted to 20 · 107 conidia/ml were mixed with 950 µl of different EW products for 0, 15, 30, 60 or 300 s. Exposure was stopped by adding 9 ml of neutralizing buffer at pH 7.2. Drops (20 µl) of the spore suspension were plated on water agar and incubated at 25°C for 24 h. After incubation, the drops were observed for conidia germination quantification. All experiments were repeated once. The effect of the different products on the mycelial growth and conidial germination was variable according to the products, treatment times and fungal species. In general, the longest treatments were more effective to reduce mycelial growth and conidia germination. A significant reduction of *Dt*, *El* and *Np* mycelium survival was observed after 5 min treatments with one of the products. Regarding conidia, all product-time combinations showed more than 93 % germination inhibition relative to the untreated control. Electrolyzed water treatments showed promising results *in vitro* and further research will be developed to evaluate their effectiveness under nursery conditions.

P.34 The impact of sanitary status of scions regarding grapevine trunk diseases and various disinfectants on phenolic compounds in different parts of grapevine grafts of 'Cabernet Sauvignon'.

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The aim of this research was to study the responses of phenolic metabolisms in different parts of the grapevine graft (scion, rootstock cane and roots) after callusing and rooting to the different scion sanitary status, regarding grapevine trunk diseases (GTD) of cv. 'Cabernet sauvignon', and to the various disinfectants against GTD. Scions were (*i*) healthy (HLT) from heathy vines and (*ii*) asymptomatic (ASYM, without any visual symptoms) and (*iii*) symptomatic (SYM, visual necroses on scions) from GTD infected mother vines. Before grafting, the scions were treated with Beltanol, Serenade, Remedier, Bioaction, sodium bicarbonate and with a combination of Beltanol and thermotherapy (Beltanol+TT). After callusing, the wood of SYM scions treated with Beltanol and Remedier had a significantly highest flavanol content. After rooting, the graft classification revealed the greatest yield (79 %) of the first-quality grafts at Serenade but the lowest (55 %) at Remedier treatment. The rootstock canes of rooted grafts with HLT scions had 2.2-2.4-fold higher content of total analyzed phenolics than the rootstock canes of grafts with SYM and ASYM scions. Furthermore, at rooted grafts, the significantly highest flavanol contents were found in SYM scions treated with Beltanol, Beltanol+TT, Bioaction and Serenade, while in ASYM scions treated with Beltanol and sodium bicarbonate. The significantly lowest content of total phenolics (2.05±0.08 $mg.g^{-1}$) was measured in roots of grafts with HLT scions, but the highest (3.99±0.25 $mg.g^{-1}$) in roots of grafts with SYM scions. The results showed that sanitary status of scions has a significant impact on phenolic metabolism of entire graft, while biological disinfectants used in this study did not show any positive impact against GTD pathogens. The research suggests that Beltanol+TT, Remedier and Serenade treatments have given the most promising results regarding disinfection against GTD.

P.35 Evaluation of *Talaromyces pinophilus* as an antagonist of the causal agents of *Botryosphaeriaceae* spp. in grapevine.

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Climate change and the intensive management of current viticulture are generating an early decline in grapevine plants. There has been an increase of grapevine trunk diseases (GTDs) due to the banning of some of the available molecules used to control their causal agents, such as sodium arsenite, leading to a decrease in the productivity and longevity of vineyards. This has encouraged the search for new alternatives to control or minimize the impact of GTDs in vineyards, focusing mainly on products based on beneficial endophytic microorganisms. In this context, two isolates of *Talaromyces pinophilus* obtained from different geographical areas of Chile were morphologically characterized and identified with molecular techniques, and then evaluated as biocontrol agents of the main *Botryosphaeriaceae* species present in Chile; *Diplodia seriata*, *Diplodia mutila* and *Neofusicoccum parvum*. The antagonistic capacity of *T. pinophilus* was evaluated *in-vitro*, in dual cultures, with each pathogen, and a fungal extract of *T. pinophilus* was prepared and evaluated as a growth inhibitor of the pathogens *in-vitro*. At the same time, a suspension of *T. pinophilus* conidia was evaluated in unrooted vine cuttings, where it was possible to observe a reduction of the damage caused by the pathogens. According to the results obtained, *T. pinophilus* showed a better performance as a preventive biocontrol agent against the different *Botryosphaeriaceae* species, inhibiting the growth of the pathogens when applied 24 hours before both *in-vitro* and in unrooted vine cuttings. The growth inhibition *in-vitro* ranged between 7.7 % and 14.4 %, while its damage reduction in unrooted vine cuttings ranged from 68 % to 92 %. Additional studies should be performed with *T. pinophilus* to investigate its potential as a new alternative for GTDs control.

P.36 Effects of the moss extracts on plant pathogenic fungus causing Phomopsis cane and leaf spot of grapevine.

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In recent years, intensive work has been focused on possibilities to replace synthetic fungicides due to their negative effects on human health and the environment. One of the options is the application of biological active substances. Bryophytes are known to be less damaged by pathogens due to peculiar natural chemical content. This bryophyte feature brings the idea to test bryophyte extracts against crop fungal pathogens. The influence of ten different ethanol-based moss extracts on colony growth of the fungus *Diaporthe ampelina*, the causal agent of Phomopsis cane and leaf spot disease of grapevine was studied. Extracts of the following moss species were used in the experiment: *Abietinella abietina* (two accessions), *Dicranum polysetum, Fontinalis antipyretica, Homalothecium sericeum, Isothecium alopecuroides*, *Pseudoscleropodium purum, Racomitrium elongatum, Thuidium delicatulum* and *T. tamariscinum*. Three doses of each extract (5, 10 and 15 µl) were applied to test the colony growth inhibition of the fungus grown in Petri dishes with potato-dextrose agar. All applied extracts showed statistically significant inhibition compared to the control (no extract applied). More exactly, all three doses of applied extracts expressed significant fungal inhibition with the exception of *F. antipyretica* extract where only the dose of 15 µl showed significant inhibition. There were no statistically significant differences among moss extract treatments compared to each other. However, extracts of *A. abietina* (accession II) and *T. delicatulum* demonstrated the greatest inhibition effect towards the fungus. The results obtained clearly demonstrate the huge potential of mosses as environmental friendly treatments against fungal pathogens.

P.37 *In vitro* evaluation of *Trichoderma* native strains as potential biological control agents against *Phaeoacremonium minimum*.

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Biological control agents (BCAs) could be considered as an important option for minimizing the effect of Grapevine Trunk Diseases (GTDs). These BCA can be used into an integrated pest management with the aim to obtain as healthier vine plants as possible. One of the most successful BCAs due to its capacity to grow and colonize a broad range of substrates is a green spored ascomycetes fungus known as *Trichoderma*. Recent studies have proved an irregular performance of commercial products based on this fungus, thus, *Trichoderma* native strains isolated from vineyard could be an option to test in order to improve its efficacy. A total of 25 *Trichoderma* isolates from bark of grapevine plant located in Castilla y León region (Spain) were evaluated in order to test its capacity under *in vitro* assays against a native *Phaeoacremonium minimum* (Y038-05-03a) from Castilla y León region. A dual confrontation assay among *Trichoderma* species and one of the most virulent and prevalent pathogens in Castilla y León region was performed evaluating percentage of inhibition of the pathogen, sporulation on plate by *Trichoderma*, sporulation on pathogen by *Trichoderma* species and production of yellow pigment by *Trichoderma* in an agar medium as possible indicative of production of diffusible antibiotic compounds (DACs). *Trichoderma* strains that obtained a higher percentage of growth inhibition were T72, T74, T75, T77, T78, T105, T79, T80, T82, T84, T85 and T154. Among these previous *Trichoderma* selected, the ones that were able to sporulate over pathogen and plate significantly were T75, T79, T84 and T154. The *Trichoderma* isolate T106 produced a yellow pigment in agar medium and it could be interesting for further antibiosis assays. This experiment revealed the importance of identifying native strains *Trichoderma* as biocontrol agents and its potential of mass production regards to spore production and identification of other modes of action for future assays.

P.38 Effectiveness of Mamull® (*Trichoderma* spp. and *Bionectria* spp.) in the control of trunk wood disease in table grape and blueberry.

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Grapevine and blueberry trunk diseases are one of the main diseases, that causes severe plants losses in Peru in the last few years. Anamorphs of *Botryosphaeria* species, represent the major causal agents that infect pruning cuttings. In Peru, *Lasiodiplodia theobromae* dominates over other *Botryosphaereaceae* anamorphs. Chemical spray lacks of prolonged protection over time, and use of paste formulations is an expensive practice, has logistic complexity and is time consuming. Both factors have driven the development of sprayed biological control formulations. In this aspect, the aim of this work was to evaluate the effectiveness of a commercial multi-strain formulation of *Trichoderma* spp. and *Bionectria* spp. (Mamull®, Chile) in comparison with a chemical alternative. Greenhouse trials were established for each crop evaluating effectiveness of preventive and retroactive treatments. Results of preventive trials demonstrate a similar efficacy between biological and chemical products, being both statistically better than the control treatment ($P < 0.01$). In addition, protection time was extended up to 45 days. The retroactive trials showed that biological products controlled the pathogen infections with retroactive effect up to 48 hours, being statistically different from the chemical and control treatments ($P < 0.05$). These results reveal a high feasibility of using biological control agents, such as Mamull, for preventive and retroactive control of trunk disease, with better retroactive control compared to the chemical products, and avoiding the logistical problem of the pastes.

P.39 Efficacy of Tachigaren® (hymexazol 360 g/L) SL to suppress branch lesions caused by *Lasiodiplodia theobromae* in grapevine plants.

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Peru is currently the first fruit exporter of fresh table grapes in the world. *Lasiodiplodia theobromae* causes severe plant losses in the main producer areas of grapevine and other fruit crops. A field study was conducted to determine the efficacy of hymexazol in the control of this pathogen. Two isolates of *L. theobromae* recovered from affected grapevine plants were used in field experiments. The fungicide was used in three doses: T1: 2.0, T2: 2.5, and T3: 3.0 L ha ^{1}, applied by drip irrigation with post-plant inoculation. For inoculation, a 5-mm-diameter plug of the bark of the branch was removed with a cork borer, and a PDA plug of the same diameter colonized by *L. theobromae* was placed onto the exposed cambium. The inoculated area was covered to prevent wound desiccation. Nontreated plants, inoculated with each isolate, were used as controls. Results were evaluated 45 days after fungicide application. The area of the lesion developed in inoculated branches was measured. Lesions registered in plants treated with hymexazol were significantly lower than the nontreated control with both isolates. The factor “fungicide treatment” was significant ($P < 0.05$). The average reduction in lesion size for the treatments that displayed significant differences from the nontreated control ranged from 64 % for T1, 77 % for T2, and 82 % for T3. The use of hymexazol in an integrated management program may represent an interesting tool to control *L. theobromae* in grapevine plants.

P.40 Epidemiological survey of grapevine trunk diseases in the Eger Wine Region.

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The grapevine trunk diseases (GTDs) cause significant loss in the vineyards worldwide. Different fungal species have been recognized as GTDs pathogens, including polyphagous pathogens of forest trees. There is no completely effective plant protection management method for GTDs, therefore prevention has high importance. The aim of this study was to identify favourable environmental conditions associated with disease incidence (DI) to be considered before planting. More specific objectives were to confirm the relationship between DI and (1) cultivar susceptibility, (2) the proximity of forest vegetation, and (3) the vertical position on the slope. Twelve vineyards older than eight years were surveyed in two season in the Eger Vine Region, North-Eastern part of Hungary. Altogether 14,500 plants were monitored including 16 different cultivars. DI was surveyed separately for (1) “tiger strip” leaf symptoms or diebacks and (2) samples on 100 plants of 4 randomly selected, non-marginal rows in every surveyed cultivar of a vineyard. Season 2020 had a hectic weather with extremely high amount of precipitation in June and October, while season 2021 was more balanced. The DI ranged between 17 and 66 % in the Eger Wine Region. The lowest DI was detected in ‘Merlot’, while the highest DI was detected in ‘Olaszrizling’, ‘Sauvignon Blanc’ and ‘Olaszrizling’ white cultivars, as well as ‘Alibernet’ and ‘Syrah’ red cultivars. Ten samples were randomly collected for identification from each surveyed vineyard. Based on morphological characteristics species of *Botryospheriaceae* were mainly detected from symptomatic plants. Regarding the relationship with environmental conditions, DI was significantly higher in vineyards with close proximity to forest or located at the lower parts of slope.

P.41 Potential GTDs antagonist microfungi isolated from grapevines.

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The antagonistic microorganisms provide alternative control methods to control grapevine trunk diseases. However, there can be significant differences between the known and the newly discovered antagonistic species regarding their efficacy, safety and the cost of production. Marketed and recently isolated Ascomycetes mould species with potential biocontrol activity were studied. One *Fusarium*, two *Trichoderma* species (*Trichoderma afroharzianum* and *Trichoderma simmonsii*), and two *Clonostachys rosea* strains were tested. All strains were isolated from wood tissues of grapevine plants from Hungarian commercial vineyards. Strains were identified based on morphological characters and molecular sequences (ITS). Trichoderma species were identified based on tef1 similarities. Their growth and spore production were studied *in vitro*. Phytotoxic effect was monitored with detached leaf soaking in filtered broth following one-week growth of fungi in batch potato dextrose (PD) broth medium. Biocontrol potential of the strains was tested in plate confrontation assay against GTD pathogens (*Eutypa lata*, *Neofusicoccum parvum*, *Phaeoacremonium* sp., *Fomitiporia* sp.) on PD agar. The two *Trichoderma* strains showed 100 % biocontrol efficacy against each GTD pathogen in plate confrontation tests. The *Fusarium* sp. strain showed weak biocontrol potential, moreover phytotoxic effects of its metabolites were detected on plant detached leaf tests. All of the tested strains grew well both on solid media and in batch cultures, producing minimum 108 up to 109 spore.mL^{-1} on the fourth day.

P.42 Interactive effects of *Dactylonectria macrodidyma* inoculation on the rhizosphere and root microbiome of grapevine.

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Black-foot disease is a soil-borne disease caused by a broad range of "*Cylindrocarpon*"-like asexual morphs, including the widely distributed species *Dactylonectria macrodidyma*. In this study, we investigated the composition and the interaction of the rhizosphere and endosphere fungal microbiome of grapevine grafted plants inoculated with *D. macrodidyma* using ITS high-throughput amplicon sequencing. Root tissues and their associated rhizosphere soil of each plant were sampled at 0, 3, 9 and 16 months after *D. macrodidyma* inoculation. Pathogen inoculation did not affect species richness, diversity or community structure, but induced changes in the relative abundance of several microbial taxa. Inoculated plants increased the proportion of *Diaporthe*, *Cadophora* and *Glomus* in the roots but reduced that of *Trichoderma*. In the rhizosphere, SparCC network maps showed almost equal number of positive (n = 122) and negative (n = 118) correlations with *D. macrodidyma*. The black-foot pathogenic genus Ilyonectria had positive correlation with *D. macrodidyma* while the arbuscular mycorrhizal genus *Glomus* had negative correlation with the pathogen. In the roots, most of the major OTUs had positive correlation with *D. macrodidyma* (n = 49), including *Ilyonectria*, but the biocontrol agent *Trichoderma* had negative correlation. These findings have important implications for further studies on the grapevine/pathogen/microbiome interactions.

P.43 Evaluation of *Trichoderma atroviride* SC1 and *Bacillus subtilis* PTA-271 combination against grapevine trunk diseases pathogens in nursery propagation process.

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Fungal grapevine trunk diseases (GTDs) still represent a threat to viticulture, leading to important economic losses worldwide. In nurseries, grapevine planting material is very susceptible to infection by GTDs pathogens due to several cuts and wounds made during the different steps in the propagation process. Without effective chemical treatments, a combination of biological control agents (BCAs), could improve the plant material protection against GTDs pathogens in the nursery process. In this study, we evaluated the effect of single or combined treatments with *Bacillus subtilis* PTA-271 and *Trichoderma atroviride* SC1 to reduce GTDs pathogens infection in grapevine planting material during the propagation process. Our results showed a reduction in Botryosphaeria dieback (BD) incidence and severity on grapevine propagation material treated with Ta SC1 and the combination Ta SC1 + Bs PTA-271, and a reduction in Black foot (BF) incidence and severity on grapevine propagation material treated with Ta SC1, Bs PTA-271, and the combination of both. Therefore, the *Trichoderma atroviride* SC1 and *Bacillus subtilis* PTA-271 combination, showed the potential to reduce infections caused by some GTD pathogens in the nursery propagation process. This combination of biocontrol agents could be an asset to an integrated preventive approach where various strategies are combined to reduce GTD infections from nursery stages.

P.44 Incidence of newly described mycoviruses in *Diaporthe* sp.

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Diaporthe species are considered causal agents of Phomopsis dieback and one of the fungal groups of the grapevine trunk disease complex (GTD). Some types of mycoviruses may reduce the virulence of their fungal hosts, which provides a valuable tool for the development of biocontrol strategies. Our research is focused on detection and description of mycoviruses present in *Diaporthe* isolates collected mainly from *Vitis vinifera* L. Using traditional methods (dsRNA purification) and high-throughput sequencing (Small and Total RNA Seq), two RNA viruses were detected. Diaporthe mitovirus 1 (DrMV1), a putative member of the family *Mitoviridae*, has a (+)ssRNA genome and an approximate length of 2455 nt containing one ORF encoding the RNA-dependent RNA polymerase (RdRp). The second virus, Diaporthe negative-stranded RNA virus 1, (DNSRV1), seems to belong to the family *Mymonaviridae*, its (-)ssRNA genome is about 9372 nt long and has five ORFs, the largest encodes the RdRp and the other four ORFs have unknown function. Based on both viral RdRp sequences, specific primers were designed to screen the occurrence of DrMV1 and DNSRV1 by RT-PCR in a collection of 35 *Diaporthe* isolates from different countries and different species composed of 24 *Vitis* sp., 7 *Junglans* sp. and 4 Prunus sp. Thus, viral coinfections were detected in one *Diaporthe* isolate collected from Czech *V. vinifera*; one *Diaporthe* isolate from Austrian *V. vinifera* L., one from Czech *Juglans regia* L. and four isolates collected in *Prunus domestica* L. from Slovakia were positive for DNSRV1. Two isolates from Czech *V. vinifera* L. and *J. regia* L. were detected for presence of DrMV1. Our results show that 28.6 % of surveyed *Diaporthe* isolates contain a virus, and DNSRV1 appears to be more abundant within isolates from different plants than DrMV1.

P.45 Biological and physical protection of grapevine propagation material from trunk disease pathogens.

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Control of fungal trunk pathogens in grapevine nurseries is based on the application of an integrated management program (IPM), which includes several chemical, biological and cultural strategies. The application of hot-water treatment (HWT) and/or biological control agents has been recommended over the past years due to the restrictions and difficulties that chemicals are facing in many countries around the world. The aim of this study was to evaluate the most adequate time of HWT and *Trichoderma atroviride* SC1 (TCH) application, as well as their effect on fungal trunk pathogen infection. Two experimental trials were established: (*i*) HWT, TCH or HWT+TCH applications to cuttings in hydration tanks before grafting, and (*ii*) HWT, TCH or HWT+TCH applications to grafted plants after rooting in field nurseries. In both trials, treated plants were planted in a commercial vineyard. Black-foot and Petri disease fungal isolations were performed from the base of the rootstock and the roots at two different moments: after rooting in field nurseries and after one season in the vineyard. Black-foot disease control was low in both experimental trials. The application of HWT or HWT+TCH significantly reduced Petri disease infection mainly in grafted plants uprooted from field nurseries.

P.46 Composition of phytopathogenic fungal communities in grapevine leaves differ among sampling months, but not between organic and conventional management.

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Plant health is highly dependent on plant-associated microbes and despite recent advances, we still lack a systematic overview of the diversity of grapevine-associated fungi and how the grapevine microbiome is influenced by cultivation methods. In this study, we compared the diversity and composition of plant pathogenic fungal communities in grapevine leaves under an organic and conventional management. We hypothesized that the differences in fungicides used in conventional and organic vineyards would have a significant impact on the diversity and composition of the leaf-associated fungal community in grapevine. We generated DNA meta -barcoding data from leaf samples of the cultivar Bianca collected throughout the growing season at the experimental vineyard of the Eszterházy Károly Catholic University, Hungary. Quality-filtered sequences were grouped into Amplicon Sequence Variants (ASVs) that were assigned to taxonomic and functional groups using the latest version of the UNITE. In the rarefied dataset, 911 ASVs of plant pathogenic fungi represented 88 genera. We found representatives of 15 fungal genera known to be associated with grapevine trunk diseases (GTDs), e.g., *Phaemoniella*, *Diplodia*, *Stereum*, *Trametes*, and *Botryosphaeria* showed the highest ASV richness. Among non-GTD pathogens, *Cladosporium*, *Aureobasidium*, *Alternaria*, *Vishniacozyma*, and *Epicoccum* were the most diverse. The observed richness and abundance of GTD-associated fungi in leaves are noteworthy and confirm recent findings that many of these fungi are part of the core mycobiome of grapevine. Differences in richness and composition of plant pathogenic fungi did not differ between organic and conventionally managed grapevines, but significant changes were apparent among months, explaining the greatest compositional variation. We found that this strong temporal turnover of leaf-associated plant pathogenic fungi likely is caused partly by the application dates of different fungicides and possible differences in sensitivity among fungal species, particularly in mid-summer, and partly by seasonality, i.e. leaf maturation and the gradual onset of senescence by September.

P.47 Bismuth Subsalicylate, a fungistatic compound and plant defenses stimulator, with potential for the treatment of grapevine trunk diseases.

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Since the use of sodium arsenite was banned in 2001, we observe an increasing number of grapevines affected by grapevine trunk diseases (GTDs). The lack of highly effective control products leads to our research on bismuth subsalicylate (BSS). This compound has been used in pharmaceuticals like PeptoBismol® for decades. Here we evaluated the antifungal potency of BSS for grapevine trunk diseases pathogens, as well as its ability to stimulate plant defense genes, since BSS contains salicylic acid. The objective was to design of an appropriate formulation for BSS which is quite insoluble in water. We set up a suitable formulation based on polyethyleneglycols (PEG), with particle sizes small enough to penetrate plant vessels, an important feature for future developments. We confirmed, *in vitro*, the antifungal potency of the newly formulated BSS on fungi involved in GTDs, through the growth inhibition of *N. parvum* (isolates Bt 67 and Bourgogne), *D. seriata* (98.1) and *F. mediterranea* (PHCO36). The stimulation of defense genes was analysed by RT-qPCR on grapevine callus (VvPAL, VvEDS1, VvHSR1 overexpressed) and we confirmed the non-toxicity of BSS on grapevine cells by fluorescence microscopy techniques. BSS was then evaluated *in planta* by vertical plant endotherapy technique developped in the laboratory. This method consists of drilling the grapevine into the rotten wood and directly inject BSS. The overall goal was to apply BSS into the rotten wood where mycelial complexes are concentrated. A panel of 100 symptomatic grapevines have been treated for 2 years in Alsace and preliminary observations will be presented, taking into account the complexity of GTDs regarding the expression of symptoms.

P.48 Trunk BioCode – A deep metagenomic study of Grapevine Trunk Diseases in Portuguese vineyards and biosensor adaptation.

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Increasing the knowledge on the incidence of Grapevine Trunk Diseases pathogens and their interactions with the surrounding environment may bring new insights into vineyard protection strategies. This study aimed to investigate if grapevine cultivars influence certain fungal species prevalence in symptomatic grapevine wood. Two grapevine cultivars, Touriga Nacional and Aragonez, were prospected in Portuguese vineyards belonging to the different continental wine Appellations. The sampling consisted of symptomatic grapevine wood of Touriga Nacional (n=36) and Aragonez (n=61) grapevine cultivars. Necrotic woody tissues were cultured onto PDA medium amended with chloramphenicol, and the presence of GTD fungi colonies was assessed based on morpho-cultural characters and further purified. A distinct pattern was found among the two cultivars: *Botryosphaeriaceae* fungal isolates seem to be more prevalent in Touriga Nacional symptomatic wood, whereas in Aragonez the most abundant are fungal taxa related to Esca disease. Such results indicate that cultivars may contain specific fungus within the GTDs complex related to the presence of resistant/tolerant genes; however, further studies must be conducted to attain such conclusions. A metagenomic analysis is being applied to the same samples to validate the data obtained by traditional means and serve as the base for biosensor development, by identifying the most relevant fungus within the GTDs complex affecting Portuguese vineyards. This work is integrated in the TrunkBioCode project, which main aim is to develop molecular tools suitable for the early identification of GTDs in grapevine. One of the strategies is based on a Biosensor platform that is aimed to be used for the detection and identification of pathogenic taxa directly in field conditions.

P.49 *Sporocadaceae* species associated with grapevine trunk diseases in Cyprus.

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Grapevine trunk diseases (GTDs) are currently considered the most destructive biotic factor of grapevines, threatening the economic sustainability of the grape industry globally. Besides well-known diseases, there is an increased interest in wood-colonizing fungi that infect the woody tissues of grapevines, thus, recently new species are being described as causal agents of GTDs. To date over 140 fungal species have been reported in association with GTDs worldwide, however the degree of involvement for many GTD-related species remains elusive. In 2017, during a survey conducted in Cyprus, wood samples were collected from vines exhibiting typical GTD symptoms including decline, dead cordons and spurs. Based on morphological and multilocus phylogenetic analyses (ITS, LSU, TEF1, and TUB2) four species in the *Sporocadaceae* family were found in association with GTDs; two of the genus of *Seimatosporium*: *Seim. marivanicum* and *Seim. vitis-viniferae* and two in the genus of *Sporocadus*: *Spo. kurdistanicus* and *Spo. rosigena*. Pathogenicity trials with seven selected isolates, representative of each species, were conducted on woody stems of two-year-old potted grapevines of the cv. Xynisteri. All isolates were pathogenic, causing dark brown to black vascular discoloration of the wood tissue below the bark, extending upward and downward from the point of inoculation. However, *Sporocadus* isolates were significantly more aggressive than *Seimatosporium* with lesions length ranging from 9.24 to 6.90 and 4.13 to 4.00 cm, respectively. Successful re-isolations were made only from the inoculated vines with recovery percentages of 40 % for *Seim. marivanicum*, 35-67 % for *Seim. vitis-viniferae*, 28-44 % for *Spo. kurdistanicus* and 25 - 32 % for *Spo. rosigena*. This is the first report of *Seim. marivanicum*, *Spo. kurdistanicus* and *Spo. rosigena* in Europe causing symptoms on *Vitis vinifera* and suggesting a potential role of *Sporocadaceae* in GTDs complex.

P.50 Grapevine trunk diseases of cold-hardy varieties grown in Northern Midwest United States vineyards coincide with wounds and winter injury.

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Surveys to characterize the composition of grapevine trunk diseases have been conducted for most major grape growing regions of the world. Many of these major grape growing regions share similar mild Mediterranean-like climates that mainly grow traditional *Vitis vinifera* cultivars. Northern Midwest United States vineyards present a stark contrast to many of these other growing regions with an atypical climate that has cold (as low as -35 °C) snowy winters, wet springs, humid summers, wet autumns, and a short growing season. We conducted a survey to identify the most prominent fungal pathogens associated with the unique climate and cold-hardy interspecific hybrid grapevine varieties grown in the Northern Midwestern USA. From the 172 samples collected, 640 isolates obtained by culturing were identified by ITS sequencing and represent 420 sample-unique taxa. Of these taxa, opportunistic fungi of the order *Diaporthales* including species of *Cytospora* and *Diaporthe* were most frequently identified. Species of *Phaeoacremonium*, *Paraconiothyrium*, and *Cadophora* were also prevalent. Species of *Xylariales* and *Botryosphaeriales* which are frequently isolated in many other growing regions where only isolated in small numbers in our study. Additionally, no taxa in the *Phaeomoniellales* were isolated. The compounding effects of winter injury, pathogens isolated, and management strategies will be discussed as well as some of the difficulties researching, understanding, and effectively communicating scientific findings about grapevine trunk diseases with growers and stake holders.

P.51 The effect of dual inoculation (*Seimatosporium* species with/without GTD fungi) on lesion length (symptom expression) in Sauvignon Blanc vines.

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In a survey of the endophytic fungal diversity associated with grapevines symptomatic or asymptomatic for grapevine trunk-diseases (GTDs) carried out in Marlborough, New Zealand in 2018, several fungal pathogens were isolated. Among these, members of the Botryosphaeriaceae family, *Neofusicoccum parvum* (from symptomatic vines) and *Diplodia seriata* (from both symptomatic and asymptomatic vines) were recovered. These pathogens are considered latent and virulent GTDs. Additionally, two *Seimatosporium* species, *S. vitis* and *S. lichenicola,* were recovered for the first time associated with GTD fungi in New Zealand vines. Both species were isolated from symptomatic and asymptomatic tissues, but their role as pathogens and interaction within GTD complexes is unclear. This study investigated the interaction between these *Seimatosporium* spp. and *N. parvum* or *D. seriata* in the GTD complex and the effect on symptom expression. The outcomes of *in planta* dual inoculation experiments between *Seimatosporium* spp. and *N. parvum* or *D. seriata* isolated from the same wood cankers were evaluated. Detached Sauvignon blanc grapevine green shoots and two-year-old woody stems of potted grapevines were wounded and co-inoculated with mycelial colonised agar discs of *S. vitis* or *S. lichenicola* and *N. parvum* or *D. seriata*. Controls consisted of each fungal species inoculated alone. After 2 weeks for detached shoots and 4 months for attached shoots, lesion length and colonisation distance by re-isolation were assessed. In both assays, there were differences in the lesion lengths and pathogen movement for co-inoculation of both *Seimatosporium* spp. with *N. parvum*. In contrast, co-inoculation of either *Seimatosporium* spp. with *D. seriata* did not develop a lesion, although *D. seriata* were recovered at a distance of 5 cm upward and downward from the inoculation point. No lesions developed with *D. seriata, S. vitis,* or *S. lichenicola* inoculation alone. Our finding confirm that *Seimatosporium* spp. are involved in the GTD complex.

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