

ABSTRACTS

**Abstracts of oral and poster presentations given
at the 11th International Workshop on Grapevine Trunk Diseases,
Penticton, British Columbia, Canada, July 7–12, 2019**

The 11th International Workshop on Grapevine Trunk Diseases (IWGTD) was held in Penticton, British Columbia, Canada, on July 7–12, 2019. This workshop was chaired by Dr. Úrbez-Torres and organized by the Agriculture and Agri-Food Canada Plant Pathology Laboratory at the Summerland Research and Development Centre (SuRDC). The 11th IWGTD also marked the 20th Anniversary of the workshop, which has been organized every two years since 1999. Delegates were presented the first day with an overview of the Okanagan Valley wine grape growing region of British Columbia by Dr. Bowen, Research Scientist and Plant Physiologist at the SuRDC.

The workshop was attended by 151 participants from 21 countries. In total, 64 oral and 48 poster papers were presented in four different sessions, including Pathogen Detection and Identification, Epidemiology, Host-Pathogen Interactions, and Disease Management. In addition, a special session on Grapevine Trunk Diseases (GTD) control was organized by the organizing committee the morning of Friday July the 12th with the goal to provide the industry and grape-growers with a practical summary and overview of the current management options against GTD as well as the economic impact they cause. The invited speakers for this session were Dr. Gramaje from the “Instituto de Ciencias de la Vid y el Vino (ICVV)” in Logroño, La Rioja, Spain, who presented an overview on GTD management in nurseries and newly established vineyards; Dr. Sosnowski from the South Australian Research and Development Institute (SARDI), who presented an overview on GTD management in mature vineyards; Dr. Kaplan from the California State University in Sacramento, who presented an overview on the economic consequences of GTD and the potential gains from adopting preventative pruning practices and vine surgery; and Dr. Úrbez-Torres from Agriculture and Agri-Food Canada SuRDC, who presented an overview of the current status of GTD in Canada and their control.

Two field trips were organized during the workshop. The first field trip took place on the afternoon of July 9th with visit to vineyards in the North Okanagan. Delegates were presented with an overview of grapevine production in the Okanagan Valley followed by discussions on the main diseases affecting grapevines in the Valley, including GTD and visit to experimental trials where the SuRDC Plant Pathology team were conducting GTD spore trapping studies within the epidemiological research being conducted in British Columbia for GTD. The second field trip took place all day of July 11th and delegates visited two vineyards located in the South Okanagan Valley. The field trip was focused on presenting the current situation of GTD in The Okanagan Valley and management strategies being put in place.

It is worth to highlight that a student competition with awards to best oral and poster presentations was organized at the 11th IWGTD. This is the first time that such competition has been organized for the IWGTD. A total of 20 graduate students (MSc or PhD) participated in the competition. For best poster presentations, 1st place went to Pierluigi Reveglia, a joint PhD student between Charles Sturt University, Wagga Wagga, New South Wales, Australia and the University of Naples, Italy, for his paper “Isolation and characterisation of phytotoxins produced by the Botryosphaeriaceae and their role in grapevine trunk diseases”; 2nd place went to Edelweiss Rangel Montoya from the “Centro de Investigación Científica y de Educación Superior de Ensenada”, Baja California, Mexico for her paper “Characterization of species of Lasiodiplodia associated with grapevines in Mexico”; and 3rd place went to Daina Grinbergs from the “Instituto Nacional de Investigaciones Agropecuarias”, Chillán, Chile for her paper “Fungal pathogens associated to grapevine trunk diseases from patrimonial vineyards in Chile”. For best oral presentations, 1st place went to Jinxz Flamand from Agriculture and Agri-Food Canada Summerland RDC for his paper “Identification and characterization of potential biocontrol agents for the management of grapevine trunk diseases in British Columbia”; 2nd place went to Clément Labois from “Université de Haute-Alsace” Colmar, France for his presentation “Metabolomic studies of two V.

vinifera subspecies during infection by Neofusicoccum parvum, a Botryosphaeria dieback pathogen – On the road to identify infection and resistance biomarkers”; and 3rd place went to María del Pilar Martínez-Diz from the “Estación de Viticultura e Enología de Galicia” Ourense, Spain for her paper “Natural infections of pruning wounds by fungal trunk pathogens in mature vineyards in Galicia (northwest Spain)”.

The next 12th IWGTD will be held in Czech Republic in 2022 as the Council Members of the ICGTD decided that from now on the IWGTD will be organized every three years.



Invited lecture to celebrate the 20th anniversary of the International Council on Grapevine Trunk Diseases and Workshops

The mystery was young vine decline - the clue was black goo. LUCIE MORTON. *Morton Viticulture, PO Box 5607, Charlottesville, VA 22905, USA. E-mail: mortonviticulture@gmail.com*

A detective story unfolded in the world of viticulture that resulted in us being here today in beautiful British Columbia. Black goo was the term I coined to describe a dark, gummy ooze in xylem vessels that looks black to the naked eye. Without knowing the cause, my observation was that vines with the black goo symptom could appear normal for many years, decline prematurely, show intermittent dieback, or die suddenly. In 1990, I was asked to explain what would cause a very young vine to grow very vigorously up the stake, suddenly collapse in August, and grow very poorly, if at all the next year. Latent viruses and graft incompatibility, all supposedly linked to the susceptibility of the rootstock

variety were common diagnoses. Although no specialist in grape pathology, I was deep into research on grapevine rootstocks and did not find these explanations satisfactory. I thought that fungi should be given consideration along with viruses and viroids. Little did I know that opening the mind to fungi can be like opening Pandora's Box! Had the black goo symptom simply remained one of many in the complex syndrome of esca in old vines, it might never have caught my attention. But when it appeared in the rootstocks of young grafted vines, it was impossible to ignore. Wherever my literature searches and travel took me around the world, I sought out plant pathologists who might provide insight into the symptom of black goo. A stroke of good fortune came one day in 1995 after complaining to a friend at UC Davis about the poor-quality images in a photocopy Luigi Chiarappa's "Wood decay of grapevine and Black Measles (1959)." There was one grainy photo of sectioned arms inoculated with *Cephalosporium* sp. that looked as if it might show black goo streaking. She suggested that I contact Luigi directly as he should be had just retired to Davis. It was at Luigi's California home where the

International Council on Grapevine Trunk Diseases was founded on July 21, 1998. And it was his coordination with Laura Mugnai and Giuseppe Surico that led to the next ICGTD meeting coinciding with the International Workshop on Grapevine Trunk Diseases Esca and Grapevine Declines hosted by the *Mediterranean Phytopathological Union* in Tuscany, Italy, October 1-2, 1999. This 20th anniversary meeting is a testament to our founding vision that a voluntary and well-coordinated research effort is the best way to bring the swiftest resolution to any problem.

ORAL PRESENTATIONS

Pathogen Detection and Identification

Ten years of investigation on the fungi associated with GTDs in west Sicily. VINCENZO MONDELLO¹, SELENE GIAMBRA², GAETANO CONIGLIARO², L. SANTOS³, A. ALVES³ and SANTELLA BURRUANO².

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After the first report of *Lasiodiplodia mediterranea* in 2008, as causal agent of a grapevine decline, epidemiological surveys on western-Sicilian vineyards with symptoms of decline highlighted the widespread presence of several GTD-associated pathogens. To date, these diseases represent a serious menace for the grape-growing regions worldwide, since no efficient and simple method is available for their control. We report the results of a 10-year study (2008-2018) on the taxonomy, pathogenicity and phylogeny of fungi associated with GTDs in West Sicily. In detail, observations were carried out in seven vineyards (cv. Alicante Bouchet, Grecanico, Grillo, Inzolia, Merlot, Muller Thurgau) located in three different provinces (2 in Agrigento, 1 in Caltanissetta and 5 in Trapani). Vines were visually inspected for GTDs symptoms, all the diseased vines were uprooted and submitted to laboratory for analyses. Fungal isolates were collected and identified at species level both traditionally and by molecular methods, using ITS and/or EF1- α regions. The latter were also used in phylogenetic analyses of Botryosphaeriaceae, the most abundant group isolated during this study. The pathogenicity of the col-

lected strains, including different isolates of the same species were studied through *in planta* artificial inoculations. The results of our study, confirming the presence of various GTDs also in western Sicilian vineyards, showed a great abundance of Botryosphaeriaceae such as *Diplodia seriata*, *Neofusicoccum parvum*, *N. vitifusiforme* and *L. mediterranea* associated to wood necrosis in mature vineyards. As expected, the four Botryosphaeriaceae species showed different virulence producing significantly different vascular discoloration length. In young vines *Phaeoconiella chlamydospora*, *Phaeoacromonium* sp., *N. parvum*, *Neonectria* sp., *Ilyonectria* sp. and *Cadophora luteo-olivacea* were found associated to wood necrosis/dicoloration. Interestingly, these fungi were selectively associated to specific symptoms and/or trunk parts, especially in the areas at root level and around the grafting point. Pathogenicity tests of some of the other fungi collected are still in progress. To our knowledge, this is the first report of *C. luteo-olivacea* on grapevine in Italy.

Identification of new pathogens associated with grapevine trunk diseases in Hungary: a review. KALMAN ZOLTAN VACZY. Food and Wine Research Institute, Eszterházy Károly University, 3300 Eger, Eszterházy tér 1., Hungary. E-mail: vaczy.kalman@uni-eszterhazy.hu

During the past years, more and more fungal pathogens have been described as associated with grapevine trunk diseases (GTDs). Similarly, new results on GTD have been recently published in Hungary. During an extensive study of fungal species associated with GTDs, roots and trunks of grapevine plants exhibiting typical GTD symptoms were collected at random from diverse cultivars in different Hungarian vineyards. Several hundreds of fungal strains were isolated from these samples and their identification was carried out based on colony morphology, conidiogenesis and conidial morphology for sporulating cultures and routine DNA sequencing and analysis for non-sporulating cultures. The objectives of this work were to identify the isolates obtained either by morphological characterization or based on DNA barcode sequences, and to assess or confirm the pathogenicity of selected strains on grapevine. New results obtained in this five-year study included (a) first report of pathogenic strains of *Seimatosporium vitis* associated with GTD symptoms in Hungary and worldwide; (b) first European record of *Dothiorella omnivora* and *Cytospora viticola* from grapevine, and associated with GTDs; (c) description of *Diaporthe hungariae* as a new *Diaporthe* species associated with several major diseases

of grapevines; and (d) the first report of *Neofabrea kiehnholzii* as GTD related pathogen. The results obtained revealed a great diversity of fungal pathogens that are linked to GTDs, and the complex background of this disease complex.

Wood-rotting Basidiomycetes associated with Esca in North America. KENDRA BAUMGARTNER¹, ALBRE BROWN¹, RENAUD TRAVADON² and DANIEL P. LAWRENCE². ¹United States Department of Agriculture - Agricultural Research Service, Davis, California, USA. ²Department of Plant Pathology, University of California, Davis, California, USA. E-mail: Kendra.Baumgartner@ARS.USDA.GOV

Phaeoemoniella chlamydospora and wood-rotting Basidiomycetes, namely *Fomitiporia* spp., are known Esca pathogens. The importance of their sequence of infection in disease development, however, is not clear. To determine the impact of dual inoculations on wood symptoms, we inoculated potted *Vitis vinifera* 'Crimson Seedless' with *P. chlamydospora*, either alone or in combination with one of the following Basidiomycetes: *Coprinellus radians*, *Fomitiporia langloisii*, *F. polymorpha*, and newly described *Tropicoporus texanus*. All isolates originated from vines with leaf symptoms of Esca in California or Texas. In plants inoculated only with a Basidiomycete, wood lesions were characterized by orange discoloration. Such lesion in plants inoculated with *C. radians*, *F. langloisii*, or *T. texanus*, species not previously reported from grape, were not significantly different in length from that of known Esca pathogen *F. polymorpha* (ranging from 45 to 100% of the length of the woody stem, after 1 year), and all were larger than that of the wounded, non-inoculated controls. In dual inoculations, we tested the effects of sequential infection (*P. chlamydospora* first, Basidiomycete 6 months later, and vice versa), compared to simultaneous infection. After 1 year, plants inoculated with *P. chlamydospora* either alone or in combination with a Basidiomycete did not differ significantly in the length of lesions typically associated with *P. chlamydospora*, specifically dark black lines in the wood. Further, there was no effect of sequence of inoculation on the length of such *P. chlamydospora* lesions or Basidiomycete lesions, both of which were present in dual-inoculated plants. Because leaf symptoms were rare, it is difficult to determine how sequence of infection by *P. chlamydospora* and Basidiomycetes affects them. Nonetheless, our finding of no differences between sequential or simultaneous dual-inoculations, compared to single inoculations, in development

of wood symptoms suggests that infection by Basidiomycetes may not require initial infection by *P. chlamydospora*.

Early season symptoms on grapevine stem and inflorescences associated with Botryosphaeriaceae species.

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Botryosphaeria dieback is one of the most important grapevine trunk diseases affecting vineyards worldwide. Several species of the Botryosphaeriaceae family in the genera *Neofusicoccum*, *Diplodia* and *Lasiodiplodia*, have been associated with *Botryosphaeria dieback* symptoms. These fungi are xylem inhabiting and attack the framework of grapevines, causing typical wedge-shaped perennial cankers in the wood that can lead to death of vines. These pathogens were also reported to cause early season symptoms including leaf and berry symptoms. However, these symptoms may be mistaken with symptoms from other diseases such as downy mildew. Currently, studies on what Botryosphaeriaceae species are commonly associated with early season symptoms in grapevines are limited. Therefore, during the 2018 season, when grapevines were on the E-L 23 - 25 phenological stage, samples showing necrosis on green shoots and dried inflorescences were collected in several vineyards spread throughout the central regions of Portugal. Fungal isolations were performed by plating symptomatic tissues onto potato dextrose agar (PDA) amended with chloramphenicol. DNA were extracted from the 23 Botryosphaeriaceae isolates recovered from the samples using a Qiagen® extraction kit. DNA sequence analyses of ITS region and part of the elongation factor 1- α genes revealed that the two major species frequently associated with the symptoms were *Diplodia seriata* and *Neofusicoccum parvum*. Representative isolates of each species that were selected for pathogenicity tests were able to reproduce the early season symptoms in 1-year old grapevine plants confirming these species were the primary pathogens of the disease. Since the appearance of these early season symptoms have not yet

been described, thus, further studies is needed to further understand the importance of these pathogens the early seasons symptoms observed in the vineyards.

Grapevine trunk diseases symptoms present in rootstocks and scions cuttings in the main grapevine nursery in Uruguay. MARÍA JULIA CARBONE, MATÍAS GELABERT, PEDRO MONDINO and SANDRA ALANIZ. *Department of Plant Protection, Faculty of Agronomy, University of the Republic, Av. Garzón 780, CP 12900, Montevideo, Uruguay. E-mail: mariajulia.93@hotmail.com*

Grapevine trunk diseases are one of the most important problems for grapevine nurseries. Rootstock and scion mother fields are usually infected by the fungi included in this complex. The symptoms present and their incidence on cuttings just after being cut from rootstocks and scions mother vines was investigated for the first time in the main grapevine nursery of Uruguay. The rootstocks evaluated were SO4, Gravesac, 1103P, 3309C and RR101-14 and the scions were Tannat, Marselan, Albariño, Merlot and Lágrima Christi. Fifty cuttings 110 cm long of each rootstock and scion were carefully observed by cross-sectional and longitudinal cuts. Isolations from symptomatic and asymptomatic samples were performed and identified by morphological and DNA analysis. The incidence of symptoms observed in the basal and distal parts of each rootstock cuttings were S04 16% and 15%, Gravesac 18% and 4%, 1103 P 6% and 4%, 3309 C 38% and 26% and RR 101-14 4% and 8%, respectively. In the scions were Tannat 48% and 2%, Marselan 18% and 6%, Albariño 30% and 18%, Merlot 14% and 4% and Lágrima Christi 20% and 8%, respectively. Presence of dark xylem vessels and necrotic streaks was the main symptom observed and secondly it was internal necrotic wedge-shaped staining in the cross section. Several Botryosphaeriaceae species, *Diaporthe* species and *Phaeo-monniella chlamydospora* were the fungi identified. This work confirms the presence of grapevine trunk diseases in cuttings used in the main grapevine nursery in Uruguay, emphasizing the importance of developing management strategies to minimize the incidence of these diseases.

Characterization and pathogenicity of black-foot disease pathogens isolated from symptomless grapevine nursery stock in Spain. MARCOS ANDRÉS-SODUPE¹, CARMEN BERLANAS¹, REBECA BUJANDA¹, EMILIA DÍAZ-LOSADA², BEATRIZ LÓPEZ-MANZANARES¹, MARÍA DEL PILAR MARTÍNEZ-DIZ², SONIA OJEDA¹

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In this study, 3,426 grafted plants ready to be sold to producers were surveyed from 15 grapevine nursery fields in northern Spain from 2016 to 2018. In all, 1,427 black-foot pathogen isolates were collected from the non-necrotic inner tissues of surface sterilized symptomless secondary roots and characterized based on morphological features and comparison of DNA sequence data of the nuclear ribosomal DNA-internal transcribed spacer region, histone H3, translation elongation factor 1- α and β -tubulin genes. Eleven species belonging to the genera *Dactylonectria*, *Ilyonectria*, *Neonectria* and *Thelonectria* were identified, namely *D. alcacerensis*, *D. macrodidyma*, *D. novozelandica*, *D. pauciseptata*, *D. torresensis*, *I. liri-odendri*, *I. pseudodestructans*, *I. robusta*, *N. quercicola*, *N. sp. 1* and *T. olida*. In addition, two species are newly described, namely *D. riojana* and *I. vivaria*. Twenty-four isolates representing the 13 black-foot species were inoculated in grapevine seedlings cultivar Tempranillo. The pathogenicity tests detected virulence diversity among fungal species and between isolates within each species. The most virulent species was *D. novozelandica* isolate BV-760, followed by *D. alcacerensis* isolate BV-1240 and *I. vivaria* sp. nov. isolate BV- 2305. The present study improves our knowledge on the etiology and virulence of black-foot disease pathogens, and opens up new perspectives in the study of the endophytic role of these pathogens on grapevines.

Diversity of *Cylindrocarpon*-like species associated with roots of grapevine and other fruit and nut crops in California. DANIEL P. LAWRENCE¹, MOHAMED T. NOURI² and FLORENT P. TROUILLAS². ¹*Department of Plant Pathology, University of California, Davis, CA, 95616, USA.* ²*Department of Plant Pathology, University of California, Kearney Agricultural Research and Extension Center, Parlier, CA, 93648, USA. E-mail: dlawrence@ucdavis.edu*

Black foot disease is a common and destructive root disease of grapevine caused by taxonomically diverse cylindrocarpon-like fungi in many viticultural areas of the world. This study identified 12 cylindrocarpon-like fungal species from five genera associated with black

foot disease of grapevine and other root diseases of fruit and nut crops in the Central Valley Region of California. Morphological assessments paired with multi-locus sequence typing of four loci, internal transcribed spacer region of nuclear rDNA ITS1–5.8S–ITS2 (ITS), beta-tubulin (*TUB2*), translation elongation factor 1-alpha (*TEF1*), and histone (*HIS*), revealed ten previously described species; *Campylocarpon fasciculare*, *Dactylonectria alcacerensis*, *D. ecuadoriensis*, *D. macrodidyma*, *D. novozelandica*, *D. torresensis*, *D. valentina*, *Ilyonectria capensis*, *I. liriiodendri*, *I. robusta*, and two new species, *Neonectria californica* sp. nov., and *Thelonectria aurea* sp. nov. Phylogenetic analyses of the ITS+*TUB2*+*TEF1* combined dataset, a commonly employed dataset used to identify filamentous ascomycete plant pathogens, was unable to assign some species, with significant support, in the genus *Dactylonectria*, while all other species in other genera were confidently identified. The *HIS* marker was essential either singly or in conjunction with the aforementioned genes for accurate identification of most *Dactylonectria* species. Results from isolations of diseased plant tissues revealed potential new host associations for almost all fungi recovered in this study. This work is the basis for future studies on the epidemiology and biology of these important and destructive plant pathogens.

Genetic analysis of *Dactylonectria torresensis* populations from grapevine using microsatellite markers.

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The soil-borne fungus *Dactylonectria torresensis* is the most common causal agent of black-foot disease of grapevine in Europe. Despite the importance and wide distribution of *D. torresensis*, no information is still available on the pathogen's population structure. In this study, the genetic diversity and subdivision among and within populations of *D. torresensis* was investigated in Spain and other Mediterranean countries. In total, 190 isolates from two regions in northern Spain (La Rioja and Navarra), Algeria, Italy, and Portugal were genotyped. Eleven newly developed polymorphic simple-sequence repeat markers revealed 144 multilocus genotypes (MLG) in the Spanish population, and 112 MLGs globally. Discriminant analysis of principal components (DAPC) showed that populations from northern Spain are structured into six groups. Globally, five distinct genetic clusters were observed. Two of these clusters included only Spanish MLG, other two clusters consisted on Spanish and Portuguese MLG and the last cluster included Spanish, Algerian and Italian MLG. In general, pairwise G'st values showed very low genetic differentiation, especially among populations from Spain and Portugal and within Spanish populations. High genotypic diversity indicated that sexual reproduction might occur among *D. torresensis* isolates. However, linkage disequilibrium analyses did not support the hypothesis of random mating. The population genetic structure of *D. torresensis* in Europe is likely associated with the spread of isolates by infected propagation material.

Global patterns of genetic diversity and population subdivision in *Phaeomoniella chlamydospora*.

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Phaeoconiella chlamydospora is a Petri disease and Esca pathogen reported from all major grape-growing regions of the world. This ascomycete fungus colonizes xylem vessels, impairing the translocation of water and nutrients. Sexual fruiting bodies of the fungus have not been found in vineyards. Asexual spores are thought to be the cause of infections in the vineyard and nursery. As such, the pathogen population presumably displays low levels of genetic diversity. Using 18 published microsatellite markers, we genotyped approximately 250 isolates collected from distant regions of the world: Brazil, British Columbia in Canada, South Africa, Spain, and the northeastern and western United States. Preliminary findings from analyses of all North American isolates revealed a high haplotypic diversity, suggesting that sexual reproduction generates new allelic combinations. Clustering analyses revealed three genetic groups of isolates. Two genetic groups spanned the California and British Columbia collections, whereas the third group was comprised mainly of northeastern US isolates. High genetic subdivision ($F_{st} = 0.28$; $P < 0.001$) suggests that genetic and/or ecological factors may maintain genetic differentiation among these three groups. Significant differences in levels of genetic diversity were found among the groups, with the highest gene diversity ($H = 0.83$) and allelic richness ($A = 9.4$) in the northeastern US. A high diversity of *Vitis* species in this region, and possibly differences in pathogen life history, may explain these findings. With the current, additional genotyping of isolates from Brazil, South Africa, and Spain, we will determine a more accurate picture of the distribution of genetic diversity of this pathogen at a multi-continental scale, in order to pinpoint centers of genetic diversity in the pathogen population and potential introduction routes among grape-growing regions.

Sensing grapevine trunk disease using metabolic markers. DION MUNDY¹, BHANUPRATAP VANGA², NIGEL JOYCE², PETER JAKSONS², CATHERINE SAN-

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Grapevine trunk disease (GTD), caused by a variety of different pathogens, including *Eutypa lata* and *Phaeoconiella chlamydospora*, is a major problem for commercial vineyards. Non-destructive detection using technologies such as field sensors that detect metabolic markers associated fungal infections could allow for targeted disease management. Understanding the chemical profiles during infection is the first step for developing these sensors. Mature Sauvignon blanc plants with presence or absence of one or more GTD-causing pathogens (confirmed by next generation sequencing) and grapevine cuttings inoculated with *E. lata* were analysed for changes in metabolite compounds. Non-volatiles were measured by solvent extraction and ultra-high performance liquid chromatography – high resolution mass spectrometry (LC-MS), and volatiles by headspace - solid phase microextraction - gas chromatography - mass spectrometry. A range of potential non-volatile markers were identified. Some were likely to be produced from the fungal pathogens causing disease, and others would be plant metabolites associated with disease response or vine stress. *E. lata* infection correlated with the unique eutypines and metabolites detected, whereas *P. chlamydospora* infection correlated with coumaryl derivatives and benzoic acid markers. Stilbene isomers and polymeric forms upregulated in response to all fungal pathogens. Vine stress was additionally shown by high ratios of sucrose to fructose or glucose with fungi present. No volatile marker compounds associated with fungal infection were found. The changes in leaf metabolite profiles identified in this study could be used to develop sensing technologies for detection of GTD in vineyards. These tools would allow non-destructive detection of infections, aiding vineyard and greenhouse decision-making for disease management.

Into the wood – monitoring fungal pathogen’s development and impact on wood tissues through non-destructive imaging. CÉDRIC MOISY¹, ROMAIN FERNANDEZ¹, MAÏDA CARDOSO², SAMUEL

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Imaging approaches and image processing have considerably improved plant phenotyping and are nowadays increasingly used for phenotypic trait measurement. Magnetic Resonance micro-Imaging (MRI) and X-ray computed micro-tomography are non-destructive imaging techniques based on the magnetic properties of atomic nuclei, and the relative radio-density, respectively. They are widely used for medical diagnosis although these techniques remain rare in plant studies. However, they could enable the study of complex plant-pathogen interactions, as observed in grapevine trunk disease (GTD). Our research aims to estimate the benefits of imaging approaches for detection of GTD, and to evaluate the impact of fungal pathogens on host tissues through non-destructive monitoring directly into the wood. Using μ -MRI and X-ray μ -CT, we performed a dynamic monitoring of wood colonization by fungi artificially inoculated under controlled conditions. Interesting results were thereby collected on both the progression of each fungus in the different tissues and their impact on the live plant. In parallel, we were also able to detect, localize and quantify different types of degraded tissues in old grapevine plants collected in vineyards. Altogether, these results demonstrated that non-destructive imaging provides informative techniques to monitor pathogenic fungi progression in the wood. They could lead to the development of new markers for monitoring trunk diseases, and for phenotyping genetic resources for their level of tolerance. Imaging approaches open new perspectives for increasing our knowledge on GTD. These new tools could also be developed for evaluating varietal tolerance in breeding programs, and for measuring the real effect and efficacy of new molecules or biocontrol agents onto the pathogen propagation in

the wood. Non-destructive imaging approaches would then benefit both the wine growing industry and the researchers.

Molecular detection of Grapevine Trunk Diseases fungi in Chilean vineyards. FELIPE GAINZA-CORTÉS, PAULINA ARRAÑO-SALINAS, ROSA ROA-ROCO, PABLO RODRIGUEZ-HERRERA, IVO AGNIC, DANIELA TORRES, PAMELA ESPINOZA, CARLOS VALDIVIA and ALVARO GONZALEZ. *Viña Concha y Toro S.A, Center for Research and Innovation, Fundo Pocoa s/n, Km10 Ruta K-650, Penciahue, Región del Maule, Chile.* E-mail: felipe.gainza@conchaytoro.cl

Fungi associated with Grapevine Trunk Diseases (GTDs) have received increased attention in vineyards worldwide during recent years. It has been widely established that mother-plant block sanitary status and nursery practices are both implicated in the spread of GTDs, affecting vineyard productivity, longevity and grapes quality. In this context, the implementation of an early diagnosis system would complement and positively impact the management and mitigation of this problem. Since 2016, The Center for Research and Innovation of Viña Concha y Toro (VCT) have implemented PCR based methods (quantitative and end-point) for the detection of the 5 main worldwide GTDs-associated fungi (*Diplodia seriata*, *Neofusicoccum parvum*, *Phaeo-*mon*iella chlamydospora*, *Phaeoacremonium minimum* and *Eutypa lata*) and the 12 main viruses associated to grapevines. In this work, we present the results of the analysis of more than 1600 plants (five rootstocks and 24 clonal selections belonging to five cultivars) in VCT vineyards. From the total plants analyzed for GTDs, it was observed a higher incidence of *Diplodia* complex and *Eutypa lata* showed the highest incidence of detection. Additionally, a 73% of *Diplodia* complex and 40% of *Eutypa lata* incidence was observed for Cabernet sauvignon; 39% and 19% for Sauvignon blanc, respectively; 13% and 21% for Chardonnay, respectively; and 37% and 25% for the evaluated rootstock, respectively. As a result of this survey, it was possible to identify 907 plants free of four of the most detrimental viruses, among these, 313 plants were free of all 12 viruses. Within the 313 high standard virus-free plants, we identify 184 GTD-free plants. Those virus/GTD-free plants have been multiplied and used in the plantation of new, healthier mother-plant blocks that will allow VCT to increase the phytosanitary status and longevity of its vineyards.

The potential of LAMP assays for the detection of grapevine trunk disease pathogens. JAMES WOODHALL¹, ALEXANDRA OROPEZA¹, GUIDO MARCHI² and LAURA MUGNAI². ¹University of Idaho, Research and Extension Center, Parma, Idaho, USA. ²Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant pathology and Entomology section, University of Florence, P.le delle Cascine, 28, 50144 Firenze, Italy. -mail: jwoodhall@uidaho.edu

Early, accurate diagnosis is essential for successful treatment of a plant disease. Although diagnosis can sometimes be made from symptoms alone, laboratory testing is often required. Molecular tests such as real-time PCR are sensitive and accurate but are frequently confined to the laboratory and take several hours to complete. Loop mediated isothermal amplification (LAMP) offers a rapid and portable alternative for molecular testing. In this study, separate LAMP assays were developed for *Phaeoemoniella chlamydospora* and *Eutypa lata*, causal agents of a vascular disease within Esca complex including Petri disease in young vineyards, and of *Eutypa* die-back respectively. For *P. chlamydospora*, primers were designed from the rDNA ITS region, whilst for *E. lata*, primers were designed from sequences derived from unique RAPD fragments. Assays were specific to the target pathogen and tested against DNA originating from 30 isolates representing closely related species and other grapevine trunk disease pathogens. The assays were sensitive, with a time to positive with DNA from pure culture within ten minutes on a Genie III (Optigene, UK), although the equivalent TaqMan real-time PCR assays were ten times more sensitive. Detection in grapevine wood was possible using an on-site DNA extraction method. Wood was sampled using a cordless drill and transferred to a 5 mL tube containing 1 mL of PEG/sodium hydroxide buffer and a ball bearing. After vigorous shaking by hand, the lysate was diluted tenfold and used in the LAMP reaction. Wood samples using the in-field extraction method were positive within 10-30 minutes. In conclusion, LAMP offers a rapid and portable diagnostic and detection tool. Although, real-time PCR is suited to high throughput testing where sensitivity is paramount within a fully equipped laboratory, LAMP is a viable alternative for use in low resource labs and when on-site testing is necessary.

Loop mediated isothermal amplification (LAMP) assays to detect *Diplodia seriata* and *Neofusicoccum parvum* isolated from Chilean grapevine wood samples. FELIPE SÁEZ CORTEZ^{1,2}, ÁLVARO CASTRO

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Members of Botryosphaeriaceae family of phytopathogenic fungi have been reported to cause grapevine trunk diseases (GTD) worldwide, causing a devastating impact in fruit yield and the lifespan of the plants. In Chile, *Neofusicoccum parvum* and *Diplodia seriata* are two members of the Botryosphaeriaceae that are commonly isolated and identified as trunk disease causing agents. Early detection of these pathogens, particularly in the stages of clonal propagation of the grapevines could help screen out infected but asymptomatic material. Loop mediated isothermal amplification (LAMP) is a molecular technique performed at a constant temperature of 60 to 65°C using either two or three sets of primers and a polymerase with high strand displacement activity. This technique has the potential to be fast, sensitive and highly specific. It can also be used in low resource labs or on-site as DNA amplification can be observed visually, through either the increase in the turbidity when amplification occurs, or with the aid of colorimetric indicators such as hydroxynaphtol blue (HNB). In this study, we successfully developed LAMP assays to detect *Neofusicoccum parvum* and *Diplodia seriata*. Amplification was determined through turbidity evaluation and HNB colorimetric indicator. The assays were highly specific and did not detect DNA extracted from a range of other GTD fungi. This study shows the potential of LAMP for the rapid detection of two GTD fungi in grapevine material.

Droplet digital PCR technology for detection and quantification of black-foot disease pathogens and *Cadophora luteo-olivacea* from grapevine nursery stock and vineyard soil. MARÍA MERCEDES MALDONADO-GONZÁLEZ¹, MARCOS ANDRÉS-SODUPE¹, MÓNICA BERBEGAL², REBECA BUJANDA¹, EMILIA DÍAZ-LOSADA³, MARÍA DEL PILAR MARTÍNEZ-DIZ^{3,4} and DAVID GRAMAJE¹. Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de La Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain. ²Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain. ³Estación de Viti-

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Cylindrocarpon-like asexual morphs and *Cadophora luteo-olivacea* are fungal pathogens frequently isolated from planting material in grapevine nurseries and young vineyards, causing black-foot and Petri disease, respectively. Accurate, early and specific detection and quantification of these fungi are essential to alert growers and nurseries about their presence in soil, and to prevent their spread in grapevine using certified pathogen-free planting material. We comparatively assessed the accuracy, efficiency, and specificity of droplet digital PCR (ddPCR) and real-time PCR (qPCR) techniques for the specific detection and quantification of *Cylindrocarpon*-like asexual morphs in grapevine root, rhizosphere and vineyard soil, and *Cadophora luteo-olivacea* in vineyard soil and grapevine wood. In both cases, ddPCR was more sensitive to lower target concentrations than qPCR. Plant-soil fraction did not affect the abundance of black-foot associated pathogens in vineyards. *Cadophora luteo-olivacea* was detected in 31.7% of analyzed plants with variable abundances. Few samples tested positive for the target fungus in soil. Roots of asymptomatic vines were found to be a microbial niche that is inhabited by black-foot disease fungi, which opens up new perspectives in the study of the endophytic role of these pathogens on grapevines. Further research is needed to determine minimum infection thresholds needed for trunk disease fungi to start infection in the field and to understand which planting conditions will enhance disease proliferation and benefit pathogen colonization.

Characterization and detection of grapevine fungal pathogens using Fatty Acid Methyl Ester analyses (FAME). CHRISTOPHER M. WALLIS. *U.S. Department of Agriculture, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, Crop Diseases, Pests and Genetics Research Unit, Parlier, CA 93648, USA. E-mail: christopher.wallis@usda.gov*

Grapevines can become infected by a variety trunk disease-causing fungi including those from genera such as *Diplodia*, *Neofusicoccum*, *Lasiodiplodia*, and others. These diverse pathogens can vary greatly in disease severity and symptom expression. Therefore, proper identification is necessary to determine the best management strategy. Although sequence-based genotyping can distinguish these fungal canker pathogens, there

is a need for faster, cheaper ways to distinguish casual agents of trunk diseases. To address this, fingerprinting of fatty acids that comprise cell membranes of each pathogen was performed using gas chromatography on methyl esters (FAME). This was done to create profiles for over 20 different fungal species, with additional analyses that followed to verify accuracy of identification. With FAME, a total of 20 samples from culture can be analyzed in as little as two hours, and costs far lower than that of DNA-based techniques at less than 10 cents a sample. Ongoing work is examining ways to extract and analyze fungal FAMES directly from plant tissues to avoid the need for culturing. FAME allows an alternative to DNA-based identification and could be employed in cases where nucleic acid degradation is a concern, or if an alternative methodology is required for regulatory actions.

Epidemiology

Incidence and severity of Botryosphaeria dieback in Chilean vineyards cv. Cabernet Sauvignon assessed in 2010 and 2018. XIMENA BESOAIN¹, CAROLINA TORRES¹, MICHAEL SEEGER² and ALEJANDRA LARACH¹. ¹*Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, San Francisco s/n La Palma, Quillota 2260000, Chile.* ²*Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Chemistry Department & Centro de Biotecnología Daniel Alkalay Lowitt, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso 2340000, Chile. E-mail: ximena.besoain@pucv.cl*

In Chile, one of the main crops is the grape (*Vitis vinifera*), for the production of both table grapes and wine. For wine production there are around 140,000 hectares and Cabernet Sauvignon is the main cultivar. Grapevine trunk diseases (GTDs), have become relevant in recent years, both in the world and in Chile, due to the greater appearance of symptoms associated with these type of disease. The main consequence of the plant dieback is the loss of productivity of the vineyards, due to the death of a part and/or the entire plant. Therefore, the objectives of this study were to estimate the incidence and severity of the GTDs and the impact on vineyard production during two years, 2010 and 2018. For this reason, eight blocks from five vineyards in O'Higgins Region and six blocks from five vineyards in Maule Region were sampled, each block consisting of 100 plants. In the O'Higgins Region, there was an increase in both incidence and severity from 2010 to 2018, with

an 82.4% and 43.6%, respectively in 2018. On the contrary, in the Maule Region, a decrease in the incidence and severity between 2010 and 2018 was observed, with an incidence of 87.2% and severity of 51.6% in 2018. In relation to the yield, in 2010-11 an average loss of almost 5 tons/ha was estimated for the O'Higgins Region and 6 tons/ha for the Maule Region. Average loss is currently being measured for the 2018-2019 harvest in both vineyards. Our results clearly demonstrated the importance and impact of GTDs in central Chile.

The diversity and epidemiology of Botryosphaeriaceous grapevine trunk disease pathogens occurring on other woody hosts in the winelands of South Africa. FRANCOISE HALLEEN^{1,2}, IHAN L. DU PLESSIS^{1,2}, LIZEL MOSTERT², BERNARD SLIPPERS³ and JAN H. NAGEL³. ¹Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa; ²Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa; ³Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0001, South Africa. E-mail: halleenf@arc.agric.za

Species from the Botryosphaerales are the most common and widespread pathogens of woody hosts globally and often affect fruit industries. These pathogens affect grape industries by causing yield losses and shortening the productive lifespan of infected grapevines. Farmers are limited to using good management practices to manage these fungi. Such management strategies are primarily aimed towards preventing pathogens from spreading between infected and healthy grapevines, but disregard the possible movement of pathogens from other host species. The broad host ranges of many Botryosphaeriaceae species, however, mean that alternative hosts of these grapevine pathogens can serve as sources of inoculum. To investigate this, a Botryosphaeriaceae species diversity survey was performed on 37 different host species that commonly occur throughout the winelands in Western Cape Province of South Africa. In total 950 Botryosphaeriaceae strains were isolated from visibly diseased hosts. These represented twenty-six different Botryosphaeriaceae species, six of which could not be identified to species level and represent new species. The grapevine pathogen, *Neofusicoccum Stellenboschiana*, was amongst the most common and was isolated from 24 different host species. Populations of *N. Stellenboschiana* from different hosts at three different locations were subsequently characterized using seven microsatel-

lite markers. The results showed shared genotypes of *N. Stellenboschiana* on the grapevines and other hosts at all of the locations, indicating that this species can spread between these hosts. These findings suggest that alternative hosts of grapevine trunk disease pathogens pose a risk to vineyards, and that these are not addressed by current management practices.

Standardizing Botryosphaeriaceae infection levels in experimental grapevine plant materials. REGINA BILLONES-BAAIJENS¹, MARK SOSNOWSKI^{2,3}, MATTHEW AYRES² and SANDRA SAVOCCHIA¹. ¹National Wine and Grape Industry Centre, Faculty of Science, Charles Sturt University, Wagga Wagga, NSW, Australia. ²South Australian Research and Development Institute, Adelaide, SA, Australia. ³School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond, SA, Australia. E-mail: rbaaijens@csu.edu.au

Grapevine nursery plants have been reported with latent infections of grapevine trunk disease (GTD) pathogens. However, the link between these latent infections and GTD symptoms observed in vineyards is still unclear. Studies have also shown that water-stress can increase the susceptibility of young vines to GTDs. Accurate quantification of infection levels from naturally-infected young vines is difficult since latent infections have no internal or external symptoms and may be randomly distributed within a vine. Thus, investigations on the effects of water stress on young vines artificially inoculated with three different conidial concentrations of GTD pathogen may provide insight on the infection thresholds that result in disease expression in vines. The objective of this study was to develop an inoculation method to standardize GTD infection levels in young vines. A published vacuum-inoculation method was evaluated for inoculating Shiraz cuttings with 300 (low), 3,000 (moderate) and 30,000 (high) conidia of *Neofusicoccum luteum* while Ringer's solution was used to inoculate control vines. The vacuum-inoculation was a reliable method as evidenced by qPCR analyses, resulting in the conidia infecting the basal, middle and apical part of the inoculated canes. The qPCR analyses further differentiated the low, moderate and high infections with the highest amount of pathogen detected from canes inoculated with 30,000 conidia and lowest from those inoculated with 300 conidia. No pathogen was detected in any of the control canes. This study showed the vacuum-inoculation method was suitable for introducing different concentrations of *N. luteum* spores into dormant canes without significant impact on plant viability.

The method was used to standardize infection levels of *N. luteum* in ~400 Shiraz rootlings in newly established glasshouse and shade house experiments. Observations on the effect of water stress on *Botryosphaeria dieback* symptom expression is on-going.

Saprophytic colonization of the bark by *Neofusicoccum* species mediates subsequent infection of grapevines through wounds.

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Botryosphaeriaceae species infect grapevines via wounds. A previous study isolated *Botryosphaeriaceae* at higher frequencies from the bark than the underlying wood of asymptomatic grapevines canes from vineyards, suggesting they were latent on surface tissues. This study investigated the colonization of the bark as a saprophytic link to infection of the underlying wood. The bark of trunks of Sauvignon blanc and Pinot noir potted vines were inoculated by spraying an area of 3 cm length with ~1 mL of a *Neofusicoccum luteum* or *N. parvum* conidial suspensions (10^4 /mL). Control vines were inoculated with sterile water. After 1 hour (T1), 2 days (T2) or 7 days (T3) a cut was made in the bark and through to the wood 1 cm above the inoculation area using a sterile scalpel. After 24 h, isolations were carried out from surface sterilized bark and wood. Infection incidence did not differ significantly between species or grapevine cultivar. Infection incidence of the bark was 100% and associated wood of the central inoculated section was 76.3%, 83.3% and 90.2% for T1, T2 and T3, respectively indicating bark infection progressed rapidly into the adjacent wood. Infection of bark and wood 1 cm above the inoculation point increased with incubation time, being 25% for bark and wood at T1 and 71% and 67% for bark and wood, respectively at T3. Infection of the bark and wood 1 cm below the inoculated area was 0%. This study showed that the pathogens remained latent in the bark and, when the cane was wounded, that the pathogen progressed towards the wound. Fluorescent microscopic observations of bark and underlying wood sections of shoots inoculated onto the bark, but without wounding, showed germinating conidia and mycelium

in the bark near lenticels, and mycelia in the underlying wood, indicating that the pathogens had entered through lenticels.

Early detection project - detection and quantification of *Phaeoconiella chlamydospora* and *Botryosphaeria* spp. in *Vitis vinifera* wood samples. SZABINA LENGYEL¹, RANDALL E. GOLD², JOCHEN FISCHER¹, ALEXANDER YEMELIN¹, ECKHARD THINES¹ and ANNETT KÜHN².
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Phaeoconiella chlamydospora (PCH), associated with esca, and *Botryosphaeriaceae* spp. (BOT), associated with dieback, are among the most important pathogens causing grapevine trunk diseases (GTDs) and therefore severe economic losses in wine-growing regions all over the world. The control and management of these diseases are challenging since the related visible symptoms occur mostly after a long latency period, when the wood is already compromised. The aims of this project were to detect and quantify fungi via their DNA in wood samples before the symptoms appear and to evaluate the efficacy of the BASF wound protectant Tessior® under field conditions. The method was developed using samples collected from grapevine plants showing typical esca foliar symptoms (“tiger-stripes”) of different levels. Wood chips were received from the trunks using a 5 mm diameter drill bit. After evaluating various DNA extraction protocols, the most successful one was optimized for grapevine wood tissue. Quantitative Real-Time PCR (qPCR) method to detect and quantify PCH and BOT in wood samples. Comparing the severity of symptoms with the results obtained by qPCR, a clear correlation was observable: the more symptomatic the plant appeared, the higher the amount of PCH DNA was determined in the samples. BOT DNA was also found in some samples, the highest amount in the dead plant. Long-term field trials were established between 2014 and 2015 in several countries in Europe, where each year a treatment was performed with Tessior® after pruning. In order to increase the infection pressure, the wounds were artificially inoculated with spores of PCH and BOT in some vineyards. In 2018, samples were collected from the spurs below pruning wounds of the previous year and the DNA amounts of PCH and BOT were determined by qPCR. The above described method has

been proved to be fast and accurate to detect and quantify DNA amounts of PCH and BOT in grapevine wood. Furthermore, the efficacy of Tessior® wound protectant has been also verified.

Minimal vs. intensive pruning: does the training system influence occurrence of Esca (GLSD) foliar symptoms? CHRISTIAN KRAUS^{1,2}, RALF VOEGELE² and MICHAEL FISCHER¹. ¹Julius Kühn-Institute, Institute for Plant Protection in Fruit Crops and Viticulture, 76833 Siebeldingen, Germany. ²University of Hohenheim, Department of Phytopathology, 70599 Hohenheim, Germany. Email: christian.kraus@julius-kuehn.de

The Esca complex is one of the most destructive grapevine trunk diseases (GTDs) worldwide. Several factors, such as plant age, grapevine cultivar, or pattern of precipitation have been identified as possible driving forces of the disease. In the present study, a four years monitoring of Esca foliar symptoms (namely grapevine leaf stripe disease [GLSD]) in vineyards subdivided into minimally (Semi minimal pruned hedge) and intensively (Vertical shoot positioned) pruned sections was conducted to investigate a possible impact of the training system on the incidence of GLSD. In addition, the occurrence of GLSD over the season and a possible influence of cultivar, plant age and precipitation on symptom development were studied. All investigated parameters, i.e. cultivar, plant age and precipitation, were shown to have at least some influence on symptoms incidence, even though some of the results were inconsistent over the period of monitoring. Concerning the influence of the training system, in 2015 no differences between minimally pruned (1.8%) and intensively pruned (1.9%) vines were found. However, in the following year minimally pruned vines (6.9%) expressed significantly more symptoms than intensively pruned vines (4.9%). In the years 2017 and 2018 the opposite was the case: 2.6% and 2.4%, respectively, of the minimally pruned vines showed GLSD symptoms, while for the intensively pruned vines the mean values were 4.5% and 3.6%, respectively. The seasonal patterns of symptom appearance were identical for the years 2015, 2016 and 2017. In 2018, however, the maximum peak of newly symptomatic vines was reached four weeks earlier, which correlated with the phenological development of the vines. The training system did not affect the seasonal symptom appearance. Our data should help to better understand the relation between incidence of GLSD in the course of the year and possibly influencing parameters.

Natural infections of pruning wounds by fungal trunk pathogens in mature vineyards in Galicia (north-west Spain). MARÍA DEL PILAR MARTÍNEZ-DIZ^{1,2}, ALES EICHMEIER³, MARCOS ANDRÉS-SODUPE⁴, REBECA BUJANDA⁴, ANGELA DÍAZ-FERNÁNDEZ¹, EMILIA DÍAZ-LOSADA¹, MARÍA MERCEDES MALDONADO-GONZÁLEZ⁴, SONIA OJEDA⁴ and DAVID GRAMAJE⁴. ¹Estación de Viticultura e Enología de Galicia (AGACAL-EVEGA), Ponte San Clodio s/n 32428-Leiro-Ourense, Spain. ²Universidade da Coruña, Facultade de Ciencias, Zapateira, 15071 A Coruña, Spain. ³Mendel University in Brno, Faculty of Horticulture, Mendeleum - Institute of Genetics, Valticka 334, 69144, Lednice, Czech Republic. ⁴Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain. E-mail: pilar.martinez.diz@xunta.gal

Information on the natural infection rates of pruned canes caused by fungal trunk pathogens is scarce. This study aimed to determine the pathogenic fungal microbiome infecting the pruning wounds in six vineyards in Galicia, Spain, belonging to three Denomination of Origin (D.O. Valdeorras, D.O. Ribeiro and D.O. Rías Baixas) in 2017–2018. Vines were pruned in each vineyard in mid-autumn (November) leaving six buds. Fungal microbiome was identified by ITS high-throughput amplicon sequencing (HTAS) from this pruning wood in 150 canes chosen at random in each vineyard. Three months later (late winter, February), two-node canes were cut leaving four buds and fungal microbiome was identified again by HTAS from this pruning wood. Vines were then definitively pruned to two buds in mid-spring (May), and identification of the fungal microbiome by HTAS was repeated from this pruning wood. The main fungal pathogens identified in this study belonged to the genera *Cadophora*, *Cytospora*, *Diaporthe*, *Phaeoacremonium*, *Phaeomoniella* and the families Botryosphaeriaceae and Diatrypaceae. A strong seasonal effect on pathogen infections was observed for most species, with a higher fungal abundance detected after the late pruning (February-May) as compared with that of the early pruning (November-February). Under the environmental conditions and the geographical locations of this study, our results showed that the rate of natural infection of pruning wounds was lower following early pruning (mid-autumn) than late pruning (late winter). Thus, early pruning could be used in combination with other control measures to reduce the infections caused by the grapevine trunk pathogens during the pruning season in Galicia, Spain.

Temporal dispersal pattern of *Phaeomoniella chlamydospora* in Spanish vineyards. ELISA GONZÁLEZ-DOMÍNGUEZ¹, CARMEN BERLANAS², DAVID GRAMAJE², JOSEP ARMENGOL³, VITTORIO ROSSI⁴ and MÓNICA BERBEGAL³. ¹*Horta srl., Via Egidio Gorra 55, 29122 Piacenza, Italy.* ²*Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain.* ³*Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain.* ⁴*Department of Sustainable Crop Production - DI.PRO.VE.S., Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84, 29122 Piacenza, Italy.* E-mail: mobermar@etsia.upv.es

Phaeomoniella chlamydospora is one of the most important pathogens associated with esca disease of grapevine. This fungus reproduces asexually by conidiophores produced in the mycelia or by pycnidia. In this work, the dispersal of aerial inoculum was studied to improve the knowledge about *P. chlamydospora* epidemiology. The main objectives were to monitor the dispersal pattern of this pathogen in two wine producing regions in Spain and to associate this pattern with the environmental conditions. Sampling was performed from October to May in two vineyards affected by grapevine trunk diseases, located in Valencia and Logroño provinces. Five spore traps (microscope slides coated with silicone) were replaced weekly during two seasons in Valencia (2015-2017) and three seasons in Logroño (2015-2018). The DNA concentration of *P. chlamydospora* was determined using a previously validated real time PCR-based protocol. Inoculum of *P. chlamydospora* was detected consistently throughout the investigated months at both locations in all the seasons, except in Logroño 2017/2018. DNA of *P. chlamydospora* was detected in 71 of the 139 sampling periods with rain (96.6% of the total DNA captured) and in 20 of the 31 periods without rain (3.4% of the total DNA). The dynamic of *P. chlamydospora* inoculum release during the season was best described by a Gompertz equation when time was expressed as hydrothermal time (i.e., a combination of accumulated temperature and relative humidity), with $R^2 > 0.7$ and $CCC = 0.87$. The information obtained and the equation developed will help to identify the periods with high risk of inoculum dispersal, and to adopt efficient management strategies based on this information.

Spore dispersal patterns of Diatrypaceae and Botryosphaeriaceae species in Australian vineyards. REGINA BILLONES-BAAIJENS¹, SANDRA SAVOCCHIA¹, MEIFANG LIU¹, MATTHEW AYRES² and MARK R. SOSNOWSKI^{2,3}. ¹*National Wine and Grape Industry Centre, School of Agricultural and Wine Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga NSW 2678, Australia.* ²*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001,* ³*School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond SA 5064, Australia.* E-mail: rbaaijens@csu.edu.au

Eutypa dieback (ED) and *Botryosphaeria dieback* (BD) are considered the two most important grapevine trunk diseases in Australia, causing significant yield reduction and threatening the sustainability of Australian vineyards. Spores of the causal pathogens are generally dispersed by rain splash and wind and infect pruning wounds resulting in cankers, dieback and eventually death of vines. Thus, understanding the spore dispersal patterns of these pathogens will help determine the critical times of the year when spores are abundant in Australian vineyards. This will assist growers in making decisions on optimal timing of pruning and wound treatment. This study investigated the spore dispersal patterns of ED (Diatrypaceae) and BD (Botryosphaeriaceae) pathogens in four wine growing regions in Australia. Burkard spore traps were deployed in South Australia (Barossa Valley and Coonawarra) and in New South Wales (Hunter Valley and Griffith) between 2014 and 2016. Spore trap tapes were collected and replaced monthly at each site and analysed for Diatrypaceae and Botryosphaeriaceae spores using qPCR assays. The 3-year study showed spore dispersal of ED and BD pathogens was sporadic and varied between regions, season and year. In South Australia, ED and BD spores were primarily detected in late winter and early spring while in New South Wales, a high number of spores were trapped over summer. Spores were generally recorded during or immediately after rain but not all rain events resulted in spore detection. The spore numbers and frequency of detection varied between years with the highest number of spores being recorded in 2016, particularly for Diatrypaceae species. Additional spore traps were deployed in 2017 across Australian wine regions and will be monitored for a further 3 years. Computer modelling of data will also investigate the role of other weather factors (temperature, relative humidity, dew point, wind direction) on the detection of these spores.

Development and implementation of a droplet digital™ PCR assay for epidemiological studies of *Botryosphaeria dieback* of grapevines in British Columbia.

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Botryosphaeria dieback (BD) is one of the most predominant grapevine trunk diseases in British Columbia (BC). The fungal species associated with BD infect grapevines primarily through pruning wounds. Pycnidia, found on previously infected vines, produce spores which are released under favourable environmental conditions. Identifying the conditions that favour spore release and the corresponding infection periods is key to developing control measures. The two main objectives in this project were: firstly, to adapt a qPCR assay originally developed to detect BD species, for use with droplet digital™ PCR (ddPCR™, Bio-Rad Laboratories, Inc.); and secondly, to quantify BD spores in commercial vineyards using the newly adapted ddPCR method. Burkard spore traps (Burkard Manufacturing Co. Ltd.) were placed in five different vineyards in the Okanagan Valley of BC and were set to collect either daily (2 sites) or weekly (3 sites). Total genomic DNA was extracted using a modified protocol of the DNeasy PowerSoil Kit (MO BIO Laboratories). BD spore counts were quantified using ddPCR with either *Botryosphaeria*-specific primers used alone or, *Botryosphaeria*-specific primers and probe. Data generated using the assay with *Botryosphaeria*-specific primers only were compared with data using *Botryosphaeria*-specific primers and probe. Although trends in the resulting data were similar, the ddPCR assay using primers and probe was shown to be more specific and had a higher level of sensitivity. Resulting trends in both datasets showed BD spore release events occurring from late March to October and were absent or greatly reduced through the winter. Generally, the first spore release was detected at the end of winter when average daily temperatures climbed above zero to around 5°C or higher and often correlated, though not always, with rainfall. These results will assist future research projects aimed to develop BD control strategies including timing for both cultural practices (i.e. pruning) and biological and chemical product applications.

Host-Pathogen Interactions

Metabolic markers of grape infection with Esca disease.

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Grapevine trunk diseases are amongst the major challenges for viticulture. Among these, *Botryosphaeria dieback*, Esca complex, *Eutypa dieback* and *Phomopsis dieback* are the most common. The main causal agents of the Esca Proper are *Phaeoacremonium* spp., *Phaeo-moniella chlamydospora* and *Fomitiporia* spp. Because the pathogens cannot be detected in leaves and berries of infected plants, the discovery of molecular and metabolic markers of the disease may lead to early detection of infected plants and elucidate on the factors responsible for the progression of the disease. Berries and leaves from control and symptomatic grapevines were collected in 2016 at harvest stage (five biological replicates) from a 17 years old vineyard of Portuguese cultivar Aragonez (= Tempranillo) that had been monitored for three years. The samples were used for metabolomics and targeted qPCR analysis of genes involved in hormonal and phenylpropanoids metabolism and biotic stress response. Principal component analysis of GC-MS data revealed that infected samples were clearly discriminated from controls. However, metabolic reprogramming due to infection was more evident in berries than in leaves. Several volatiles, fatty acids, triterpenoids and phenylpropanoids, putatively involved in defense, were present in higher amounts in infected berries. On the other hand, undergoing qPCR analysis suggested the involvement of salicylic acid signaling in the stress response. Molecular data is being integrated with metabolomics data in order to provide insights on the mechanisms involved in Esca disease affecting grapevines worldwide. Additionally, these results will contribute to predict the impact of infection on wine quality.

A comparison between constitutive and inducible chemical defenses against the esca disease in grapevine.

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Plants are able to counteract microorganisms by establishing physical and chemical barriers, which can be preformed/constitutive or induced. Phenolic compounds are among the most recognized chemical defenses in plants. This study was undertaken to test the hypothesis that grapevine responds to wood infection by esca fungi by systemically inducing the biosynthesis of phenolics in asymptomatic leaves. In a naturally infected vineyard, the occurrence of esca syndromes was annually evaluated on *Vitis vinifera* L. cv. Malvasia, and brown streak, petri disease, leaf stripe, apoplexy and black measles vines identified. Plants reacted to esca through the production of phenolics in a diversified way, depending on the presence or absence of foliar symptoms. In asymptomatic leaves of brown streak vines, total phenolics decreased relative to healthy vines, independent of the class assayed i.e., phenolic acids, anthocyanins, flavonoids, and proanthocyanidins. These decreases were concomitant with those of several phenolics identified by liquid chromatography e.g., caftaric acid, kaempferol-3-O-glucoside, *trans*-resveratrol. A declining trend was also observed in asymptomatic leaves of leaf stripe and black measles vines, which did not support the hypothesis of a systemic induction of phenolic defenses. Plants responded to foliar symptom development by increasing levels of phenolics and inducing compounds undetected in healthy leaves, and these changes correlated with the extent of lesions on the leaf surface. For example, an increase of 75% between healthy and apoplexy leaves was observed for kaempferol-3-O-glucuronide. It appeared that the sequence of events in the defense response of grapevine include first the recruitment of preformed phenolics before visible browning of leaves, and second the synthesis of specific phenolics with the onset of symptoms. Decreased phenolic levels in asymptomatic leaves suggest that high amounts of constitutive compounds might be associated with the long latency time and lack of symptoms between continuous years, which are characteristic traits of esca disease.

Metabolomic studies of two *V. vinifera* subspecies during infection by *Neofusicoccum parvum*, a *Botryosphaeria dieback* pathogen – on the road to identify infection and resistance biomarkers. CLEMENT LABOIS^{1,2}, MARY-LORENE GODDARD^{1,2}, HELENE LALOUE¹, PETER NICK³, CELINE TARNUS¹, CHRISTOPHE BERTSCH¹ and JULIE CHONG¹. ¹Laboratoire Vigne,

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In the last decades, vineyards worldwide have been facing the resurgence of wood diseases with dramatic impact on viticulture sustainability and wine production. *Botryosphaeria dieback* is one of the main grapevine trunk diseases. Unfortunately, to date, no efficient treatment is convenient to prevent, protect or limit the progression of these diseases. We have previously shown that some wild grape *Vitis vinifera* subsp. *sylvestris* accession were less susceptible to the infection of *Botryosphaeriaceae*. This result is of great interest to study *Botryosphaeria dieback* resistances. In this context, grape canes from different accessions of *Vitis vinifera* subsp. *sylvestris* and different cv. of *Vitis vinifera* subsp. *vinifera* were infected with *Neofusicoccum parvum*. Necrosis area were measured seven days after infection. Targeted metabolomic studies were further performed to quantify the primary metabolites by GC-MS (sugars, organic acids and amino acids) at different times after infection. In the same way, untargeted metabolomics studies of secondary metabolites were achieved by LC-MS/MS. This approach identified biomarkers of the fungal infection. In addition, several secondary metabolites showed a specific accumulation pattern between the two-grapevine subspecies (*vinifera* and *sylvestris*). Specific accumulation pattern of secondary metabolites could be linked to their differences in trunk disease pathogen resistance.

Clone-dependent expression of grapevine esca disease. FLORIAN MORET¹, CHRISTELLE LEMAÎTRE-GUILLIER¹, CLAIRE GROSJEAN², GILLES CLEMENT³, CHRISTIAN COELHO⁴, LUCILE JACQUENS¹, JONHATAN NEGREL¹, REGIS GOUGEON⁴, GUILLAUME MORVAN⁵, GREGORY MOUILLE³, SOPHIE TROUVELOT¹, FLORENCE FONTAINE⁶ and MARIELLE ADRIAN¹. ¹Agroécologie, AgroSup Dijon, CNRS, INRA, Univ. Bourgogne, Univ. Bourgogne Franche-Comté, F-21000 Dijon, France. ²Chambre Régionale d'Agriculture de Bourgogne Franche-Comté, 21110 Bretenière, France. ³Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78000 Versailles, France. ⁴UMR PAM Université de Bour-

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The occurrence of grapevine trunk diseases (GTDs) increases gradually within vineyards, leading to important economic losses. Several factors could affect grapevine susceptibility to GTDs, especially climate, vine age, soil fertilization, and also rootstock, cultivar and clone. For a cultivar, the level of disease expression can vary with region and from year to year. In this context, our objective was to assess if the expression of esca disease, one of the most widespread GTDs, was also clone-dependent. Two clones (76 and 95) of the Chardonnay cultivar, grown in the same plot, were compared according to their developmental and physiological traits, metabolome, and esca foliar symptom expression. Agronomical data and symptom expression were recorded during summer 2015, and metabolome analyses were performed in leaf samples collected from visually healthy vines as control (C), and from both symptomatic (D+) and asymptomatic (D-) shoots of esca-affected vines. The bud burst percentage and the fertility ratio were significantly lower for clone 76 than for clone 95, whereas the plant vigor was similar. The percentage of vines expressing the apoplectic and chronic forms of esca disease was low but slightly higher for clone 95. Global GC-MS analysis highlighted a clone-dependent metabolic fingerprint of disease expression. Additional targeted HPLC analyses showed opposite variations in the accumulation of *trans*-caffeoyltartaric acid, quercetin-O-glucoside, quercetin-O-galactoside, and kaempferol-O-glucoside (higher levels in control leaves of clone 76 than in diseased ones, and the opposite for clone 95). An original approach, 3D fluorescent analysis, was also used to compare samples and also pointed out significant differences in disease expression between clones. Altogether, this study highlights a clone-dependent metabolic response related to esca-disease expression, and the potential of the 3D fluorescence analysis as a new method to detect it. It would be interesting to extend the analysis to other clones and varieties.

Two infections, one grapevine: How infection by one pathogen may impact that of a second pathogen via induced changes in host physiology. CHRISTOPHER M. WALLIS¹, KENDRA BAUMGARTNER² and ERIN GALARNEAU³. ¹U.S. Department of Agriculture, Agri-

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Grapevine trunk diseases are often characterized by mixed infections resulting from colonization of different pruning wounds by spores of a variety of trunk pathogens. Despite being located on different branches, a first infection may induce systemic changes in host physiology that could alter progress of an independent infection elsewhere on the vine. However, studies examining multiple fungal infections on the same grapevine host are lacking. To investigate the interactions of two separate infections in a single vine, a trunk pathogen was inoculated two months after initially infecting the vine with a trunk pathogen on a different branch. The lesion growth of the second infection was assessed and compared with the growth of infections on plants not previously infected. Furthermore, we analyzed amino acid, sugar, phenolic compound, and terpenoid levels in grapevine stem tissues just prior to the second infection to observe if the initial infections shifted host physiology to disfavor pathogen establishment and growth. Initial inoculation with *Botryosphaeria*-dieback pathogen *Diplodia seriata* was associated with reduced lesion lengths of subsequent inoculation with *D. seriata*, *Botryosphaeria*-dieback pathogen *Neofusicoccum parvum* and Esca pathogen *Phaeomonliella chlamydospora*. Preliminary results suggest these reductions were associated with changes in host physiology including shifts in amino acid, sugar, phenolic compound, and terpenoid levels. Follow-up studies are underway to confirm this phenomenon and examine other pathogen combinations. Results should provide information on how grapevine physiology could be manipulated to better increase disease resistance and allow fine-tuning of integrated pest management programs based how presence of one pathogen may affect another.

Genetic basis of xylem morphology in grapevine: impact on hydraulic conductivity and resistance to *P. chlamydospora*. JEROME POUZOULET¹, SABINE GUILLAUMIE¹, REMI CHARBOIS¹, LAURENT LAMARQUE³, GREGORY A. GAMBETTA¹, SYLVAIN DELZON³, CHLOE E. L. DELMAS² and NATHALIE OLLAT¹. ¹UMR 1287 EGFV, ISVV, INRA, 33882 Villenave d'Ornon, France. ²UMR 1065, SAVE, INRA, 33140

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Vascular diseases of cultivated grapevine, *Vitis vinifera* L. *sativa*, are factors that can considerably limit vineyards productivity and longevity. Among these diseases, Esca disease is one of the most prevalent and destructive worldwide. While complete resistance toward Esca causal agents does not exist within the *Vitis* genus, differences in the ability of cultivars to limit the movement of pathogens have been reported. Previous studies provided clues about the role of xylem vessel diameter in the ability of some commercial cultivars to respond to the infection of an Esca causal agent, *Phaeoemoniella chlamydospora*. Here, we tested this concept within a grapevine rootstock experimental progeny (F2, *V. riparia* cv. Gloire de Montpellier x *V. vinifera* cv. Cabernet Sauvignon). Two hundred and sixty-one genotypes from the progeny were characterized for various xylem morphological traits and a QTL analysis was performed over 2 years of observation. Our results showed that strong and stable QTLs associated with various xylem morphological traits can be found in this progeny, including for the diameter of vessels. Based on this analysis, subsets of genotypes were further characterized for functional traits (hydraulic conductivity, resistance to *P. chlamydospora*). A QTL found for vessel diameter was seen to impact xylem hydraulic conductivity significantly. In addition, our results confirmed that within this progeny as well, the density of vessels of wide diameter was positively correlated with the level of susceptibility to the pathogens. This study provides useful insights about genetic basis responsible for xylem morphological traits in perennial plants, and their potential impact on vascular diseases resistance.

Correlating xylem vessel size of grapevine cultivars and esca disease incidence in the field. ENZO FOGLIA, LUCIA LANDI and GIANFRANCO ROMANAZZI. Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, I-60131 Ancona, Italy. E-mail: g.romanazzi@univpm.it

Esca is one of the most important grapevine trunk diseases. This disease seriously affects vineyard productivity and longevity, being the main cause of the death of vines in most viticultural areas. Previous studies suggest that wide xylem vessel diameter favor the development of *Phaeoemoniella chlamydospora*, one of the fungi involved in esca. The aim of this work was to determine the anatomical measurements (diameter and frequency)

of xylem vessels of grapevine cultivars with different susceptibilities to esca. In this study 27 white-berried and 24 red-berried grapevine cultivars, grown in the same experimental vineyard were analyzed and the vessel dimension characteristics were correlated with esca incidence previously detected. The cultivars showed significant differences in vessel parameters. However, no linear relationship was detected among the vessel size and esca incidence in the field. Overall, white-berried cultivars showed wider vessel diameter than red-berried cultivars. From our investigations there is no clear relationship between vessel size and esca incidence. Vessel anatomy profiles can be useful for further investigations on sensitivity of grapevine genotypes to grapevine trunk diseases.

Exploring the hydraulic failure hypothesis on esca leaf symptom formation. GIOVANNI BORTOLAMI¹, GREGORY A. GAMBETTA², SYLVAIN DELZON³, LAURENT J. LAMARQUE³, PASCAL LECOMTE¹, JEROME POUZOULET² and CHLOE E. L. DELMAS¹. ¹Santé et Agroécologie du Vignoble, INRA, 33140 Villenave d'Ornon, France. ²Bordeaux Science Agro, Institut des Sciences de la Vigne et du Vin, Ecophysiologie et Génomique Fonctionnelle de la Vigne, INRA, 33140 Villenave d'Ornon, France. ³BIOGECO, INRA, Univ. Bordeaux, 33615 Pessac, France. E-mail: chloe.delmas@inra.fr

Plant vascular disorders are sometimes identified by conspicuous leaf scorch symptoms but the exact mechanisms driving leaf symptoms remains unknown. Two of the main hypotheses, presence of air embolism or tyloses/gels, rely on the disruption of xylem integrity but they still need to be investigated. In this study we explore the xylem integrity in esca symptomatic leaves of naturally field-infected grapevines (*V. vinifera* cv. Sauvignon) using traditional light microscopy and non-invasive, *in vivo* imaging via x-ray microtomography (microCT). This method allows for the visualization and quantification of embolism and vessel functionality in esca symptomatic leaf petioles and midribs. We survey annual stems and leaves using qPCR to determine if two of the main pathogen species associated with esca, *Phaeoemoniella chlamydospora* and *Phaeoacremonium minimum*, were present in these annual organs. Our results demonstrated that symptomatic leaves are not associated with air embolized xylem conduits. In symptomatic leaves, high percentages of xylem vessels were not functional due to nongaseous embolisms formed by gels and/or tyloses. However, the severity of esca leaf symptoms was not correlated to the proportion of non-functional ves-

sels. *P. chlamydospora* and *P. minimum* were undetected in the vine's distal organs confirming that the symptoms and vascular occlusions likely occur at a distance from the trunk where fungal infections occur. Studying xylem water transport and vessel integrity during esca pathogenesis is fundamental and critically important if we are to understand esca etiology. Our observations inform new perspectives on esca symptom expression where two of the underlying hypotheses (elicitor/toxin and hydraulic failure) are not necessarily mutually exclusive

Effect of water and heat stresses on the physiology of Ugni blanc infected by *Neofusicoccum parvum*.

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Botryosphaeria dieback is one of the most widespread and prevalent grapevine trunk diseases (GTDs) in Europe. Symptoms include necrosis of perennial organs of grapevine while external symptoms include bud necrosis, leaf discoloration and dead arms although incidence and severity vary from year to year. The causal agents of this disease are Botryosphaeriaceae species with *Neofusicoccum parvum* being one of the most prevalent. Their aggressiveness were reported to be influenced by environmental factors particularly temperature increasing disease severity. Thus, our study investigated the influence of heat and water stress on the grapevine / *N. parvum* interaction. Cuttings of Ugni Blanc, a cul-

tivar known to be susceptible to GTDs, were artificially infected by *N. parvum* and subjected to two soil water status and two heat conditions (three days at 35°C or no heat stress). Plant growth, photosynthetic activity and the necrosis induced by *N. parvum* were monitored. The whole plant responses against these biotic and abiotic stresses, alone or in combination were investigated using transcriptomic and metabolomics analyses. Our results showed the two abiotic stresses altered the photosynthetic activity of the vines. The response to infection differed depending on the abiotic stresses applied as revealed by specific transcriptomic and metabolomic profiles. The impact of heat and water stresses seems greater when they occurred simultaneously due to reduced plant growth and increased aggressiveness of *N. parvum*. The impact of infection on the metabolism was greater after a heat stress, compared to water stress, but highest severity was observed when these two stresses were present. The lipids and secondary metabolites were the two plant compounds greatly impacted by the infection. Globally, these results may partly explain the seasonal variability of GTD symptom expression in the vineyards that may be influenced by abiotic stresses that can occur separately or simultaneously.

Investigating the production and translocation of phytotoxic metabolites by Australian Botryosphaeriaceae spp. in artificially-inoculated and naturally-infected vines.

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Grapevine trunk diseases (GTDs) caused by pathogenic fungi are considered a serious problem to worldwide. Some GTD pathogens produce phytotoxic metabolites (PMs) that play an important role in their virulence. It is hypothesized that these PMs are translocated into the leaves causing foliar symptoms. However, foliar symptoms in vines affected with Botryosphaeria dieback have never been reported in Australian vineyards. A previous investigation demonstrated that different PMs, including R(-)-mellein, were produced *in vitro* by Australian Botryosphaeriaceae isolates. To gain insight into the role of PMs in the virulence and symptomatology of the pathogens, production of PMs *in planta* were investigated by

molecular and analytical chemistry techniques. Wood samples from vines naturally-infected with *Botryosphaeria dieback* were collected from three vineyards in New South Wales, Australia. For artificially-inoculated vines, one-year-old rootlings (cv. Chardonnay and Cabernet Sauvignon) were inoculated with *Diplodia seriata*, *Spencermartinsia viticola* and *Dothiorella vidmadera*. All symptomatic and asymptomatic wood samples were analysed by cultural isolations and qPCR to detect and quantify the pathogens. An LC-MS/MS protocol was optimized for the detection of spencertoxin, protocatechuic alcohol and *R*-(-)-mellein from infected wood. *R*-(-)-mellein was detected by LC-MS/MS but was only present in symptomatic wood and its amount correlated with the amount of pathogen DNA detected by qPCR. These results suggest that this PM was not translocated as previously hypothesized. The inability to detect the other two PMs could be explained by the following: they could be under the detection limit, they could be produced during the later stages of infection, they could be broken down into other compounds or detoxified by the plant. Our findings suggest that *R*-(-)-mellein may not be directly involved in the development of foliar symptoms and these symptoms may be caused by a combination of diverse factors which require more in-depth studies.

Mechanistic overview of the chelator-mediated Fenton (CMF) system in brown rot fungi and its potential role in fungal grapevine trunk diseases. BARRY GOOD-ELL¹, JODY JELLISON² and E. PETIT¹. ¹*Department of Microbiology, 102 Morrill Science Center IV-N, 639 N. Pleasant St., University of Massachusetts, Amherst, MA 01003 USA,* ²*Center for Agriculture, Food and the Environment, 319 Stockbridge Hall, University of Massachusetts, Amherst, MA 01003 USA. E-mail: bgoodell@umass.edu*

Brown rot fungi comprise only 6% of all known Basidiomycota species, yet they degrade approximately 80% of wood, by mass, in terrestrial environments. Unlike white rot fungi, brown rots do not possess lignin degrading enzymes and have abandoned both peroxidases and many carbohydrate-active enzymes (CAZymes) as they evolved from white rot progenitors. Concurrently, many brown rot fungi evolved a unique low molecular weight mechanism for initiating decay in wood known as the chelator-mediated Fenton (CMF) system to depolymerize both cellulose and lignin components of wood. In this system, low molecular weight iron-binding fungal metabolites are produced, and at the correct pH these metabolites also redox-cycle to generate a stream

of hydroxyl radicals which have been demonstrated to be responsible for much of the depolymerization of the plant/wood cell wall components in brown rot. These metabolites can diffuse through cell walls rapidly to depolymerize both lignin and holocellulose components. It is this depolymerization, particularly of the cellulose backbone of the elementary wood fibrils, that dramatically reduces the mechanical properties of wood undergoing brown rot attack. It is unknown if the stem necrosis observed in some grapevine trunk diseases (GTD) is associated with low molecular weight fungal metabolites or similar types of CMF reactions. However prior research has demonstrated that *Phaeoconiella* and *Phaeoacremonium* genera produce iron-binding redox-cycling compounds that are capable of generating hydroxyl radicals associated with cellulose degradation. Further, *Fomitiporia mediterranea* is an intermediate species between white rots and brown rots and may possess CMF capability. We propose, with French and Italian collaborators, that examination of the role of low molecular weight iron-binding fungal metabolites may permit a better understanding of the mechanisms behind some types of fungal GTDs, and that this understanding may lead to new targets for therapeutic treatments and IPM to combat GTDs.

Non-Enzymatic *in lignum* degradation mechanism: a way to control Grapevine Trunk Disease? SAMUELE MORETTI², ROMAIN PIERRON¹, ANDREA PACETTI², MELANIE GELLON¹, CELINE TARNUS¹, STEFANO DI MARCO³, LAURA MUGNAI², BARRY GOOD-ELL⁴, ERIC GELHAYE⁵, CHRISTOPHE BERTSCH¹ and SIBYLLE FARINE¹. ¹*Laboratoire Vigne Biotechnologies et Environnement UR-3991, Université de Haute Alsace, 33 rue de Herrlisheim, 68000 Colmar, France.* ²*Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant pathology and Entomology section, University of Florence, P.le delle Cascine, 28, 50144 Firenze, ITALY.* ³*CNR IBIMET, Via Gobetti 101, 40129 Bologna, Italy.* ⁴*Microbiology Department, University of Massachusetts. 102 Morrill, Science Center IV-N, 639 North Pleasant St. Amherst, Massachusetts 01003, USA.* ⁵*Unité Mixte de Recherches 1136 Université de Lorraine/INRA, Interactions Arbres / Micro-organismes, Faculté des sciences, Bd des aiguillettes, 54500 VANDOEUVRE-Lès-NANCY France. E-mail: sibylle.farine@uha.fr*

For many years wood decay by fungi was assumed to be caused exclusively by extracellular cellulases and lignin-degrading peroxidase enzymes produced by certain

Ascomycota and Basidiomycota species. It is now recognized that enzymatic action alone does not explain how “brown rot” wood decay occurs. A non-enzymatic pathway generating hydroxyl radicals deep within wood cell walls has now been demonstrated to be responsible for the depolymerization of both cellulose and lignin during brown rot wood decay, and extracellular cellulases are then produced secondarily to permit further deconstruction of the plant cell walls. Because iron-binding compounds mediate this degradation of wood, the mechanism observed has been termed the “chelator-mediated Fenton” (CMF) reaction. Decay species such as *Fomitiporia mediterranea* (*Fmed*) are intermediate between brown and white rot fungi and this species is known to be involved in Esca disease in *Vitis vinifera*. However, it is unknown whether *Fmed* or other grapevine trunk disease fungal species produce low molecular weight (LMW) iron-binding compounds to promote CMF reactions and wood decay. The aim of our work is to better understand the role of CMF reactions *in lignum* during Esca infection in order to develop control strategies. Indeed, assuming that the iron-binding and the redox capacity of the LMW metabolites from the pathogen may be important in disease development, any Biological Control Agent (BCA) that could play a role in interfering with the fungal CMF mechanism may help to reduce the pathogenic effects and spread of the causal fungi. For this purpose, we are investigating the ability of the fungi associated with Esca to produce iron-binding metabolites and oxalic acid; both playing an essential role in the CMF reaction. We are also considering the degradation capacity of oxalic acid by different *Trichoderma* species to explore if oxalate oxidase increases the activity of these biocontrol agents.

Ecological role of phytopathogens in vineyard microbial ecosystem: pathogenic synergies and antagonisms from global mycobiome studies. RÜDIGER ORTIZ-ÁLVAREZ¹, CÁTIA PINTO¹, CRISTINA PEÑAS¹, AVA MEHRPOUR¹, LUIS LOPEZ¹, CHARLES RAVARANI¹, IGNACIO BELDA^{1,2} and ALBERTO ACEDO¹. ¹Biome Makers, 890 Embarcadero Drive West Sacramento, CA 95605, USA. ²Unit of Biodiversity and Conservation, Rey Juan Carlos University, 28933 Móstoles, Spain. E-mail: catiapinto@biomemakers.com

Soil and vine are naturally colonized by a complex microbial ecosystem, and its balance is critical for plant health and productivity. However, understanding how such microbial networks interact with the plant is a primary challenge not only to develop a model to predict

plant disease scenarios but also to identify microorganisms with beneficial potential. In this context, the aim of this study was to explore the fungal communities from 404 vineyard soil samples by using ITS metabarcoding DNA sequencing. For this, samples from vineyards under different management regimes (conventional, organic, and biodynamic) and from different geographic and climate regions of Spain and USA were compared, and a deep analysis of the fungal pathogen species related with grapevine trunk diseases (GTDs) was performed. Results from the probabilistic method to infer co-occurrences and co-exclusions in two soil metacommunities (Spain and USA) showed both positive and negative associations between microorganisms and grapevine. Thus, the list of all the significant pairs included potential positive associations between groups of pathogens, acting as potential co-infection complexes (such as *Ilyonectria liriodendri* and *Ilyonectria robusta*); and, also negative associations of multiple pathogens and other microorganisms present in the soil, which may act as broad biocontrol agents (i.e., 21 species linked to both *Cadophora luteo-olivacea* and *Campylocarpon fasciculare*). Quantifying per sample the number of species negatively associated to plant pathogens, may help to assess: (1) the risk of a plant to harbor a disease, (2) the biocontrol potential of the soil, and (3) if there are links between the soil community structure and the presence of plant pathogens. Altogether, this model contributes to the understanding of the role of the microbial structure and functioning in the vulnerability of a vineyard soil ecosystem. We are convinced that by understanding these ecological roles this would foster the innovation on vine sector.

Synergistic interactions between *Fomitiporia mediterranea* and wood-inhabiting bacteria promote grapevine-wood degradations. RANA HAIDAR^{1,2,3}, AMIRA YACOUB^{1,2}, ANTOINE PINARD^{1,2}, JESSICA VALLANCE^{2,1}, STEPHANE COMPANT⁴, ANTOINE LOQUET⁵ and PATRICE REY^{1,2}. ¹INRA, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), F-33140 Villenave d'Ornon, France. ²Université de Bordeaux, ISVV, UMR1065 SAVE, Bordeaux Sciences Agro, F-33140 Villenave d'Ornon, France. ³Tichreen University, Faculty of Science, Biology Department, PO Box 2231, Latakia, Syrian Arab Republic. ⁴AIT Austrian Institute of Technology GmbH, Bioresources Unit, Konrad-Lorenz-Straße 24, Tulln 3430, Austria. ⁵Institut de Chimie et Biologie des Membranes et des Nanoobjets, CNRS, Université de Bordeaux, 33077 Bordeaux, France. E-mail: amira.yacoub@inra.fr

Nowadays, viticulture worldwide is confronted with heavy economic losses caused by Grapevine Trunk Diseases (GTDs). Fungi have been described as the main cause of grapevine trunk diseases. Among these fungi, *Fomitiporia mediterranea*, is considered as the main white wood rotting Basidiomycota associated with GTDs. Bacteria diversity is very high in the wood of grapevine but little information is available about their functions and their interactions with fungi inhabiting the wood. We therefore isolated bacterial strains from different anatomical parts (i.e. trunk and cordon) and different type of tissues (i.e. necrotic or not) of GTD-symptomatic grapevines. Two hundred thirty seven bacterial strains were isolated from grapevine-wood samples and, based on their 16S rRNA genes, assigned to bacterial species, OTUs belonging to *Xanthomonadaceae* were the dominant taxonomic groups, at the family level. Fifty nine bacterial strains representing the various OTUs were screened for their ability to degrade the three main components of wood, i.e. cellulose, hemicellulose and lignin, by using different selective media. Their ability to inhibit *F. mediterranea* was also studied. Based on a microcosm experiment, the hypothesis that some bacterial strains inhabiting wood interact with *F. mediterranea* to promote grapevine wood degradation was tested. *Results demonstrated*, for the first time, a synergetic interaction between *F. mediterranea* and the two bacterial strains: *Chryseobacterium* sp. and *Paenibacillus* sp., to degrade the grapevine-wood structures. The whole-genomes of the 2 bacterial strains were sequenced because of their interest in wood degradation. *After wood samples inoculations with F. mediterranea* alone, the bacteria alone, and *F. mediterranea* plus *Paenibacillus* sp., analysis of the wood-degradations that occurred in the cellulose, hemicelluloses, and lignin, were investigated by Solid-State Nuclear magnetic resonance (NMR) method. The contribution of bacteria and fungi alone, or in synergy, in wood degradations will be discussed with regards to GTDs.

The control and control basis of grapevine trunk diseases in China. JIYE YAN, WEI ZHANG, QIKAI XING, JUNBO PENG, MEI LIU, YING ZHOU and XINGHONG LI. *Beijing Key Laboratory for Environmental Friendly Management on Pests of North China Fruits, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China. E-mail: jiyeyan@vip.163.com*

Grapevine trunk diseases (GTDs) are widely spread in almost all grapevine cultivating countries, causing serious economic losses. At present, six species of *Botryosphaeriaceae*, including *Botryosphaeria dothidea*, *Lasiodip-*

lodia theobromae, *Neofusicoccum parvum*, *Diplodia seriata*, *L. pseudotheobromae* and *N. mangiferae*, were proved to be associated with GTDs in China. Recent studies have established a correlation between environmental factors and GTDs occurrence; however, less is known about the factors that can trigger these diseases. Therefore, de novo sequencing of *L. theobromae*, one of the most prevalent species in China, was conducted and resequencing was done for *B. dothidea* and *N. parvum* in this study. Our data showed that gene families associated with cell wall degradation, nutrient uptake, secondary metabolism and membrane transport, which contribute to adaptations for wood degradation were expanded in botryosphaeriaceous genomes. Further, transcriptome analysis was performed and the results revealed that genes involved in carbohydrate catabolism, pectin, starch and sucrose metabolism, and pentose and glucuronate inter-conversion were induced during the infection process of *L. theobromae*. Furthermore, genes involved in carbohydrate-binding modules and the lysine motif domain and glycoyl hydrolase gene families were found that can be induced by high temperature. Among these genes, over-expression of two selected putative lignocellulase genes led to increased virulence in the transformants. These results demonstrate the importance of high temperatures in the opportunistic infections of *Botryosphaeriaceae* species. The current study also presents a set of *Botryosphaeriaceae* specific effectors related to pathogenicity. In conclusion, these findings significantly improve our understanding of the determinants of pathogenicity or virulence in *Botryosphaeriaceae* species and provide new insights for developing new strategies to control them.

***Lasiodiplodia gilanensis* used a model for understanding the pathogenicity of Botryosphaeriaceae.** EDELWEISS AIRAM RANGEL-MONTOYA¹, MARCOS PAOLINELLI^{2,3} and RUFINA HERNANDEZ-MARTINEZ¹. ¹*Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Carretera Ensenada-Tijuana, 3918, Zona Playitas, 22860, Ensenada, B.C. México.* ²*Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria de Mendoza, San Martín 3853 (5507), Luján de Cuyo, Mendoza, Argentina.* ³*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290 (C1425FQB) CABA, Buenos Aires, Argentina. E-mail: erangel@cicese.edu.mx*

Members of the *Botryosphaeriaceae* are one of the primary fungal pathogens causing trunk diseases on grapevines. Among them, *Lasiodiplodia* spp. are reported as

the most aggressive, causing degenerative diseases, die-back, and plant death. To understand the pathogenicity of the Botryosphaeriaceae we are using *Lasiodiplodia gilanensis* as a model. A transcriptional study suggested that this fungus is capable of using the phenylpropanoid precursors and salicylic acid to avoid the host defense response of the plant. Several genes encoding enzymes involved in different melanin synthesis pathways, 3,4-dihydroxyphenylalanine (DOPA)-melanin, 1,8-dihydroxynaphthalene (DHN)-melanin, and pyromelanin were identified, and their production evaluated, concluding that the fungus use different types of melanin to overcome environmental stress. An *in silico* analysis shows the presence of those genes in all the available genomes of Botryosphaeriaceae in GeneBank, evidencing the importance of the melanin in this family. *Lasiodiplodia gilanensis* also produces siderophores of catechol of hydroxamate-type, as well as naturally esterified fatty acids; those compound might have a role in plant growth regulation. A plethora of hydrolytic enzymes is also produced, including xylanases, ligninases, cellulases, pectinases, cutinases, and hemicelluloses. An organic compound also produced is oxalic acid, reported as a pathogenicity factor in other fungi; its role in *L. gilanensis* is under evaluation. Microscopical observations showed that the fungus uses the starch deposited in the ray cells as carbon source, induces the production of suberin and phenolic compounds, and colonizes the vascular cambium, ray parenchyma, and the vascular bundles. In summary, these studies extend our understanding of the pathogenicity of a widely distributed pathogenic fungus.

Screening of biocontrol agents against black-foot and Petri diseases under field conditions.

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Experimentally, most of the studies on biocontrol agents (BCAs) have been applied so far in vines under con-

trolled conditions and little information is still available about the effectiveness of this strategy under field conditions. In this study, two field experiments were established to evaluate the effect of five BCAs (*Streptomyces* sp. E1+R4, *Pythium oligandrum* Po37, and commercial products containing *Trichoderma atroviride* SC1, *T. koningii* and *Pseudomonas fluorescens*+*Bacillus atropheus*) root treatments on black-foot and Petri disease fungal infection in one-year-old dormant grafted plants prior to dispatch. In April 2017, vines were hot-water treated at 53°C for 30 min and roots were immediately soaked in BCAs suspensions for 24 h. Two additional applications of BCAs were applied by drip irrigation in May 2017 and 2018. In each field, 50% of the vines were evaluated in February 2018 and the remaining 50% in February 2019. The fungal incidence and severity in roots and at the base of the rootstock in all vines and the total root mass and shoot weight in 3-year-old vines were determined. The effectiveness of some BCAs in reducing the incidence and severity of both diseases was dependent on the plant zone analysed and the plant age. *Streptomyces* sp. E1+R4, *Pythium oligandrum* Po37 and *Trichoderma atroviride* SC1 were able to reduce significantly fungal incidence and severity in specific scenarios. BCA treatments had no effect on the shoot weight, and root weight was significantly lower in all BCA treatments with respect to the control. This study represents the first approach to evaluate the effectiveness of different antagonistic microorganisms (bacteria, fungi and oomycete) to control black-foot and Petri disease under field conditions. Investigation of BCA able to prevent or at least reduce the development of GTDs should be considered a research priority based on the restriction and difficulties that chemicals are facing in most countries around the world.

Identification and characterization of potential biological control agents for the management of grapevine trunk diseases in British Columbia.

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Species within the *Trichoderma* genus are widely used biocontrol agents (BCA) capable of protecting plants by actively antagonizing plant pathogenic fungi, including grapevine trunk disease (GTD) pathogens, through a wide array of mechanisms. Despite this, no BCA prod-

ucts are registered in Canada for the control of GTD. Accordingly, the main objectives of this research were i) to characterize *Trichoderma* spp. from the Okanagan Valley in British Columbia (BC) by means of morphological, biological, and molecular studies and ii) to screen for isolates that can be used as BCA against *Botryosphaeria dieback* fungi in BC. A total of 29 *Trichoderma* isolates were obtained from grapevines in BC and phylogenetic analyses of the ITS1-5.8S-ITS4 and TEF-1a genes allowed us to identify eight species in BC, including *T. asperellum*, *T. atroviride*, *T. harzianum*, *T. koningii*, *T. koningiopsis*, *T. viride* and two *Trichoderma* sp. novel. Characterization studies included experiments to determine optimum temperature for mycelial growth and spore germination. The antagonistic capabilities of *Trichoderma* isolates against *Diplodia seriata* and *Neofusicoccum parvum* were screened in vitro via dual culture assay and results revealed 26 isolates to provide more than 50% mycelium growth inhibition against both fungi. The best performing isolate from each *Trichoderma* sp. was then selected for screening their potential as BCA *in plantae* via a detached cane assay (DCA) under greenhouse conditions. Canes were pruned and treated with a total of 50,000 spores from each individual *Trichoderma* sp. Pruning wounds were then challenged with a total of 5,000 conidia of *D. seriata* or *N. parvum* 24h, 7d and 21d after treatment. The three best performing isolates were re-evaluated as double and triple species combination suspensions. Fungal re-isolation results show 100% reduction of infection from 1d to 21d post treatment with combined BCAs compared to inoculated controls. This research represents the first steps towards developing BCA for management of GTD in Canada.

Disease Management

Ozone dissolved into water: an innovative tool in grapevine nursery for young plants production? ANA ROMEO-OLIVAN^{1,‡}, MARIELLE PAGÈS-HOMS^{1,‡}, HUBERT CROS², OLIVIER YOBREGAT³, CORALIE BRETON¹, ROMAIN PIERRON^{1,*}, FRÉDÉRIC VIOLLEAU⁴ and ALBAN JACQUES¹. ¹Unité Pathologie, Physiologie et Génétique Végétales (PPGV), INP PURPAN, Université de Toulouse, France. ²Pépinière Daydé, Montans, France. ³Institut Français de la Vigne et du Vin, pôle Sud-Ouest, Lisle sur Tarn, France. ⁴Laboratoire de Chimie Agro-Industrielle, INP-PURPAN, Université de Toulouse, France. * Present Address: LVBE, Université de Haute Alsace, Colmar, France. ‡ These authors contributed equally to this work. E-mail: ana.romeo-olivan@purpan.fr

Grapevine trunk diseases (GTDs) can cause severe

symptoms and eventual death. GTDs can infect nursery plants at different propagation stages and symptoms may appear one year later. Grapevine cultivation is in need of a high volume of phytosanitary products. However, a reduction on pesticide use is a requirement for a sustainable viticulture. Ozone is a promising alternative for controlling microbial infections in plant production processes. The goal of this study was to evaluate the efficiency of ozonated water against different fungal species associated with GTDs *in vitro*, *in planta* and in nursery conditions. The effect of ozone on the plant growth was also studied. *In vitro*: Spore solutions of *Phaeoacremonium minimum* (*P.min*) and *Phaeomoniella chlamydospora* (*P.ch*) were treated with ozonated water or water (control). Spore germination was evaluated after 5 days. *In planta*: Cuttings of *Vitis vinifera* L. Cabernet-Sauvignon clone 15 were injured and infected by *P.min* and *P.ch*. Immediately after infection, injuries were treated with ozonated water or water (control). Fungal development was assessed by q-PCR. Nursery-like assays: Plants were treated with ozonated water or antifungal products within the different steps of the plant propagation process. Viability and contamination with *P.min* and *P.ch* were evaluated. Irrigation tests: Cuttings were irrigated with ozonated water or demineralized water (control) for 6 weeks. We assessed scion bud sprouting and rootstock root growth. The results obtained were: *In vitro*: While spores on control samples germinated normally, no spore germination was observed in the ozone treated samples, showing a powerful sporicidal effect. *In planta*: Results showed a lower number of copies of both fungi in treated plants in an early evaluation after infection compared to control plants, suggesting that ozone would retard the fungal colonization in cutting-wounding conditions. Nursery-like assays: The viability and sanitary quality of plants was comparable for both treatments. Irrigation test: Ozone treatments accelerated bud sprouting and the stimulation root growth, suggesting that ozone might favor plant development.

Hybrid trunk disease evaluation: a serendipitous opportunity. PAUL E. READ, BENJAMIN A. LOSEKE and STEPHEN J. GAMET. Department of Agronomy and Horticulture, University of Nebraska Viticulture Program, University of Nebraska, Lincoln, NE 68583 USA. E-mail: pread@unl.edu

Declining yields in Midwest and Nebraska vineyards have been variously attributed to aging of the vines, winter injury or other environmental factors. Only recently have grapevine trunk diseases (GTD) become suspect.

A serendipitous opportunity arose for the University of Nebraska Viticulture Program (UNVP) when a 20-year-old research planting was required to be terminated in August, 2018. Because of the projected termination, UNVP personnel “harvested” and evaluated for GTD multiple complete vines of over 25 cultivars of hybrid grapevines, most of which were at or approaching 20 years of age. All were trained to a high-wire double cordon system. Evaluations were based upon observations of the visible symptoms illustrated by cross-sections at five locations: 15 cm from the distal end of the cordon, mid-cordon, crown, 90 cm above the ground, and 10 cm above-ground. All of the evaluated vines exhibited recognizable symptoms of one or more GTD, with some showing text-book symptoms of *Eutypa* and/or *Botryosphaeria*. In general, the severity of symptoms progressed from a low rating at Position 1 (distal part of cordon) to greater severity at Position 5 (10 cm above the ground) for 18 of the cultivars examined. However, two cultivars showed a discernible decrease in severity from position 5 to Position 1 (‘Norton’ and ‘Brianna’). In addition to these two cultivars, ‘Petite Pearl’, ‘Frontenac Gris’, ‘Frontenac Blanc’ and ‘Sabrevois’ ratings were considerably better than the 18 cultivars that exhibited the progression of severity from position 1 to Position 5. It is tempting to speculate that the apparent tolerance of ‘Norton’, which also had significantly higher yields than any of the other cultivars, may have been conferred by its purported *Vitis aestivalis* ancestry. Furthermore, ‘Sabrevois’ had far less GTD than its sibling ‘Saint Croix’, while ‘Brianna’ exhibits differing morphological traits than otherwise similar cultivars. We postulate that genetic relationships may play a part in understanding the tolerance of some cultivars to GTD and should be studied further.

Special Session on Grapevine Trunk Diseases control

Disease Management 101: Grapevine trunk diseases control in nurseries and young vineyards. DAVID GRAMAJE. *Instituto de Ciencias de la Vid y del Vino, Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de la Rioja, Logroño 26071, Spain. E-mail : david.gramaje@icvv.es*

A sizeable but unknown percentage of existing grapevine nursery stock produced under conventional nursery practices is likely to be infected with a broad range of taxonomically unrelated pathogens associated with several grapevine trunk diseases (GTDs), namely black-foot and Petri diseases, and *Botryosphaeria* dieback. These infections can eventually debilitate and kill infected plants after they are planted in the vineyard. Production

practices in nurseries provide many opportunities for infection, through poor sanitation practices or introducing infected asymptomatic cuttings from mother vines. Infected plants may initially have no visible symptoms, but they may become apparent after a certain period of time when exposed to field stresses and depending on the level of infection. Detection and identification of these pathogens is challenging, as it requires destructive sampling from different plant parts due to their uneven distribution within the host. In this talk, I evaluate the currently known management strategies applied in nurseries and young vineyards. I will also give an overview of how to minimize the economic impact of these pathogens and to improve the quality of planting material.

Disease Management 101: Grapevine trunk diseases, control in mature vineyards. MARK R. SOSNOWSKI. *South Australian Research & Development Institute, GPO Box 397, Adelaide SA 5001, Australia, School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, SA 5005, Australia. E-mail: mark.sosnowski@sa.gov.au*

Grapevine trunk diseases (GTDs) have become a major concern worldwide, causing significant economic impact by reduced production and vineyard longevity. *Eutypa*, *Botryosphaeria* and *Phomopsis* dieback, and esca affect mature vineyards. These diseases are caused by a wide range of fungal pathogens producing a range of symptoms including: leaf and shoot distortion and discoloration, wood cankers and dieback, internal wood necrosis and staining, poor growth, and eventually vine death. The prevalence of GTDs has significantly increased with changes in production practices, loss of effective chemicals, predominance of susceptible cultivars and ageing of vineyards. Pruning wounds are the main infection portal for these pathogens and inoculum sources include a wide range of alternative hosts such as fruit crops and many introduced and/or native tree species. A good understanding of the etiology, biology and epidemiology of GTD fungi has led to the development of effective management strategies in the vineyard. Cultural practices, such as the timing of pruning, can influence the likelihood of infection and disease control can be achieved through early adoption of preventative strategies to protect pruning wounds. Paints and pastes are used for protection of large reworking wounds. Fungicide and biocontrol products, used for annual pruning wounds, can be applied most efficiently with spray machinery. Removing infected wood by remedial surgery and retraining grapevines can provide curative control.

The economic consequences of grapevine trunk diseases and the potential gains from adopting preventative pruning practices and vine surgery. JONATHAN D KAPLAN¹, RENAUD TRAVADON², MAX NORTON³ and KENDRA BUAMGARTNER⁴. ¹*Department of Economics, California State University, Sacramento, 6000 J St. Sacramento, CA 95819, United States of America.* ²*Department of Plant Pathology, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States of America.* ³*Vancouver School of Economics, University of British Columbia, 6000 Iona Drive, Vancouver, BC, Canada.* ⁴*United States Department of Agriculture, Agricultural Research Service, Davis CA 95616, United States of America. E-mail: kaplanj@csus.edu*

Grapevine trunk diseases threaten the economic viability of vineyards worldwide. In California, the causal fungi are thought to infect pruning wounds with rain, mainly during the dormant season. Infections can reduce yield by over 90% during what would otherwise be a vineyard's most productive years. Revenue falls precipitously as well, leading to significantly shortened profitable lifespans. Preventative practices (pruning-wound protectants, delayed pruning) adopted before symptoms appear (<10 years old) can potentially reduce yield losses and extend a vineyard's profitable lifespan. After symptoms are apparent (~10 years old), vine surgery (or trunk renewal), involving the retraining of a vine from a shoot just above the graft union, can be performed as well to mitigate further losses. To estimate the economic benefits from adopting preventative practices and vine surgery alone or in tandem, we simulated alternative scenarios depicting their adoption on symptomatic vines in a representative California 'Cabernet-Sauvignon' vineyard, which faces time-varying yield effects from infection. We found that preventative practices adopted early (≤ 5 years old) and vine surgery adopted in mature vineyards (>10 years old) significantly reduced yield losses, raised revenue, and extended vineyard profitable lifespans by over 100%. Adoption of preventative practices in year 10, however, showed limited economic value. The analysis also showed that vine surgery alone outperformed early-adopted preventative practices, but in tandem they performed best, with greater gains the earlier preventative practices began. Further, the greatest gains from vine surgery occurred when performed on all symptomatic vines beginning in year 11 (when no preventative practices are adopted earlier) and up to year 14 (when adopting preventative practices in year 3). As such, we recommend growers pursue preventative practices in young vineyards and then vine surgery after symptoms appear in approximately 20% of vines or

before year 15, to maximize vineyard profitability and longevity.

From nursery to vineyard: working towards the development and implementation of management strategies against grapevine trunk diseases in Canada. JOSE RAMON ÚRBEZ-TORRES, DANIEL T. O'GORMAN, JULIE BOULÉ, MELANIE WALKER and JINXZ POLLARD-FLAMAND. ¹*Agriculture and Agri-Food Canada, Summerland Research and Development Centre, Summerland, British Columbia V0H1Z0, Canada. E-mail: joseramon.urbeztorres@canada.ca*

The long term economic viability of the grapevine industry relies on healthy planting material and practical disease management strategies in vineyards. Nowadays, grapevine trunk diseases (GTD) are considered one of the most important biotic factor limiting both grapevine production and lifespan of vineyards worldwide. Etiological, biological and epidemiological studies conducted by the Plant Pathology laboratory at the Summerland Research and Development Centre (SuRDC) since 2010 have significantly contributed to better understand the current status of GTD diseases in British Columbia (BC), laying the foundation for the development of effective disease control strategies. However, contrary to most grape-growing countries around the world, neither cultural practices nor registered products are currently available to mitigate the impact of these diseases in Canada. This presentation will give an overview of the research conducted to date with regard the status of GTD in BC and how research findings are being put into practice to develop and implement the first management strategies against GTD in both young and mature vineyards in Canada.

Trial with an innovative product based on a biocompatible drug delivery system on GTDs incidence, disease development and vine physiology. VINCENZO MONDELLO, OLIVIER FERNANDEZ, PATRICIA TROTEL-AZIZ, CHRISTOPHE CLÉMENT and FLORENCE FONTAINE. *SFR Condorcet FR CNRS 3417, Université de Reims Champagne-Ardenne, Résistance Induite et Bioprotection des Plantes, RIBP EA 4707, BP 1039, Reims, Cedex 2 51687, France. E-mail: vincenzo.mondello@univ-reims.fr; florence.fontaine@univ-reims.fr*

CA3356 is a control product based on plant extracts and oligo elements (Zn and Cu) under registration in Italy, France and Spain. Its specificity lays in the low Cu con-

tent (35g/L) and in the presence of the hydroxyapatite, a “biomimetic” drug carrier able to be absorbed, to move and delivers active ingredients inside the plant. Previous greenhouse tests showed its efficiency in controlling *Plasmopara viticola* and *Phaeoconiella chlamyospora* infections on grapevine. The new regulation of Cu treatments in vineyards (4kg/Ha/year) imposed by the European Union in 2019, encouraged us in testing this product also towards control of GTDs. We analysed its effects (5 treatments/year) on Esca incidence in the field (cv Chardonnay) and on the *D. seriata* and *N. parvum* disease development in greenhouse (cv Chardonnay and Cabernet sauvignon, 2 post-infection treatments). For the latter trials, we studied the effect of CA3356 on plant physiology through measures of the main biometric and photosynthetic parameters, as well as the impact on primary metabolism and plant defense response, by the transcriptomic analyses of thirteen-targeted genes. Results showed a certain reduction trend in cumulate Esca incidences that need to be confirmed by additional experimental replicates. In the greenhouse trial, CA3356 was able to reduce the presence of the target pathogens, but without reducing necrosis length. CA3356 showed a positive role on vine physiology by increasing the photosynthetic activity for both cultivars and of the fresh weight on cv. Chardonnay. At transcriptomic level, CA3356 induced the most of the targeted genes, especially those related to plant defense response such as chitinase, glucanase and other PR-related genes. These inductions were higher than those determined by ASM (S-methyl benzo [1,2,3] thiadiazole-7-carbothioate), a molecule able to activate systemic resistance (SAR) in plant, present in commercial products and here utilized as positive control.

The present research is funded by Nufarm and Natural Development Group.

Evaluation of the fungicide Tessior (boscalid and pyraclostrobin) for control of grapevine trunk diseases in Greece. A. SAMARAS¹, P. NTASIOY¹, S. TESTEMPASIS¹, S. THEOCHARIS², S. KOUNDOURAS² and G. KARAOGLANIDIS¹. ¹Plant Pathology Laboratory, Aristotelian University of Thessaloniki, Thessaloniki, 54124, Greece. ²Laboratory of Viticulture, Aristotelian University of Thessaloniki, Thessaloniki, 54124, Greece. E-mail: gkarao@agro.auth.gr

Grapevine trunk diseases (GTDs) are among the most important grape diseases affecting yield, wine quality and vineyard longevity. Due to the lack of availability of chemical products with high efficacy, the control of

GTDs in Europe relies mainly on cultural measures and some biofungicides. Recently, a new chemical product was released into the European market for the control of GTDs, under the commercial name Tessior®, containing the fungicides boscalid and pyraclostrobin. A series of field experiments were established in Greece aiming to investigate the effectiveness of this new product in protecting pruning wounds from infections caused by *Phaeoconiella chlamyospora* and *Diplodia seriata*. Applications were conducted before (protective) or after (curative) wound inoculation with the pathogens following pruning. Results showed that the applications of Tessior contributed to a significant reduction of disease incidence and severity when applied either on the day of pruning or at least 6 days after pruning. In contrast, delayed applications of 13 or 29 days after pruning and inoculation did not contribute to pathogen control, leading to a disease incidence similar to that observed in the untreated control plants. To further investigate the effectiveness of the protective activity of Tessior in controlling GTDs, two long-term experiments were established in newly planted vineyards, in 2015. Applications of Tessior were initiated from the 1st year of planting, while artificial inoculations with either *P. chlamyospora* or *D. seriata* were initiated 2 years after establishment. Preliminary results of these long-term experiments confirm the high efficacy of the product. This study highlights the effectiveness of Tessior and the appropriate application time to achieve the highest efficacy.

Factors affecting progress of grapevine trunk disease in New Zealand vineyards. MARK R. SOSNOWSKI^{1,2} and DION C. MUNDY³. ¹South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia. ²School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond SA 5064, Australia. ³The New Zealand Institute for Plant and Food Research Limited, Marlborough Wine Research Centre, PO Box 845, Blenheim 7240, New Zealand. E-mail: mark.sosnowski@sa.gov.au

Eutypa and Botryosphaeria dieback are major grapevine trunk diseases (GTDs) in New Zealand, causing significant economic losses, as they do worldwide. This has led to concerted efforts, with support from the newly established Bragato Research Institute, to better understand the progress of GTDs over time and some factors affecting disease incidence. In December 2013, 697 vineyard blocks were selected to represent a cross-section of ages (4 years and older) and varieties planted in the major growing regions of Marlborough and Hawke's Bay. In a

randomly selected section of each block, 200 vines were visually assessed and incidence of vines with dieback and foliar symptoms recorded. Information on variety, clone, and rootstock, along with pruning and disease management strategies, was collected. In November 2018, the same blocks were revisited and assessed as previously, although 95 blocks (14%) had been removed and 14 blocks (2%) reworked. The overall mean incidence of dieback increased from 8 to 20% and foliar symptoms 0.1 to 0.3% over the 5 years across all of the blocks assessed, which corresponded with an increase in average vine age from 12 to 17 years. Overall trends in 2018 revealed variation in dieback incidence between vine ages, varieties, clones, rootstocks and pruning styles, with signs of short-term success using remedial surgery to control GTDs. Details of this study will be presented and implications of the results discussed, with particular reference to management of GTDs and future research requirements.

Factors involved in the failure of grapevine nursery stock in Israel. DAVID EZRA, MENACHEM BORENSTEIN, RAN SHULHANI and DANI SHTIENBERG. *Department of Plant Pathology & Weed Research, ARO, The Volcani Center. P.O.Box 15159, Rishon LeZion, 7528809, Israel. E-mail: dezra@volcani.agri.gov.il*

Grapevine nurseries across Israel experience difficulties producing healthy, robust table grapes and wine grapes during the last year. The causes for this failure were unknown. Due to the importance of these industries in Israel, a national project was initiated aiming at identifying the factors involved and developing a practice that would enable the production of healthy nursery stock. Pathogens were not found to be a factor. Instead, one of the main causes of the failure was the timing of collection of rootstock cuttings. Cuttings were collected too early, before canes on the mother vine became fully dormant, or too late, after the mother vine had terminated their dormancy period, reduced the rooting capabilities of the cuttings significantly. Lack of inappropriate rooting of the rootstocks caused poor development of the rootings and subsequently resulted in their degeneration and decay. Factors related to the handling of the propagation material in the nurseries were of significance as well. In Israel, some of the nurseries store cuttings at 4°C before grafting and callusing. It was found that keeping the rootstock propagation material at 4°C for an appropriate time increases their rooting potential, whereas keeping them too long decreased their rooting potential. Further, the optimum time of cold storage var-

ied based on when during the dormant season cuttings were collected from the mother vines. This presentation will report the procedures used to identify the factors involved in failure to produce healthy table grape and grapevine planting material in Israel and describe the practices for producing healthy plants.

Physiological and qualitative consequences of curettage on grapevine grape berry and young wine of Sauvignon variety from the Bordeaux region. CELINE CHOLET¹, EMILIE BRUEZ¹, CECILE THIBON¹, PASCALINE REDON¹, PASCAL LECOMTE², PATRICE REY², TOMMASO MARTIGNON³, MASSIMO GIUDICI³, PHILIPPE DARRIET¹ and LAURENCE GÉNY¹. ¹EA 4577, *Unité de recherche œnologie, INRA, USC 1366 Œnologie, Université de Bordeaux / Institut des Sciences de la Vigne et du Vin, 210 Chemin de Leysotte - CS 50008, F-33882 Villenave d'Ornon, France.* ²UMR 1065 *Santé et Agroécologie du Vignoble, Institut National de Recherche Agronomique/ Bordeaux Sciences Agro / Institut des Sciences de la Vigne et du Vin, 71, Avenue Edouard Bourleaux, INRA Domaine de la Grande Ferrade - BP81, 33883 Villenave d'Ornon Cedex, France.* ³Simonit&Sirch, *maitres tailleurs de vigne, 1 Rue Porte des Benauges, 33410 Cadillac - Bordeaux, France. E-mail: celine.cholet@u-bordeaux.fr*

Esca, one of the most devastating grapevine trunk diseases, has increased in incidence worldwide over the past decade. Currently, there are no chemical products registered for Esca, as sodium arsenite was banned in 2001 in France and in 2003 in Europe. As a non-chemical therapeutic treatment, curettage has been adopted by growers, but published studies evaluating its efficacy are rare. Curettage consists of physical removal of the white-rotted wood (i.e., amadou), caused by Basidiomycete wood-rotting fungi associated with Esca. It is thought to preserve the healthy part of the vine from being contaminated by these fungi. The aim of this study was thus to examine the influence of curettage on vine growth, nutrient content, and fruit and wine quality. This work presents a synthesis of 3-year experiment of Sauvignon vines curetted in 2014 in a plot in Bordeaux. There were three treatments: (i) untreated, asymptomatic vines, (ii) untreated vines with leaf symptoms (control), and (iii) treated vines (in 2014) with no leaf symptoms following treatment. For each treatment, fertility and growth, chemical composition of the fruit and wine, and sensory quality of the wine were evaluated. Our results showed that in comparison with control vines, treated vines had lower vigor and fertility, but there was no effect of treat-

ment on fruit quality. Wine made with fruit from treated vines was not different from that of control vines in terms of aromatic markers. Wine made with fruit from control vines was less appreciated by a sensory panel, and was characterized by a decrease in aromatic markers and an increase in oxidative markers. Our findings suggest that curettage may minimize the impact of Esca on wine quality, albeit after 3 years. As such, curettage can be considered effective for management of Esca in the short term.

A new architecture of the vine: an original wrestling track. CHRISTOPHE BERTSCH. *Laboratoire Vigne Biotechnologies et Environnement UR-3991, Université de Haute Alsace, 33 rue de Herrlisheim, 68000 Colmar, France. E-mail : christophe.berthsch@uha.fr*

The different varieties of *Vitis vinifera* have varying sensitivities; none of them appear to be resistant or tolerant to grapevine trunk diseases. However, *Vitis vinifera* Subsp. *sylvestris*, the ancestor of our vineyard seems to have the capacity to contain the fungi implied in the disease. From a conceptual point of view, it seems difficult today, with the environmental constraints, to eradicate all the fungi involved in trunk diseases. We must therefore also exploit the resistance processes of the plant. In this context, the history of phylloxera tells us certainly the most beautiful and most effective biological approach: the use of American rootstocks resistant to the devastating insect. The weak point of our current vine about pathogens involved in trunk diseases is not the roots, as for phylloxera, but the trunk, a true bio-reactor for the fungi involved. We propose to rethink the architecture of our vine like the use of rootstocks to fight against phylloxera. It is perfectly possible to insert between the graft and the rootstock a “trunk” tolerant to trunk diseases (*sylvestris*). This innovative approach may produce vines tolerant to the diseases. The first grafting trials done are very promising. A strong point of this method is that it would contain this scourge, without using chemicals, in the same way that the use of rootstocks ended the phylloxera crisis.

An overview of lessons learnt in the application of *Trichoderma* products in grapevine nurseries. WYNAND VAN JAARVELD¹, ELODIE STEMPIEN¹, ROMAIN PIERRON¹, FRANCOIS HALLEEN^{1,2} and LIZEL MOSTERT¹. ¹Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa. ²Plant Protection Division, ARC Infruitec-

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Trichoderma spp. hold potential to be used for the management of black foot disease (BFD). Apart from hot water treatment of dormant nursery vines, no treatment is available to prevent nursery vines from becoming infected with BFD pathogens. In the past variable success of colonization of *Trichoderma* spp. were found when applied to nursery vines. Therefore, different methods of application of *Trichoderma atroviride* were tested on commercially planted nursery vines. One hundred graftlings were used per treatment, replicated five times and repeated over two seasons. To assess the efficacy of different *Trichoderma* products one hundred graftlings were used per treatment, replicated four times and repeated over two seasons. For both seasons the trials were uprooted after 7 months and isolations made from the base and roots of the vines. *Trichoderma* and BFD pathogens were recorded. For both trials the number of certifiable vines of the *Trichoderma* treatments were not significantly more than the untreated controls. *Trichoderma* treatments did not cause additional root growth, which might be due to the vigorous rootstock cultivar Ramsey that was used. When comparing tissue parts, the base of the vine and top part of roots had significantly higher *Trichoderma* colonization than the middle and bottom parts of the roots. Only in one season (2016/17) of the trial evaluating different products did all of the *Trichoderma* treatments significantly lower the black foot infections in the bases of the untreated controls from 6.5% to as low as 1.0%. The different application methods clearly showed that dipping of basal ends in the dry formulation consistently gave the highest colonization. The 1 hour soak of bases of vines was not effective. The field drenching was significantly less effective than the dry formulation application. These results provide valuable insights into the application of *Trichoderma* products in grapevine nurseries.

Evaluation of different strains of *Trichoderma* spp. against Grapevine Trunk Diseases *in vitro* and colonization of Arbuscular Mycorrhizal Fungi in roots of nursery grapevine plants. CATARINA LEAL^{1,2}, ROSA ROA-ROCO¹, PAULINA ARRAÑO-SALINAS¹, PABLO RODRIGUEZ-HERRERA¹, IVO AGNIC¹, DANIELA TORRES¹, CARLOS VALDIVIA¹, MAURICIO LOLAS³, ALVARO GONZALEZ¹ and FELIPE GAINZA-CORTÉS¹. ¹*Viña Concha y Toro S.A, Center for Research and Innovation, Fundo Pocoa s/n, Km10 Ruta K-650, Penco, Región del Maule, Chile.* ²*Universidade de Lisboa,*

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Grapevine Trunk Diseases (GTDs) impact economic sustainability of the wine industry affecting vineyards worldwide without efficient control methods. As consequence of this emerging problem, several studies aim to find eco-friendly solutions to control these diseases. *Trichoderma* spp. and Arbuscular Mycorrhizal Fungi (AMF) show high potential in decreasing the incidence of GTDs in vineyards as well as increasing the ability of vines to survive in stress conditions. During season 2016–2017, the Center for Research and Innovation of Viña Concha y Toro (VCT) have tested different *Trichoderma* strains (T1: based in *T. atroviride*; T2: *T. gamsii*; T3: *T. virens*; T4 and T5: *T. spp.*) against *Diplodia seriata*, *Neofusicoccum parvum*, *Phaeomoniella chlamydospora*, *Inocutis* spp. and *Eutypa lata* under *in vitro* assays. Moreover, grapevine nursery plants were inoculated in field nursery with commercial product based on AMF (M1: *Rhizophagus irregularis*, *Glomus. mosseae*; and M2: *G. brasilianum*, *G. clarum*, *G. deserticola*, *R. irregularis*, *G. mosseae*, *G. monosporum*, *Gigaspora margarita*) in order to evaluate the colonization capacity. By determining the occupied area over time for each strain of *Trichoderma* and its ability to inhibit the growth of each GTD, it was possible to determine that T1 was the most effective strain against all the GTDs tested, followed by T3 and T2 (respectively) that show some potential inhibition of the pathogens growth. T5 shows low capacity to cope with the GTDs evaluated. In the case of AMF evaluation, both M1 and M2 showed significant differences in frequency (F%), colonization intensity (M%) and arbuscles abundance (A%) compared with non-inoculated plants. M2 showed significant higher nitrogen and arginine contents in roots compared with M1-inoculated and control plants. Together, these results shows the potential use of this beneficial microorganisms in order to strengthen and improve the performance of VCT grapevines against GTDs.

Influence of different application times of *Trichoderma* in the nursery for the reduction of wood pathogens and quality improvement of grafted cuttings. GIUSEPPE CARELLA¹, ALESSANDRA BENIGNO¹, ELISA METRUCCIO², SAMUELE MORETTI¹, FABIO OSTI², ANDREA PACETTI¹, LAURA MUGNAI¹ and STEFANO DI MARCO². Istituto di Biometeorologia, CNR, Via Gobetti 101, 40129 Bologna, Italy. ²Dipar-

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Wood pathogens of grapevine are recognized to be already present in propagation material. These infections together with field infections through pruning wounds, which represent the main source of infection cause decline symptoms in the young vineyards. More recently, the availability of biological control products to be applied in the control of GTDs had a large increase. Application timing of a product based on *Trichoderma atroviride* SC1 (Vintec[®]) was tested for two years in a commercial nursery to establish the influence of application timing on the quality of the grafted vine and level of natural infections in the nursery. The formulation was applied at three stages: rehydration, callusing, basal callus formation followed by soil drenching. In addition, the effect of the combined applications was also tested. All treatments were compared with controls (another *Trichoderma*-based product, plants treated with synthetic fungicide and untreated plants). Surveys included assessment of plant quality and viability first, after callusing and later after a growth season in the nursery soil. At uprooting root development was analysed by video image analysis and quality assessed as percentage of certifiable plants. The final grafted vines, ready for sale, were used for isolating the mycoflora, thus analyzing the influence of application at different stages of the grafted cuttings production on the fungal mycoflora and in particular fungal colonization by wood pathogens. The rehydration stage showed to be the better performing application in reducing fungal infections, whereas the applications at the basal callus followed by soil drenching produced plants with a better root quality, and a different increase of the certifiable plants number depending on the rootstock tested. The use of biological control products requires a lot of care in detecting the most useful application timing, avoiding overlapping of needless treatments.

Effects of *Trichoderma asperellum* and *Trichoderma gamsii* and hot water treatment on *Phaeomoniella chlamydospora* in grapevine plant propagation material. STEFANIA POLLASTRO^{1,2}, DONATO GERIN¹, CRESCENZA DONGIOVANI³, GIULIO FUMAROLA³, MICHLE DICAROLO³, RITA MILIVIA DE MICCOLIS ANGELINI^{1,2}, CATERINA ROTOLO¹ and FRANCESCO FARETRA^{1,2}. ¹Department of Soil, Plant and Food Sciences and ²Selge Network, University of Bari Aldo

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Phaeomoniella chlamydospora, associated with Petri disease and Esca in young and mature plants, respectively affects plant propagation material frequently as latent infections. Curative methods are few and scarcely effective and hence proactive prevention measures are indispensable to limit the presence of the pathogen especially in young vineyards. In this study, the biofungicide Remedier™ (ISAGRO S.p.A, Milan, Italy) composed by *Trichoderma asperellum* icc012 and *T. gamsii* icc080 and hotwater treatment (HWT) were applied individually and in combination for controlling *P. chlamydospora* during the propagation process in nursery and at planting in pots. Cuttings of 1103 Paulsen (P) rootstock or grafted plants cv. Italia/1103P were used in experiments carried out in the nursery (trials 1 and 2), and grafted plants Italia/1103P in the pot experiment (trial 3). The biofungicide was applied by soaking cuttings during the hydration stage, at planting and 15, 30 and 60 days afterwards. Propagation material artificially inoculated with a conidial suspension (10^6 spores mL⁻¹) of *P. chlamydospora* strain CIA43.2, marked with benomyl resistance, was used after the first BCAs application. In trial 3, HWT plants were also compared. In all trials, the appropriate untreated and non-inoculated controls were also used. At uprooting, quality status of each plant was assessed at three different points and the presence of *P. chlamydospora* was evaluated by isolation on appropriate media. BCA-treated vines showed a significant ($P \leq 0.05$) increase in stems and roots development even as compared to untreated ones subjected to HWT. *Phaeomoniella chlamydospora* and Botryosphaeriaceae sp., the fungi isolated with prevalence higher than 10%, were significantly reduced (up to 100%) by HWT. BCAs reduced *P. chlamydospora* CIA43.2 up to 70%. These results contribute to an integrate management of esca and Petri disease in grapevine.

Evaluation of biocontrol agents against fungal pathogens associated with grapevine trunk diseases. ISIDORA SILVA-VALDERRAMA¹, DIANA TOAPANTA¹, GONZALO DIAZ², MAURICIO LOLAS² and ALVARO CASTRO¹. ¹UC Davis Chile Life Science Innovation Center. Avenida Andrés Bello 2299, piso 11, 7511303 Providencia, Chile. ²Laboratorio de Patología Frutal, Facultad de Ciencias Agrarias, Universidad de Talca. Avenida Lircay s/n, 3462227 Talca, Chile. E-mail: alvcastro@ucdavis.edu

Grapevine trunk diseases (GTDs) are a major problem for the wine industry worldwide, leading to reductions in vineyard productivity and longevity. In Chile, several fungal phytopathogens have been identified and isolated from internal GTDs symptomatic plant tissue, most of them from the Botryosphaeriaceae family. The aim of this study was to evaluate *in vitro* and grapevine canes potential biocontrol agents (BCAs) against *Diplodia seriata* and *Neofusicoccum parvum*. 387 endophyte and epiphyte fungal isolates were obtained from vines located in organic and integrated-pest management vineyards. Identification was performed by ITS sequencing and the isolates were classified into 56 different genera. A small group of fungi previously reported as BCAs and with antagonistic activity were selected. Isolates from *Trichoderma* sp., *Clonostachys* sp., *Chaetomium* sp., *Purpureocillium* sp., *Epicoccum* sp. and *Cladosporium* sp., in comparison with registered products Mamull and Tifi (*Trichoderma atroviridae* based), showed pathogen growth inhibition from 30% to 100% against *D. seriata* and *N. parvum* in dual culture agar plate evaluations. Strains of *Trichoderma* sp., *Clonostachys* sp. and *Epicoccum* sp. were evaluated in qualitative bioassays using grapevine canes. Four strains exhibited 100% growth inhibition against the two pathogens. Differences were found depending on the matrix used for the test, meaning PDA (potato dextrose agar), PA (plant agar) or grapevine pruning material. In addition, scale-up tests were made in order to allow field-level strain-efficacy evaluations. Therefore, the establishment of a novel pipe-line for biocontrol of trunk diseases, according to the internal localization of the pathogens in the plant, allowed the identification of promising endophytic fungal biocontrol agents isolated from grapevines. These experiments demonstrated potential for the development of a commercial biocontrol product which enables the establishment of prophylactic steps against GTDs fungi as an essential part of an integrated disease management program.

The use of three biocontrol agents alone or in combination to control *Neofusicoccum parvum*, a Grapevine Trunk Disease pathogenic fungus. AMIRA YACOU^{1,2}, RANA HAIDAR^{1,2}, JONATHAN GERBORE³, MARIE-CECILE DUFOUR^{1,2}, REMY GUYONEAUD⁴ and PATRICE REY^{2,1}. ¹INRA, UMR 1065 Santé et Agroécologie du Vignoble, ISVV, F-33140 Villenave d'Ornon, France. ²Université de Bordeaux, Bordeaux Sciences Agro, UMR 1065 Santé et Agroécologie du Vignoble, F-33175 Gradignan, France. ³Université de Bordeaux, ISVV, Unité de recherche Œnologie EA 4577, USC

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Neofusicoccum parvum is one of the most virulent fungal pathogens involved in Grapevine Trunk Diseases (GTDs). Using biocontrol agents (BCAs) may be a promising method to limit the extension of this pathogen, and one of the strategies for enhancing plant protection is to combine BCAs. Previous studies showed that some microorganisms such as the oomycete, *Pythium oligandrum*, and bacteria, *Pantoea agglomerans* and *Brevibacillus reuszeri*, applied individually on young vines, reduced necrosis caused by GTD pathogens. In the present experiment, *P. oligandrum*, *P. agglomerans* and *B. reuszeri* (inoculated at trunk or root levels) were applied individually or in combination to evaluate their effectiveness against *N. parvum* (inoculated at the trunk level). Results showed that 5 months after *N. parvum* inoculation, necrosis size was reduced by about 60% in most treatments. Efficacy was very similar when BCA were applied individually or in combination. However, for two modes of BCA combinations, the efficacy had decreased. These results suggest that there was no synergistic effect between these BCAs to control *N. parvum* attacks. In order to develop an adapted control strategy, the molecular events occurring during the tripartite interaction: grapevine/BCAs/*N. parvum* have been investigated. Wood samples have been collected 0 and 14 days post-inoculation and the expression of a set of 96 genes ("NeoViGen96" chip) implicated in *Vitis vinifera* defense mechanisms were analyzed. Gene expressions were quantified by real-time-PCR. Studied genes include 26 genes encoding PR proteins; 18 and 3 genes involved, respectively, in secondary metabolites and indole biosyntheses; 14 genes involved in wall thickness enhancement; 15 and 4 genes involved, respectively, in signaling and oxylipine pathways. Preliminary results will be presented.

Vertical vegetal endotherapy: a new mode of treatment to cure grapevine trunk diseases? ANDREA PACETTI², ROMAIN PIERRON¹, SIBYLLE FARINE¹, LAURA MUGNAI², CELINE TARNUS¹, CHRISTOPHE BERTSCH¹ and MELANIE GELLON¹. ¹Laboratoire Vigne Biotechnologies et Environnement UR-3991, Université de Haute Alsace, 33 rue de Herrlisheim, 68000 Colmar, FRANCE. ²Department of Agricultural, Food,

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Esca is usually described as one of the most devastating grapevine trunk diseases. This disease has been increasing in the last 30 years, and since sodium arsenite was banned in 2001, there is no solution available to effectively cure the disease. One potential technique could be plant endotherapy, which is used in fruit tree cultivation and in urban landscape trees. It has many advantages, particularly in terms of the environment as, compared to spraying, it allows a low input as for treatment doses and limits product drift in soil, air and water. This technique of vegetal endotherapy consists of drilling and injecting chemical compounds or beneficial microorganisms directly into the plant. It has many advantages, particularly for the environment. Indeed, compared to a conventional spray, this technique allows a reduction in treatment doses and limits product drift into the soil, air and water, thus reducing the impact on non-target organisms. Recently, it has been reported that the impacts of Esca could be minimized by mechanical curettage of the white rot. The recent study of the mechanisms of sodium arsenite showed that the molecule is concentrated in white rot after spray application to the wood. All of these results suggest that some fungal activity in the white rot may have a role in Esca symptoms, specifically leaf symptoms. We adapted plant endotherapy to grapevine by drilling a hole vertically in the trunk to reach and treat directly the white rot, with chemical compounds at different concentrations, and therefore the pathogens associated to it. We apply a "chemical curettage" to vines with leaf and wood symptoms of Esca. Experiments initiated in an Alsace vineyard in 2018, showed a fairly promising trend with a reduction in the expression of leaf symptoms by up to 50% in some varieties (*V. vinifera* cv Gewurztraminer and Riesling) treated by this method. The experiments need to be repeated, monitored and extended on other regions, over several years, to see if this trend is confirmed and if this method could be used to reduce the impact of Esca.

Pruning time can reduce grapevine trunk diseases infection under British Columbia environmental conditions. JOSE RAMON ÚRBEZ-TORRES, DANIEL T. O'GORMAN, JULIE BOULÉ, MELANIE WALKER and JINXZ POLLARD-FLAMAND. ¹Agriculture and Agri-Food Canada, Summerland Research and Development

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Grapevine trunk disease pathogens within the Botryosphaeriaceae and Diatrypaceae families infect grapevines primarily through pruning wounds. Airborne spores are discharged from fruiting bodies (pycnidia or perithecia) under optimum environmental conditions, land on susceptible pruning wounds and start infection after germinating. These infections develop into wood necrosis and perennial cankers, which lead to grapevine dieback and eventual death of the plant. Understanding the spore seasonal abundance of these fungi is critical to determine low risk infection periods in which pruning can be conducted. It has been shown in some grape-growing regions that either early or late pruning can reduce GTD infections in vineyards. Accordingly, the main objective of this study was to determine best pruning time to minimize GTD infection caused by the botryosphaeriaceous fungi *Diplodia seriata* and *Neofusicoccum parvum* under British Columbia environmental conditions. Chardonnay and Merlot grapevines (6 vines/treatment) were pruned the 15th of each month, including December, January, February, March, and April. Then, 50 uL of 10⁵ conidia/mL suspensions of *D. seriata* and *N. parvum* were used to artificially inoculate the pruned vines on March 16th, April 16th, May 15th, and June 15th, which correlates with the highest presence of Botryosphaeriaceae spores in the Okanagan Valley. The trial was conducted in two consecutive years (2016–2017 and 2017–2018). Results showed that pruning wounds made in mid-December and mid-January were significantly less susceptible to *D. seriata* and *N. parvum* infection compared to pruning wounds made in February, March and April. Pruning wounds made in mid-February were highly susceptible (>60% infection) to infections happening in March. However, pruning wound susceptibility decreased over time from 50% to 10% when infection happened in April, May and June. This study suggests that early pruning in winter can significantly minimize the infection cause by *D. seriata* and *N. parvum* in the Okanagan Valley.

POSTER PRESENTATIONS

Incidence and symptoms of grapevine trunk diseases on nursery finished plants in Uruguay. MARÍA JULIA CARBONE, MATÍAS GELABERT, PEDRO MONDINO and SANDRA ALANIZ. *Department of Plant Protection, Faculty of Agronomy, University of the Republic, Av. Garzón 780, CP 12900, Montevideo, Uruguay. E-mail: salaniz@fagro.edu.uy*

The occurrence of grapevine trunk diseases is one of the most important problems in young vineyards and nurseries worldwide. The large number of cuts and wounds made during the propagation process make the planting material vulnerable to infection by fungal trunk pathogens. The symptoms present and its incidence on nursery finished plants were investigated by first time in the main grapevine nursery of Uruguay. The plants evaluated were Tannat/RR101-14, Merlot/Fercal, Prosecco/SO4, Moscatel de Hamburgo/Gravesac, Tannat/3309C and Tannat/1103P. Twenty grafted plants of each combination scion/rootstock were carefully observed by cross-sectional and longitudinal cuts. Isolations from symptomatic samples were performed and identified. The incidence of symptoms was 100% in all nursery plants evaluated. The symptoms observed were black discoloration and necrosis of wood tissue developed from the base of the rootstock, necrotic streaks and internal necrotic wedge-shaped staining in the cross section. Species associated to Petri disease, Black foot, Phomopsis dieback and Botryosphaeria dieback diseases were isolated. This work confirms the presence of grapevine trunk diseases in plants produced in the main grapevine nursery in Uruguay. This emphasizes the importance of developing management strategies to minimize the incidence of grapevine trunk diseases in nurseries.

Effect of water stress and plant inoculation with wood-inhabiting bacteria on the symptoms caused by the grapevine trunk pathogen *Neofusicoccum parvum*. RANA HAIDAR^{1,2,3}, AMIRA YACOUB^{1,2}, ANTOINE PINARD^{1,2} and PATRICE REY^{1,2}. ¹INRA, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), F-33140 Villenave d'Ornon, France. ²Université de Bordeaux, ISVV, UMR1065 SAVE, Bordeaux Sciences Agro, F-33140 Villenave d'Ornon, France. ³Tichreen University, Faculty of Science, Biology Department, PO Box 2231, Latakia, Syrian Arab Republic. E-mail: amira.yacoub@inra.fr

Esca and Botryosphaeria dieback are considered to be major factors limiting grapevine productivity. In the context of these diseases, the influence of combined biotic and abiotic factors, however, is still not well understood. Thus, in the present study, the combined effect of a biotic (bacteria) and an abiotic (water stress) factors on the symptoms caused by the Botryosphaeria dieback pathogen *Neofusicoccum parvum* were evaluated. Cabernet Sauvignon cuttings were co-inoculated individually with *N. parvum* or in combination with two wood-inhabiting bacteria, *Bacillus pumilus* and *Xanthomonas* sp., in normal or reduced watering condi-

tions. Nine months after inoculation, the effect of water stress, as well as the effect of bacteria / *N. parvum* interaction on the external and internal lesions caused by *N. parvum*, were assessed. The presence of *N. parvum* in the various wood samples was confirmed by qPCR. Results demonstrated that the combination of the abiotic and biotic factors have greater influence on the cankers lesions than on the necrosis lengths. The detection and the quantification of *N. parvum* DNA by qPCR revealed that this pathogen was found in higher concentrations in water-stressed vines than in well-watered vines. Interestingly, *N. parvum* concentrations were higher in the plants co-inoculated with *B. pumilus*, compared to those inoculated only with *N. parvum*. The relationship between factor-inducing stress and growth of *N. parvum* in inoculated vines and the special interaction between *N. parvum* and *B. pumilus* will be discussed.

Botryosphaeriaceae species: the causal agents of grapevine dieback in Algeria. FAIZA AMMAD¹, MESSAOUD BENCHABANE¹, PASCAL LECOMTE² and AMEUR CHERIF³. ¹Département de Biotechnologie, Faculté SNV, Université de Blida 1, Blida. ²INRA, UMR 1065, Santé Végétale, ISVV Bordeaux-Aquitaine, France. ³Laboratoire microorganismes and biomolécules actives, Département de biologie, Faculté des sciences de Tunis, Campus Universitaire, 2092 Tunis, Tunisia. E-mail : sahraoui_a_f@yahoo.fr

Grapevine (*Vitis vinifera*) dieback is an increasing problem in Algeria. Field surveys conducted during spring season between 2006 and 2012 showed that the average incidence of diseased grapevines was from 1.43% to 25.11% and the severity index (to estimate the mean value of the dieback class in vine) was 0.65 to 2.25. Cross sections of symptomatic wood of Cabernet Sauvignon, Cardinal, Carignan, and Syrah were collected in four vineyards in Algeria showing different symptoms such as sectorial brown colored necrosis, central and sectorial gray necrosis, and central light-brown necrosis. Three Botryosphaeriaceae species, including *Botryosphaeria dothidea*, *Neofusicoccum parvum* and *Diplodia seriata* were isolated from the infected wood and identified based on morphological characters and analyses of the internal transcribed spacer region (ITS) and β -tubulin nucleotide sequences. DNA sequences revealed 99 % homology with 10 isolates of *B. dothidea*, 18 isolates of *Diplodia seriata* and 7 isolates of *Neofusicoccum parvum*. The field surveys revealed the presence of both anamorph and teleomorph stages just for *Botryosphaeria dothidea* and *Diplodia seriata* produced on dead and

infected wood. Inoculation of grapevine plantlets (Hmar Bouamr) with all three Botryosphaeriaceae species produced necrosis and vascular lesions in the wood after a 6-week incubation; *Neofusicoccum parvum* isolates were the most virulent, followed by *D. seriata* and *B. dothidea*. All species tested were re-isolated from lesions on infected plantlets. Our results represent the first report of a canker disease of grapevine associated with Botryosphaeriaceae species in Algeria.

Differences between small RNA profiles of *Vitis vinifera* cv. Chardonnay infected by *Diaporthe eres* and *Diaporthe bohemiae* as non-pathogenic on grapevines.

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Diaporthe spp. are important pathogens, also saprobes and endophytes on grapevines. Several species are known to cause cane bleaching, swelling arm and trunk cankers. In this study, we explore the differentially expressed small RNAs in response to *Diaporthe eres* (CPC 28220) as pathogenic and *Diaporthe bohemiae* (CPC 28223) as non-pathogenic in *Vitis vinifera* cv. Chardonnay cultivated *in vitro*. The group of small RNAs was investigated because of increasing evidence that a class of small non-coding endogenous RNAs, known as microRNAs (miRNAs), play an important role in post-transcriptional gene regulation during plant development and response to biotic and abiotic stresses. We cultivated cv. Chardonnay on DKW/Juglans medium, when the plants were in size of 5 cm, we inoculated them through the second upper leaf by a sterile needle inoculated with i) *Diaporthe eres*; ii) *Diaporthe bohemiae*; and iii) empty prick. Each treatment was sustained through five samples. After ten days post-inoculation, we harvested the plants and RNA was extracted with PureLink™ Plant RNA Reagent (Thermo Fisher Scientific). Small RNA libraries were constructed using TruSeq Small RNA Library Preparation Kit (Illumina). All the steps were carried out two times to evaluate two

variants important for statistics. Three treatments were sequenced twice using a MiniSeq (Illumina) and MiniSeq High Output Reagent Kit (75-cycles). Group of miRNAs appeared differentially within analyzed treatments. Subsequently, we analyzed the sequencing data to identify putative miRNA targets and explore their involvement in possible pathogen response pathways. Our results may contribute to the understanding of the role that miRNA pathways play during plant pathogenesis, and may be crucial in understanding disease symptom development in grapevines infected by pathogenic *Diaporthe eres* and non-pathogenic *Diaporthe bohemiae*.

A LysM domain-containing protein LtLYSM1 functions as a major virulence factor during *Lasiodiplodia theobromae* infection. JUNBO PENG, XINGHONG LI, WEI ZHANG, QIKAI XING, MEI LIU and JIYE YAN. *Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China. E-mail: pjb169961@163.com*

Lasiodiplodia theobromae is a plant pathogen, which has the ability to infect and cause serious damage to a diverse range of fruit crops including apple, peach, pear, blackberry, and grape in China. However, the pathogenic mechanisms of this fungus have not yet been completely explored. In this study, a LysM domain-containing protein LtLysM1 was identified in *L. theobromae*. Over-expression of *LtLYSM1* in *L. theobromae* resulted in increased pathogenicity on *Vitis vinifera*, indicating that LtLysM1 functions as an important virulence factor. Moreover, the expression profile of gene *LtLYSM1* during infectious stages was detected, and results indicated that the transcriptional level of the *LtLYSM1* gene was significantly increased at 48 hours post inoculation. Additionally, the signal peptide of LtLysM1 could bring about the secretion of yeast invertase. Furthermore, interacting proteins of LtLysM1 were obtained by yeast two hybridization library screening, and we are going to lighten the regulatory mechanisms among LtLysM1 with its interacting targets.

Ecophysiological shifts induced by Esca disease on *Vitis vinifera* L. LORIS OUADI¹, EMILIE BRUEZ³, CHLOÉ DELMAS¹, SYLVIE BASTIEN^{1,2}, PASCAL LECOMTE¹, CINDY COPPIN⁴, FLORENCE FONTAINE⁴, JEAN-CHRISTOPHE DOMEQ² and PATRICE REY^{1,2}. ¹INRA, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), F-33140, Villenave d'Ornon, France. ²Bordeaux Sciences Agro, INRA

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Esca is a widely reported Grapevine Trunk Diseases (GTDs), caused by a broad range of taxonomically unrelated fungal pathogens that colonize and damage the conductive vascular tissues, thus interfering with the vine physiology and potentially leading to plant death. However, the quantification of the effect of Esca on whole-plant water transport disruption remains unknown. Here a detailed analysis of xylem-related physiological parameters in grapevines that expressed Esca-foliar symptoms was carried out. The experiments were conducted in a vineyard from the Bordeaux region (France) on 16-year-old vines of the cultivar Cabernet-Sauvignon (*Vitis vinifera*), which have been monitored for Esca-foliar symptoms since 2005. For two consecutive years (2017, 2018), a set of healthy and Esca-infected grapevines has undergone detailed physiological measurements. Foliar transpiration, stomatal conductance, chlorophyll activity, and sap flow were regularly recorded to compare the physiological response of plant that remained healthy or developed Esca-foliar symptoms during the timespan of the experiment. To complete the two-year monitoring, healthy and Esca-symptomatic leaves were sampled throughout the season to measure the expression levels of genes involved in primary metabolic functions, using qPCR analysis. This study reveals that sap flow density and whole-plant transpiration of Esca-infected vines is significantly lower, several weeks before the first foliar symptoms appear.

Emerging patterns in the dispersal of *Botryosphaeria* and *Eutypa dieback* pathogen spores in New Zealand vineyards. ELINE VAN ZIJLL DE JONG¹, YVONNE WRUCK¹ and MARK R. SOSNOWSKI^{2,3}. ¹Linnaeus Limited, PO Box 1199, Gisborne 4040, New Zealand. ²South Australian Research and Development Institute, GPO BOX 397, Adelaide SA 5001, Australia. ³School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond SA 5064, Australia. E-mail: mark.sosnowski@sa.gov.au

Botryosphaeria dieback (BD) and *Eutypa dieback* (ED) are becoming an increasing problem in New Zealand. The incidence of dieback is rising rapidly according to

recent surveys, with *Eutypa lata* and at least six different Botryosphaeriaceae species present in New Zealand vineyards. Spores of these fungi are spread by water splash and wind and infect vines through pruning wounds leading to canker, dieback and eventual vine death. As part of a major research effort supported by the newly established Bragato Research Institute, to optimise pruning wound protection strategies, two Burkard spore traps have been placed in vineyards located in the major wine producing regions of Marlborough and Hawke's Bay to monitor the dispersal of BD and ED pathogen spores over different seasons and years. Spore tape samples collected daily from the two sites have been analysed by quantitative real-time PCR (qPCR) for the target spores, and compared against daily temperature and rainfall data. Spores of BD and ED pathogens were detected throughout the year in both regions and tended to be detected during and after rain events. Different patterns are emerging between the two regions in the frequency and quantity of spores detected for each of the pathogens during different seasons, which will be presented. Spore monitoring continues and will be expanded to other regions with differing climates, and the data will be used to inform disease management strategies for the New Zealand wine industry.

Characterization of *FmMCA* gene, a unique metacaspase from grapevine trunk disease phytopathogen *Fomitiporia mediterranea*. PEIJIE GONG¹, JULIE CHONG¹, SIBYLLE FARINE¹ and CHRISTOPHE BERTSCH¹. ¹Laboratoire Vigne Biotechnologies et Environnement, Université de Haute-Alsace, 33 rue de Herrlisheim, Colmar, 68000, France. E-mail: peijie.gong@uha.fr

In both plants and pathogens, programmed cell death (PCD) is an indispensable process that removes redundant cells during development, or genetically damaged cells during the development of multicellular organisms, or as ultimate mean to block the spread of pathogens in innate immunity and defense (so called hypersensitive response). Grapevine trunk disease (GTD) symptoms includes bud necrosis, shoot dieback, leaf spots, fruit rots. All these processes are relative with regulated cell death. However, the cause of these symptoms and their relationship with wood necrosis are far away from understood. Therefore, it is worth to consider how to conquer infection of GTD pathogens, for instance *Fomitiporia mediterranea* (*Fmed*), using the way of suppressing cell death or necrosis in grapes. In our study, a unique metacaspase (MCA) called *Fmca1*,

was identified in pathogen *Fmed*. As the unique MCA member in *Fmed*, we deduced *Fmca1* predominantly regulate the execution of cell death in most case of PCD. Multi-sequence Alignment analysis with *Fmca1* protein-sequence and other putative MCA genes (from fungi, bacteria, and plant) have been completed via MEGA software. The results showed that the fungi-associated MCA can be classified into same class. Furthermore, we revealed that *Fmca1* shares closest relationship with MCA *Eutypa lata* and *Neofusicoccum parvum*. Last but not least, we found putative 3D protein structure of *Fmca1* belongs to type I metacaspase, which contains a classical structure: beta strands in centre, surrounded by alpha helixes as outer ring.

Comparative genomics of *Dactylonectria torresensis* strains from grapevine, soil and weeds highlight potential mechanisms in pathogenicity and lifestyle.

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The soil-borne fungus *Dactylonectria torresensis* is the most common causal agent of black-foot disease in Europe. However, there is a lack of understanding on how this fungus induce symptoms on plants. In this study, we sequenced and analysed the genomes of three *D. torresensis* isolates collected from asymptomatic vine, weed, and soil. The main objectives of this study were to (i) identify the genomic characteristics of these fungi, (ii) understand the genetic variation among the sequenced species, (iii) identify genes potentially involved in niche specialization within species, (iv) identify fungal adaptations to the endophytic lifestyle, and (v) identify unique and shared genes and pathways related to fungal virulence. Genomes sequenced were further compared to those of 30 fungal species including root and aerial pathogens, white rot degraders, saprotrophs, dark septate endophytes or mycorrhiza. *Dactylo-*

nectria torresensis strains present genomes comprised between 64 and 65 Mbp with up to 18,548 predicted genes for each strain. Average nucleotide identity shows that strains are different according to genome contents. Clusters of orthologous groups were compared, and clusters of genes related to necrosis were detected in all strains (necrosis and ethylene inducing peptides, necrosis inducing proteins) as well as genes related to heavy metal resistance. Interestingly, an expanded high number of genes related to carbohydrate active enzymes were detected in each *Dactylonectria* strain, especially those related to glycoside hydrolases that could be involved in plant tissues penetration or pathogenicity. Genes of *D. torresensis* corresponding to secretome and small secreted proteins were further characterized. Antismash analysis demonstrated the presence of gene clusters such as fujikurin-like genes that have been linked to fungal pathogenicity. These results highlight several gene clusters present in *D. torresensis* strains that could be linked to pathogenicity, plant tissues maceration and degradation, adaptation to heavy metal contaminated soils, and endophytic lifestyle.

Increased levels of C18 fatty acids prior to visible leaf stripe disease symptoms in grapevine colonized by *Phaeoacremonium aleophilum* and *Phaeoconiella chlamydospora*. ISABEL CORTEZ and PIEBIEP GOUFO. ¹Centre for the Research and Technology of Agro-Environment and Biological Sciences, Departamento de Agronomia, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal. E-mail: pgoufo@utad.pt

Lipids play signaling roles in plant resistance against pathogens, modulating basal, effector-triggered, and systemic immunity. Two major mediators of defense signaling pathways are jasmonic and salicylic acids. Jasmonic acid is derived from C18:3n3, while increased levels of 18:1n9, C18:2n6, and 18:3n6 have been associated with enhanced synthesis of salicylic acid. In this study, fatty acids levels were determined in asymptomatic leaves of grapevine plants showing esca symptoms in the woods, leaves or berries, in order to characterize events preceding symptom appearance, and the duration of chemical signals with disease progression. As compared to healthy control leaves, fatty acids accumulated to varying levels in asymptomatic leaves in response to esca. The presence of brown wood-streaking in the trunk was associated with the accumulation of C18:0 and C18:3n6 in asymptomatic leaves, indicating an active communication of signals between woods and leaves. A sharp increase in

C17:1n7, C18:1n9t, C18:2n6c, C18:2n6t, C20:4n6, and C22:6n3 levels was observed in asymptomatic leaves of vines already exhibiting necrotic leaf stripes. Asymptomatic leaves of vines with black measles berries also showed esca-induced increases in C18:2n6c and C18:2n6t, which strongly support the involvement of C18 fatty acids in hormonal-mediated signaling in grapevine response to esca. Vines likely utilize alterations in fatty acids levels as a signal for inducing defense gene activation. However, the intensity of the signal is low when fungi are still in the trunk, involving only C18:0 and C18:3n6. With early appearance of foliar symptoms, the signal is amplified with more unsaturated fatty acids acting as transduction mediators. The additional knowledge on defense mechanisms in grapevine–esca interaction gained from this study could lead to ways to exploit host resistance responses to control the disease, in light of the finding that increasing C18 levels via genetic mutations upregulates the expression of resistance genes in several plants.

Population genetic analysis of *Dactylonectria torresensis* from grapevine, soil and weeds in northern Spain.

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Dactylonectria torresensis is the most common fungal species associated with black-foot disease of grapevine in Europe. The aim of this study was to analyze the genetic diversity among *D. torresensis* isolates obtained from asymptomatic grapevines, soil and weeds in two field nurseries located in Navarra (Spain) and in a young vineyard located in La Rioja (Spain). A total of 213 isolates collected from grapevine (n=199), weeds (n=4) and soil (n=10) were genotyped for single nucleotide polymorphisms (SNPs) detected by sequencing a partial region of the histone H3 (*his3*) gene. Based on the phylogenetic analysis, 12 haplotypes were found in the *D. torresensis* population. The most abundant haplotype included 141 isolates. The phylogenetic tree formed two clades, one of them containing 8 out of the 12 haplotypes. All isolates obtained from plants, soil and weeds were closely related. In addition, there were no significant differences in the haplotype distribution of

plots within Navarra region. Movement of haplotypes between both regions may have occurred repeatedly, given the low level of geographic genetic structure and the frequent retrieval of the same haplotype in distant locations. Sexual reproduction events and recombination should not be excluded due to the high genotypic diversity found within *D. torresensis* population. Our results also confirm the hypothesis that *D. torresensis* has an endophytic lifestyle on grapevine and on alternative hosts such as weeds.

The holobiont, a biological lever to manage some declines of grapevine. SOPHIE TROUVELOT¹, FLORANCE FONTAINE², HELOISE MAHE³, PAULINE BETTENFELD^{1,2}, EVERT VAN SCHAİK¹, MARIELLE ADRIAN¹, FRANCOIS DAL⁴, OLIVIER FERNANDEZ², CLAIRE GROSJEAN⁵, PHILIPPE LARIGNON⁶, GREGORY MOUILLE⁷, FABRICE MARTIN-LAURENT¹, OLIVIER ZEKRI⁸, SAMUEL MONDY¹, DANIEL WIPF¹ and PIERRE-EMMANUEL COURTY¹. ¹*Agroécologie, AgroSup Dijon, CNRS, Univ. Bourgogne, INRA, Univ. Bourgogne Franche-Comté, Dijon, France.* ²*SFR Condorcet CNRS 3417, Université de Reims Champagne-Ardenne, Unité Résistance Induite et Bioprotection des Plantes, Reims, France.* ³*CNIV, 12 rue Saint-Anne, Paris, France.* ⁴*SICAVAC Laboratoire œnologique, Sancerre, France.* ⁵*Chambre Régionale d'Agriculture de Bourgogne Franche-Comté, Bretenière, France.* ⁶*Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, Rodilhan, France.* ⁷*Institut Jean-Pierre Bourgin, INRA, AgroParis-Tech, Centre National de la Recherche Scientifique, Université Paris-Saclay, Versailles, France.* ⁸*Pépinières Mercier-Novatech, Le Gué De Velluire, France.* E-mail: sophie.trouvelot@u-bourgogne.fr, Pierre-Emmanuel.Courty@inra.fr

The concept of a multicellular organism to describe complex organisms composed of groups of analogous cells called tissues and organs with specific functions, shows currently some limitations. An organism such as a plant does not live alone, but closely associated with different microbial communities making up its microbiomes. The microbiomes, specific to a tissue, interact with the plant and modulate some of its functions, including physiology and immunity. The host and the associated microbiomes define a holobiont, whose function is influenced by the spatio-temporal dynamics of their interactions. Thus, a dysfunction of the grapevine holobiont (linked for example to inappropriate technical itineraries or to climate change) could lead to the development of diseases or physiological disorders, similarly

to the alteration of gut microbiota observed in numerous human diseases as type 2 diabetes, obesity and irritable bowel syndrome. The analysis of microbial communities associated with a plant such as grapevine has until recently been limited by the fact that most microorganisms cannot be cultured *in vitro*. New methods (named -omics) based on the analysis of genome, metabolome and proteome allow to define the taxonomic and functional characteristics of the different microbiomes associated with a plant, in particular organisms (*i.e.* bacteria, fungi) that are not cultivable. In the Holoviti project, we aim to characterize the role of microbiomes in grapevine homeostasis by describing and comparing the holobiont of i) healthy grapevines and ii) grapevines affected by three declines (Esca / BDA, fanleaf and linked to 161-49C rootstock). One of our objectives is to identify taxonomic and functional bio-indicators (linked to either microbes or the plant) of the host sanitary status. One prospect could be handle the microbial component of the holobiont to improve some ecosystemic services, such as the control of pathogens or the fight against abiotic stresses.

Characterization of species of *Lasiodiplodia* associated with grapevines in Mexico. EDELWEISS AIRAM RANGEL-MONTOYA¹, MARCOS PAOLINELLI^{2,3}, CESAR VALENZUELA-SOLANO⁴ and RUFINA HERNANDEZ-MARTINEZ¹. ¹*Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE), Carretera Tijuana-Ensenada 3918, Zona Playitas, 22860, Ensenada, B.C. Mexico.* ²*Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria de Mendoza, San Martín 3853 (5507), Luján de Cuyo, Mendoza, Argentina.* ³*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290 (C1425FQB) CABA, Buenos Aires, Argentina.* ⁴*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Costa de Ensenada, Av. del puerto 375-23 fraccionamiento playa ensenada, Ensenada B. C. Mexico.* E-mail: erangel@cicese.edu.mx

Botryosphaeria dieback is one of the most aggressive diseases of grapevine caused by fungi in the Botryosphaeriaceae family. The fungi invade the vascular tissue mainly through pruning wounds, which can lead to perennial cankers, necrotic lesions, decline and eventually death of the vine. Among these fungi, members of the genus *Lasiodiplodia* have been reported as the most virulent. Recent studies have revised the taxonomy and phylogeny of *Lasiodiplodia* and as a result, eight-

een new species have been described worldwide. This study aimed to identify the *Lasiodiplodia* species associated with grapevine in Mexico. Canker samples were obtained from ten vineyards established on the states of Sonora and Baja California and isolations were made on Potato Dextrose Agar (PDA). From colonies showing smoke-gray abundant aerial mycelia, twenty-two isolates with hyaline and pigmented longitudinally striate, dark brown conidia were selected. The phylogenetic tree generated using *EF1- α* and ITS sequences revealed the presence of six different *Lasiodiplodia* species in these vineyards. Eight isolates were identified as *L. exigua*, eight *L. theobromae*; two *L. gilanensis*, two *L. crassispora*, and one isolate for each species of *L. parva* and *L. brasiliensis*. Pathogenicity studies showed *L. parva* VSM16a and *L. gilanensis* MXCS01 isolates to be the most virulent and caused lesions of up to 7 cm in length. *Lasiodiplodia crassispora* Vid5 and VSM1b isolates as well as *L. theobromae* VSM6a isolate caused lesion below 3 cm. Except for *L. theobromae*, all the identified species are new reports for Mexico.

Grapevine cultivar variability in colonization by *Phaeoconiella chlamyospora* and *Phaeoacremonium minimum*. MARÍA DEL PILAR MARTÍNEZ-DIZ^{1,2}, MATTHEW AYRES³ and MARK R. SOSNOWSKI³. ¹Estación de Viticultura e Enología de Galicia (AGACAL-EVEGA), Ponte San Clodio s/n 32428-Leiro-Ourense, Spain. ²Universidade da Coruña, Facultade de Ciencias, Zapateira, 15071 A Coruña, Spain. ³South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001. E-mail: pilar.martinez.diz@xunta.gal

The use of tolerant cultivars is a key element in an Integrated Pest Management program and would be one of the most effective means of controlling grapevine trunk diseases, not only reducing disease losses, but also decreasing the need for chemical control strategies. The aim of this study was to evaluate the extent of colonization by fungi associated with esca disease in a detached cane assay with twenty grapevine cultivars from the South Australian Research and Development Centre germplasm collection. In June 2018, one-year-old canes were harvested and stored at 4°C. Two weeks later, dormant grapevine canes were cut into 10 cm single-node sections and placed into holes in polystyrene boards which were floated on water in plastic tubs in a greenhouse. Wounds on top of twelve canes per cultivar were inoculated with spore solutions (800 spores per wound) of either *Phaeoconiella chlamyospora* or *Phaeoacremonium minimum*. Non-inoculated controls

were also included. Canes were harvested seven weeks after establishment and the extent of colonization by the fungi was assessed by isolation onto potato dextrose agar. Both pathogens colonized the xylem vessels of all cultivars but the extent of fungal growth within woody tissue varied significantly. Canes of all cultivars were colonized between 3 and 34 mm (*P. chlamyospora*) and between 9 and 48 mm (*P. minimum*) from the wound site. This variation could suggest different disease progression depending on the cultivar and pathogen tested, indicating possible disease tolerance in some cultivars. Further investigation is required to confirm the results and better understand the way that fungi colonize the tissues and how some cultivars are able to restrict this colonization.

The most informative loci for identifying grapevine trunk disease pathogens. DAVID GRAMAJE¹, ANA CABRAL², LIZEL MOSTERT³, DANIEL P. LAWRENCE⁴, FLORENT P. TROUILLAS⁴, JOSÉ RAMÓN ÚRBEZ-TORRES⁵ and ARTUR ALVES⁶. ¹Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain. ²LEAF, Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. ³Department of Plant Pathology, Stellenbosch University, Private Bag X1, Matieland, 7602. ⁴Department of Plant Pathology, University of California, Kearney Agricultural Research and Extension Center, Parlier, CA, 93648, USA. ⁵Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Box 5000. Summerland, BC V0H 1Z0, Canada. ⁶Departamento de Biologia, CES-AM, Universidade de Aveiro, 3810-193 Aveiro, Portugal. E-mail: david.gramaje@icvv.es

Grapevine trunk disease (GTD) fungi are highly diverse and species recognition is largely dependent on multi-locus DNA phylogenetic analyses. The DNA sequences frequently used for this multi-locus approach, include the internal transcribed spacer region (ITS1-5.8S-ITS2) of the ribosomal DNA and fragments of the translation elongation factor 1- α (*TEF1*), β -tubulin (*TUB2*), histone (*HIS3*), calmodulin (*CAL*), and actin (*ACT1*) genes, depending on the taxonomic group under consideration. In this study, we provide information concerning the best loci combinations to better estimate species boundaries in GTD fungi in the families Botryosphaeriaceae, Diaporthaceae

ae, Diatrypaceae, Phaeomoniellaceae, Togniniaceae, and Nectriaceae belonging to Ascomycota, and the Hymenochaetales in the Basidiomycota. Accurate identification of these pathogens is an essential first step towards meaningful studies on epidemiology and disease management of GTDs.

Effect of pruning wounds on cones of desiccation on Ugni blanc grapevines in Charentes, France. EMILIE BRUEZ¹, CELINE CHOLET¹, TOMMASO MARTIGNON³, MASSIMO GIUDICI³, FLORIAN PRULEAU⁴, MATHILDE BOISSEAU⁴, PASCAL LECOMTE², PATRICE REY² and LAURENCE GENY¹. ¹EA 4577, Unité de recherche œnologie, INRA, USC 1366 Œnologie, Université de Bordeaux / Institut des Sciences de la Vigne et du Vin, 210 Chemin de Leysotte - CS 50008, F-33882 Villenave d'Ornon, France. ²UMR 1065 Santé et Agroécologie du Vignoble, Institut National de Recherche Agronomique/ Bordeaux Sciences Agro / Institut des Sciences de la Vigne et du Vin, 71, Avenue Edouard Bourleaux, INRA Domaine de la Grande Ferrade - BP81, 33883 Villenave d'Ornon Cedex, France. ³Simonit&Sirch, maitres tailleurs de vigne, 1 Rue Porte des Benauges, 33410 Cadillac - Bordeaux, France. ⁴Hennessy Jas et Cie, 1 rue Richonne 16100 Cognac, France. E-mail: emilie.bruez@u-bordeaux.fr

Practical methods such as pruning are used in vineyards to sustain grapevines. The aim of this study was to analyze the effect of different sized pruning wounds to limit the percentage of Esca-diseased vines in Charentes (France) that was 12.7% in 2017. The experiment focused on the closing over of the pruning wound and the appearance of the cone of desiccation in the wood. Two Ugni blanc vineyards of same age showing a low vigor and a high vigor were studied. Two different pruning wounds were made, that were respectful or not of sap flow and leave or not a knot. Four (spring) and 8 (autumn) months after pruning, the form, length and size of the cones of desiccation of five plants were studied. The cones were cut longitudinally and photographed. The images were analyzed using ImageJ software. The results showed different forms and sizes of necrosis in the shoot pruning area, depending on the type of pruning but also depending on the plant vigor. The environmental conditions, such as wind direction, landscape slope, between both vineyards could explain the differences. In conclusion, the type of pruning wound has an effect on the creation of the cone of desiccation and may have an influence the pathogen entry and particularly for Esca pathogens.

Economic impact of grapevine trunk diseases in a mature vineyard of the Protected Designation of Origin “Manchuela”, Spain. RAMONA MARÍA MUÑOZ¹, ANDREA CAMPOS¹, MARÍA LUISA LERMA¹, PURIFICACIÓN CASTILLO¹, JOSÉ ANTONIO MONREAL² and DAVID GRAMAJE³. ¹Servicio de Diagnóstico y Asistencia Fitosanitaria (SEDAF), Instituto Técnico Agronómico Provincial de Albacete (ITAP), Parque Empresarial Campollano, 2ª Avenida, 61, 02007 Albacete, Spain. ²Departamento de Producción Vegetal y Tecnología Agraria, Universidad de Castilla La Mancha, Campus Universitario s/n, 02071 Albacete, Spain. ³Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas, Universidad de La Rioja, Gobierno de La Rioja, Ctra. LO-20 Salida 13, 26071 Logroño, Spain. E-mail: david.gramaje@icvv.es

Grapevine trunk diseases (GTDs) impact grapevine health in all major grape-growing regions of the world, limiting vineyard productivity and longevity. These diseases represent one of the major threats to future economic sustainability of viticulture. In order to evaluate the impact of GTDs, approximately 1,000 vines in a mature vineyard (33 years old) of cv. Bobal, located in the Manchuela wine region, were monitored for external symptom expression including foliar discoloration and dead arms and spurs, at least once a year from 2003 to 2018. Yield and crop losses were estimated according to the number of dead vines in 2017 and 2018. Annual vine mortality rate ranged between 0 (2004) and 0.73% (2010), and the cumulative mortality at the end of the study (2018) reached 5.13%. A slight increase in dead plants was recorded in 2010, coinciding with the conversion of the vineyard from gobelet to bilateral cordons. In 2017 and 2018, the disease was estimated to cause yield losses of at least 470 and 675 kg/ha, respectively. The direct crop loss to grape grower was estimated at 429 and 266 euros/ha for the 2017 and 2018 crop year, respectively. Estimations of replacement cost of dead vines accounted for more than 630 euros/ha. Investigation on the fungal communities inhabiting the necrotic wood tissues led to the identification of *Diplodia seriata* as the most frequent pathogen associated with internal wood symptoms, followed by *Phaeomoniella chlamydo-spora*. Considering the large investments in vineyard establishment and maintenance, a long lifespan of vines is essential for economic success in vineyards.

In vitro screening of *Trichoderma* species isolates for potential bio-control of black foot disease causing pathogens in grapevine nurseries. WYNAND VAN

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Black foot disease (BFD) of grapevines is a decline and dieback disease caused by a soil-borne pathogen complex including *Ilyonectria*, *Dactylonectria* and *Campylocarpon* species. Apart from hot water treatment of dormant nursery plants, no treatment is available to prevent nursery plants from becoming infected with BFD pathogens after planting. *Trichoderma* species are well known biocontrol agents and have shown good efficacy to prevent grapevine trunk disease infections of pruning wounds. However, the efficacy of *Trichoderma* species against BFD pathogens have not been determined *in vitro*. The following *Trichoderma* species *Trichoderma atroviride*, *T. fertile*, *T. harzianum* and *T. virens* were evaluated *in vitro* against four of the most commonly occurring BFD pathogens, *Ilyonectria liriiodendri*, *Dactylonectria macrodidyma*, *Campylocarpon fasciculare* and *Campylocarpon pseudofasciculare*. The effects of volatile organic compounds (VOCs) and diffusible antibiotic compounds (DACs) produced as well as the direct antagonistic effects were determined in Petri dish assays. The percentage growth inhibition was determined for both the volatile and diffusible antibiotic assays. Microscopic observations were made of the interaction zone for the dual plate assay. Higher growth inhibition of the black foot pathogens were obtained with the DACs than the VOCs. For both classes of compounds *D. macrodidyma* were found to be more sensitive (maximum of 100% inhibition by DACs and 66.94% by VOCs). In some cases growth stimulation was observed for isolates of *I. liriiodendri*, *C. fasciculare* and *C. pseudofasciculare*, depending on the *Trichoderma* spp. isolate. Several interactions were macroscopically observed between *Trichoderma* spp. and the pathogen isolates, which included overgrowth or partial overgrowth, often associated with sporulation of the *Trichoderma* spp., and to a lesser extent arrested growth or the formation of inhibition zones between *Trichoderma* and the pathogen isolates. In general, *in vitro* efficacy was dependant on *Trichoderma* spp. isolates as well as the black foot species isolates.

Outbreak of young grapevine diseases in new table grape varieties in Chile. JAIME AUGER¹, CLAUDIO OSORIO-NAVARRO¹, LAURA POZO¹ and MARCELA ESTERIO¹. ¹Depto. de Sanidad vegetal, Fac. de

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Chile is the world's largest table grape exporter, with North America accounting for 47% of total exports in 2016–2017. Peruvian table grape expansion, which has some strategic advantages to the early growing Chilean regions, and development of late harvested varieties in California, have impacted the table grape Chilean market, causing a decline in demand. Growers are now renewing their vineyards with new varieties which hold good shipping conditions plus better size, color and flavor profiles. New vineyards were established with effective and good farming practices that avoid biological and mechanical stress. However, in the last two growing seasons grapevine decline has caused serious economic losses to the renewed vineyards with novel varieties. The symptoms include reduced vigor, xylem vessels plugging, significant delay in bud break and trunk dieback. Decline symptoms were particularly significant in some varieties with almost 30% of plants lost. In symptomatic plants *Phaeoacremonium* sp. was detected using specific primers Pm1 and Pm2. In order to ensure detection, a restriction-based strategy was developed using EcoRI enzyme, which present a conserved and unique restriction site in the DNA fragment Pm1/Pm2 in all pathogenic *Phaeoacremonium* strains reported to date. *Phaeoacremonium minimum*, *Phaeoacremonium mortoniae* and *Phaeoacremonium parasiticum* were identified. Contrasting symptoms severity suggest different defense responses, therefore we focus in studying expression of central genes involved in pathogen response (*PR* genes, flavonoid biosynthesis and hormone response). Understanding the etiology and epidemiology of the grapevines trunk diseases is a big concern for growers because of the high susceptibility of the currently planted new table grapes varieties. This problem became worse considering the absence of adequate sanitary tests, management of the propagation plant material in the Chilean nursery and the inefficiency of the vineyard control treatments.

Root colonization and host defence activation of grapevine rootstock by the biological control agent *Trichoderma atroviride*. ELODIE STEMPIEN¹, ROMAIN PIERRON¹, ILKA ADDENDORF¹, FRANCOIS HALLEEN^{1,2} and LIZEL MOSTERT¹. ¹Department of Plant Pathology, University of Stellenbosch, Private Bag XI, Matieland, 7602, South Africa; ²Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa. E-mail: lmost@sun.ac.za

Biocontrol agents (BCA) such as *Trichoderma atroviride* hold the potential to control soil borne diseases and promote plant growth. Very little is known about the colonization pattern of *T. atroviride* of grapevine roots and the activation of Induced Systemic Resistance *in planta*. Therefore, a laboratory model was developed to measure the colonization of grapevine roots and its impact on host resistance activation. Cuttings of the rootstock Richter 110 (*Vitis berlandieri* x *Vitis rupestris*), US 8-7 [Jacquez (*V. aestivalis* x *V. cinerea* x *V. vinifera*) x Richter 99] Ramsey (*V. champinii*), and Paulsen 1103 (*V. berlandieri* x *V. rupestris*) were made from one-year-old dormant canes and rooted in a mist bed. When sufficient roots and shoots developed, the plants were potted in perlite. One week after potting, plants with six fully developed leaves were drenched with a spore suspension (10^8 spores per plant) of *T. atroviride* strain T-77. Three weeks post inoculation, *T. atroviride* were re-isolated from root and crown sections. *Trichoderma atroviride* was successfully re-isolated from all the treated rootstock plants. The colonization is significantly higher in the roots (60 to 80%) than in the bases (15 to 40%), however, not significantly different between the four cultivars. The induction of defence genes were assessed with RT-qPCR in Richter 110 and US 8-7 plants three weeks post *T. atroviride* strain T-77 inoculation. Defence gene induction were normalized to the expression of two reference genes, *VvEF1y* and *VvActin*. *VvSTS* and *VvCHIT4C* genes were significantly induced by *T. atroviride* in US 8-7, but not in Richter 110. Furthermore, *T. atroviride* T-77 was transformed with a fluorescent *tdTomato* gene to allow visualization of infection structures on roots by confocal laser scanning microscopy using the same model. These results give new insights in the mechanisms of grapevine-*Trichoderma* interactions to ensure that the potential BCA is well established inside its host plant and that it can potentially be used to prevent pathogen establishment.

Metatranscriptomic approach to characterize the microbiome and identify molecular markers for early detection of “Hoja de malvón” disease in *Vitis vinifera* cv. Malbec. MARCOS PAOLINELLI^{1,2}, GEORGINA ESCORIAZA¹, CECILIA ALICIA CESARI¹, RUFINA HERNÁNDEZ-MARTÍNEZ³, SEBASTIÁN GÓMEZ-TALQUENCA¹ and SANDRA GARCÍA-LAMPASONA¹.
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Argentinian viticulture is markedly affected by the grapevine trunk disease “Hoja de malvón.” Similar to Esca disease, *Phaeoacremonium* spp., *Phaeoconiella chlamydospora* and Botryosphaeriaceae spp. are the pioneer fungi, but in a secondary stage of the disease, the Basidiomycota *Arambarria* sp. (formerly, *Inocutis jamaicensis*) was included. Currently, detection of the pathogen is done through culture-dependent techniques since molecular culture-independent methods are poorly developed. The objective of this work was to design a metatranscriptomic approach for the simultaneous exploration of the microbiome and the host gene expression, to identify molecular markers that help with the early detection and the monitoring of the disease progression in vineyards. Twenty-three year-old own-rooted *Vitis vinifera* cv. Malbec plants were selected to obtain a pool of wood chips from the trunk and arms from either symptomatic or asymptomatic plants. Total RNA was extracted and processed for RNAseq using Illumina HiSeq4000. The metatranscriptome renders an average of 37 million 100 bp PE reads per sample. Around 81% of reads were mapped on the genome of *V. vinifera* cv. Pinot Noir and used for gene expression analysis. The remaining unmapped reads were used for microbiome characterization through two bioinformatics strategies: 1) rRNA reads assembly and taxonomic assignment using SILVASSU and RDP_ITS database 2) Taxonomic assignment and relative quantification based on uniquely kmer mapping of reads on microbial genome database. Culture-based and culture-independent methods coincide in the detection of the most abundant species (i.e., *Alternaria* sp.), but culture-independent and kmer-based technique detect a vast diversity of microorganisms never identified before using culture-based strategies. Based on the differential abundance of disease-associated pathogens, host functional enrichment on differential expressed genes was evaluated. Results indicate that polyamines genes are candidates for molecular markers of the disease.

Effect of a formulation containing biocontrol agents against *Diplodia seriata*, on the development of Botryosphaeria dieback in vine canes. JAVIERA MOLINA¹, TAMARA NÚÑEZ¹, KARINA VALDÉS², KATHERINA AGUIRRE¹, LUZ MARÍA PÉREZ¹ and JAIME R. MON-

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Diplodia seriata is one of the fungal pathogens associated with Botryosphaeria dieback in *Vitis vinifera*. Chemical fungicides have been used for its control, which can impact the environment along with human and animal health. Thus, it is important to find alternatives to these chemicals such as the use of biocontrol agents. The mixture of two Ascomycota fungi named Trizian1 and Clo-seal (MBTC[®]), has proved its ability to control *D. seriata* under laboratory, greenhouse and field conditions. MBTC is the active ingredient of a new bioformulation (Formulation-MBTC), which must be tested for the biocontrol of *D. seriata*, both *in vitro* and *in vivo*, and compared to the biocontrol effect of MBTC. *In vitro* assays used dual cultures experiments, while *in vivo* assays used wounded vine canes (simulating pruning wounds) inoculated with *D. seriata*, in the presence and absence of Formulation-MBTC. The biocontrol effect of Formulation-MBTC was compared with that of MBTC. Controls were set up using the excipients of the formulation, methylthiofanate (MTF) and a commercial biofungicide containing *Trichoderma* sp. Results showed there were no differences between Formulation-MBTC and MBTC on the antagonism against *D. seriata* in *in vitro* assays, and that Formulation-MBTC maintained the biocontrol activity of MBTC on *D. seriata*, in *in vivo* assays, being identical to that of MTF. It may be concluded that Formulation-MBTC may be used for the control of *D. seriata*.

Shifts in fungal community composition during the propagation process in grapevine nurseries inferred by high-throughput amplicon sequencing. DAVID GRAMAJE¹, REBECA BUJANDA¹, ALES EICHMEIER², MILAN SPETIK², JESSICA VALLANCE³ and PATRICE REY³. ¹Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas, Universidad de La Rioja, Gobierno de La Rioja, Ctra. LO-20 Salda 13, 26071 Logroño, Spain. ²Mendel University in Brno, Faculty of Horticulture, Mendeleum - Institute of Genetics, Valticka 334, 69144, Lednice, Czech Republic. ³INRA, ISVV, UMR1065 SAVE, F-33140, Villenave d'Ornon, France INRA, ISVV, UMR1065 SAVE, F-33140, Villenave d'Ornon, France. E-mail: david.gramaje@icvv.es

The objective of this study was to investigate the changes in the composition of fungal communities at different stages of the grapevine propagation process by high-throughput amplicon sequencing (HTAS). A non-destructive method was used to collect grapevine wood from the xylem vessels of rootstocks 110 Richter and 41 Berlandieri at four stages of the propagation process: cuttings before cold storage (1), after hydration (2), grafted plants after callusing (3) and prior to dispatch (4). Grapevine wood was collected from the base, middle and apical sections of the rootstocks. DNA from the three plant parts was pooled and fungal communities from these samples were analysed by HTAS of the DNA-ITS2 region. Alpha diversity of fungal communities in the xylem vessels did not differ significantly between genotypes. Temporal shifts in fungal microbiome composition were observed when studying the genotypes separately, with fungal communities identified after hydration more diverse than in the other nursery stages. Several fungal genera associated with grapevine trunk diseases (GTDs) were identified (i.e. *Cadophora*, *Phaeoacremonium*, *Phaeoconiella*), with their populations generally increasing towards the last stages of the propagation process. This study provides new insights into the ecology of fungal communities in grapevine nurseries, and opens up new perspectives in the study of grapevine-GTDs interactions.

Pruning wound colonization of *Trichoderma* native strains from Castilla y León (Spain) in semi-field conditions in winter. GUZMÁN CARRO-HUERGA¹, SARA MAYO-PRIETO¹, ÁLVARO RODRÍGUEZ-GONZÁLEZ¹, ÓSCAR GONZÁLEZ LÓPEZ¹, JAVIER SAIZ-GADEA¹, SAMUEL ÁLVAREZ-GARCÍA¹, SANTIAGO GUTIÉRREZ² and PEDRO ANTONIO CASQUERO¹. ¹Grupo Universitario de Investigación en Ingeniería y Agricultura Sostenible (GUIIAS), Instituto de Medio Ambiente, Recursos Naturales y Biodiversidad, Universidad de León, Avenida, Portugal 41, 24071 León, España. ²Grupo Universitario de Investigación en Ingeniería y Agricultura Sostenible (GUIIAS), Área de Microbiología, Escuela de Ingeniería Agraria y Forestal, Universidad de León, Campus de Ponferrada, Av. Astorga s/n, 24401 Ponferrada, España. E-mail: gcarh@unileon.es

Trichoderma spp. are a well-known Biological Control Agents (BCA) against Grapevine Trunk Diseases (GTDs). However, nowadays we are far from controlling efficiently these pathogens, so it is necessary to evaluate the influence of weather conditions on *Trichoderma* colonization. The aim of this experiment was to assess if *Trichoderma*

can persist during winter in order to protect the plants in the most critical period of infection, twelve weeks after pruning. So that, we want to avoid the primary point of entry, annual pruning wounds, after having sprayed vines with *Trichoderma* native strains. Two isolates from vineyards of cv. Tempranillo in Castilla y León were tested; T071 isolated from soil and T154 isolated from plant. One-year-old dormant grapevine grafted plants of Tempranillo/110 Richter combination were used. A solution of 2×10^6 *Trichoderma* spores of T071 and T154 were applied within 24 hours after pruning in December. Vine plants were harvested (eight replicates per treatment) twelve weeks after *Trichoderma* native strains application and cut into 3-cm-long pieces up to 15 cm. Bark was removed and different segments were surface sterilized to isolate *Trichoderma* isolates, 7 chips per plate and three plates per each segment were cultured on Rose Bengal Chloramphenicol Agar (RBCA) and incubated during 7 days at 23°C. After twelve weeks T071 showed 70.31% and T154 showed 89.58% of recovery at 0 cm (surface). Significant differences were shown between treatments ($P=0.01$). A low percentage of recovery was obtained at 3 cm and none at 6 cm, 9 cm, 12 cm and 15 cm. In control treatments no *Trichoderma* strains were re-isolated. This study shows that good results can be obtained using native *Trichoderma* strains isolated from the same location and type of tissue in order to improve colonization and demonstrates that both strains has colonized pruning wounds.

Development of rapid molecular assays for the detection of grapevine canker pathogens from woody plant tissue. XUEWEN FENG, SVETLANA DUBROVSKY and ANNA LIISA FABRITIUS. *AL&L Crop Solutions Inc., Vacaville, CA, 95688, United States. E-mail: info@allcropsolutions.com*

Grapevine trunk diseases caused by *Botryosphaeria* spp., *Eutypa* and other closely related species, Petri disease (young vine decline or young esca) caused by *Phaeoconiella chlamydospora* and *Phaeoacremonium minimum*, and black foot disease caused by *Cylindrocarpon* spp. are the four leading disease problems in grape production regions worldwide. Symptoms include cankers, dieback, slow decline, and vine death, all of which increase production costs and yield losses. While several groups of fungi cause trunk diseases in older vineyards (>10 years), Petri disease, black foot disease-associated pathogens and *Botryosphaeriaceae* spp. also affect newly planted vineyards. Early and accurate diagnosis of the grapevine trunk diseases plays an important role in facilitating the

growers to take proactive cultural and chemical measures for disease management. Microbiological plating is one of the major approaches for plant disease diagnosis, and usually requires the pieces of infected plant tissue to be placed on various nutrient media. This study compared the sensitivity of SYBR-Green Real-time PCR (qPCR) tests in various combinations of DNA extraction and microbiological plating methods. Woody tissue chips were sampled from the graft union of seven symptomatic grapevines and were subject to DNA extraction right after sampling, 5 days after microbiological plating on microbiological media, and 10 days after microbiological plating on microbiological media respectively. SYBR-Green qPCR tests were then conducted for the detection of *Botryosphaeriaceae* spp., *Eutypa* spp., *P. chlamydospora*, *P. minimum*, and *Cylindrocarpon* spp. The results indicate that microbiological plating before DNA extraction significantly increases qPCR sensitivity for the detection of trunk disease and Petri disease pathogens.

Evaluating techniques to induce water-shoot production following remedial surgery to control grapevine trunk diseases. MARK R. SOSNOWSKI^{1,2} and MATTHEW R. AYRES¹. ¹*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia.* ²*The University of Adelaide, Waite Campus, Glen Osmond, SA, Australia. E-mail: mark.sosnowski@sa.gov.au*

Remedial surgery (also known as vine surgery or trunk renewal) is an effective method of controlling the grapevine trunk diseases *Eutypa* and *Botryosphaeria* dieback. It involves cutting visibly affected wood from vine cordons and trunks, and retraining a basal shoot (aka watershoot) into a new canopy. However, it has been reported that, for some own-rooted cultivars, shoot production may be limited to as few as 60% of vines, and even fewer in grafted vines. In 2017, a trial was established to evaluate techniques anecdotally reported to improve water-shoot production in an own-rooted 'Cabernet Sauvignon' vineyard planted in 1995 in the Clare Valley, South Australia. Vines were assessed with 100% incidence of external symptoms of *Eutypa* dieback and *Botryosphaeria* dieback. All vines underwent remedial surgery by cutting trunks with a chainsaw to just above the irrigation wire. Beforehand, cross-sectional cuts starting at the top of the trunk revealed wood symptoms of *Eutypa* dieback and *Botryosphaeria* dieback in 87% of vines, with far fewer vines, 28%, with wood symptoms at the base. Treatments for inducing water-shoots were

undertaken in an area on the lower trunk predesignated by painting a white circle. They included hitting with a hammer, making an X-shaped cut with a tomahawk, rubbing bark with a wire brush and application of a hormone used to break dormancy (Dormex®). In September 2017, a spring remedial surgery was undertaken, and treatments applied, as described above. Grafting treatments were conducted in October. Assessment in February 2018 revealed water-shoots were readily produced on 80-92% of treated and untreated control trunks, but rarely (<18%) originated within the painted white circle, suggesting little influence of most treatments. No significant differences were observed between winter and spring remedial cuts, or treatments, other than grafting, which increased reworking success from 85 to 98%. This research shows that, of the treatments tested, only grafting significantly improved water-shoot production following remedial surgery. Trials continue to investigate seasonal variability of water-shoot production and effectiveness of grafting.

High prevalence of *Diplodia seriata* in vineyards cv. Cabernet Sauvignon located in the central valley of Chile. ALEJANDRA LARACH¹, CAROLINA TORRES¹, NATALIA RIQUELME¹, MICHAEL SEEGER² and XIMENA BESOAIN¹. ¹*Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, San Francisco s/n La Palma, Quillota 2260000, Chile.* ²*Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Chemistry Department & Centro de Biotecnología Daniel Alkalay Lowitt, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso 2340000, Chile. E-mail: alejandra.larach.v@mail.pucv.cl*

Chile is currently the fourth largest exporter of wine in the world, with a planted area of more than 140,000 hectares, concentrated mainly in the regions of O'Higgins and Maule. Cabernet Sauvignon is the main variety planted with more than 30% of the total area. Botryosphaeria canker is one of the main grapevine trunk disease (GTD), with *Diplodia seriata* being the most frequently isolated species. Therefore, in Cabernet Sauvignon it is important to know the prevalence of the Botryosphaeriaceae species over the years. The aim of this work is to identify and quantify the pathogens associated with the death of vines in cv. Cabernet Sauvignon vineyards located in the regions of O'Higgins and Maule, and compare this data with records on fungal infections of 8 years earlier. During the fall and spring of 2018, samples were taken from 14 vineyards (the

same ones sampled in 2010), obtaining pieces of wood from rotten wood, cankers or necrotic stripes, which were placed on plates with acidified potato dextrose agar media and malt extract agar. Each isolate obtained was identified morphologically and molecularly (ITS and β -tubulin). In 2010 the main species detected over 39 isolates were: *D. seriata* (69.2%), *D. mutila* (12.8%), *Neofusicoccum parvum* (10.3%), *N. australe* (5.1%) and *Spencermartinsia viticola* (2.6%). In 2018, out of 44 isolates (corresponding to 98% recovery), the main species detected were: *D. seriata* (72.7%), *D. mutila* (4.5%), *Diaporthe* sp. (4.5%), *N. parvum* (2.3%) and *Neopestalotiopsis* sp. (2.3%). Thus, *D. seriata* is consistently the main isolated pathogen of cv. Cabernet Sauvignon vineyards with symptoms of GTDs in Chile.

Evaluating spray coverage and fungicide efficacy when using adjuvants for pruning wound protection. MATTHEW AYRES¹ and MARK SOSNOWSKI^{1,2}. ¹*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia.* ²*The University of Adelaide, Waite Campus, Glen Osmond, SA, Australia. E-mail: mark.sosnowski@sa.gov.au*

Eutypa dieback, which causes significant impacts to vineyards worldwide, can be controlled by applying fungicides to pruning wounds using tractor-driven sprayers, with efficacy correlated to wound coverage. In winter 2017, a vineyard trial was established in McLaren Valley, South Australia to evaluate the effect of using spray adjuvants with fungicide on wound coverage, using fluorescent dye and fungicide efficacy as assessment criteria. A recycle sprayer was used to apply two fungicide treatments; fluazinam (Emblem®) and tebuconazole (Gelseal™) with or without a wetter (trisiloxane ethoxylate; DuWett®) or a sticker (di-1-p-menthene; Flexextend®). In order to determine if adjuvants can increase coverage, spray treatments were applied at a low water rate of 200 L/ha to simulate poor coverage. Following treatment, wounds were inoculated with *Eutypa lata* ascospores. To assess wound coverage, fluorescent dye was added to the treatments and water sensitive papers were attached to four positions on a post and six randomly selected positions on cordons and spurs. A selection of treated canes was immediately removed from vines and returned to the laboratory for assessment under UV light. Digital images were captured and analysed using Image J software, revealing little difference between treatments for coverage. Image analysis of water sensitive paper indicated relatively higher wound coverage compared with fluorescent dye. After 12 months, the inoculated canes

were removed from vines and returned to the laboratory for isolation on agar media. There was no difference between treatments for recovery of *E. lata*. This preliminary data suggests there may be no advantage in using adjuvants when applying fungicides to pruning wounds with tractor-driven sprayers for protection against infection by *E. lata*. The use of fluorescent dye in treatments may be a more accurate tool than water sensitive paper for evaluating spray coverage on the pruning wound surface. The trial was repeated in winter 2018 to confirm the results.

Fungal pathogens associated with Grapevine Trunk Diseases from patrimonial vineyards in Chile. DAINA GRINBERGS, JAVIER CHILIAN, MARISOL REYES, JEAN FRANCO CASTRO, MACARENA DEL RÍO and ANDRÉS FRANCE. *Instituto de Investigaciones Agropecuarias. Av. Vicente Méndez 515, Chillán, Chile. E-mail: dgrinbergs@inia.cl*

Grapevine is one of the most important fruit crops in Chile, either for wine or fresh fruit production. There are 141,000 ha for wine production with Cabernet Sauvignon as the main cultivar. Trunk diseases are a major problem in grapevine, reducing the productivity, quality and longevity of vineyards. In Chile, the problem has been studied in commercial cultivars. In recent years, there has been an effort to make historical wines, rescuing old winemaking techniques and vines, like the patrimonial cultivars Carignan, País, Torontel and Moscatel. The objective of this work was to characterize the fungal species causing cankers in these cultivars, which has never been investigated. A survey was conducted from different ancestral vineyards in Cauquenes and Itata valleys. Diseased trunks and cordons showed yellowish-spongy-wood decay sectors with a dark brown margins and cankers, i.e. dark-brown-hard V-shaped-lesions. Wood samples were collected from symptomatic plants of 14 different patrimonial cultivars. Fungi were isolated plating wood sections that were previously disinfected using sodium hypochlorite-0.1%, ethanol-70%, rinsed with sterile-distilled-water and flamed. Wood pieces were plated on 25%-acidified-PDA or water-agar, then incubated at 25°C and darkness until mycelial development, and transferred to PDA to obtain pure cultures. The isolates were identified by their cultural characteristics on PDA and MEA, morphometry of the fungal structures, and by the analyses of nucleotide sequences of ITS1-ITS4 and Ef728-Tef1R. A total of 115 isolates were obtained and Botryosphaeriaceae spp. were the most common fungi isolated (37%), with *Diplodia* (78%)

as the most frequent genus in this group, followed by *Neofusicoccum*, *Dothiorella* and *Botryosphaeria*. Among basidiomycete isolates (21%) *Fomitiporia* was the most common genus. The third group in importance was *Phaeoacremonium* spp. (15-isolates, 13%), and *Seimatosporium* spp. (13-isolates, 11.3%). There were less frequent genera like *Phaeoconiella* and *Diaporthe*. Future studies will be developed to assess the pathogenicity and virulence of these fungi.

Evaluating natural alternative products for the control of eutypa dieback in grapevine. MATTHEW AYRES¹ and MARK SOSNOWSKI^{1,2}. ¹*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia.* ²*School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond, SA, Australia. E-mail: mark.sosnowski@sa.gov.au*

Eutypa dieback is a major grapevine trunk disease throughout the world, and the most effective means of control is to prevent infection of pruning wounds. Research has shown that synthetic fungicides are effective, and a number of products are now registered in Australia and New Zealand for this purpose. However, there are limited natural alternative products registered for pruning wound protection against *Eutypa dieback*. Detached cane assays using single node Shiraz canes were used to evaluate vanillin (at 5 and 10 mg/ml), chitosan (at 12.5 and 25 mg/mL), previously reported with activity against trunk disease pathogens, and the yeast-like fungus *Aureobasidium pullulans* (Botector®), registered for control of botrytis bunch rot. The fungicide tebuconazole (Gelseal™), registered for control of *Eutypa dieback*, was included as a reference, as well as negative (no treatment, no inoculum) and positive (no treatment, inoculated) controls. All treatments (except negative control) were inoculated with 100 ascospores of *Eutypa lata*, the primary causal agent of *eutypa dieback*, and the canes grown in tubs of water for 5 weeks prior to their removal to the laboratory for isolation on agar medium. Vanillin had no effect on recovery of *E. lata* compared with the positive control, while chitosan, particularly at the higher concentration, significantly reduced recovery, to a similar level to canes treated with Gelseal. No *E. lata* was recovered from the canes treated with Botector, and all agar plates contained *A. pullulans*. The results of this experiment indicate that chitosan and Botector may have potential as pruning wound protectants against infection by trunk disease pathogens. Ongoing research is evaluating these products against *N. luteum* (*Botryosphaeria die-*

back) and Botector will be further evaluated in vineyard trials.

Characterization of *Trichoderma* isolates from southern Italy and their potential use as biocontrol agents against grapevine trunk diseases. EUGENIA TOMASELLI¹, JULIE BOULÉ², MELANIE WALKER², JINXZ POLLARD-FLAMAND², STEFANIA POLLASTRO¹ and JOSÉ RAMÓN ÚRBEZ-TORRES². ¹*Università degli studi di Bari “Aldo Moro”, facoltà di Agraria, via G. Amendola, 165/A 70126 Bari (BA), Italy.* ²*Agriculture and Agri-Food Canada, Summerland Research and Development Centre, Summerland, British Columbia V0H1Z0, Canada. E-mail: e.tomaselli@studenti.uniba.it*

Grapevine trunk diseases (GTD) are one of the most economic important diseases on grapevines causing decline, loss of productivity and eventual death of the vine. In the last decade, the interest by the grape and wine industry in developing and implementing sustainable and biological control practices against diseases, including GTD is raising. Accordingly, the objectives of this study were to i) identify and characterize *Trichoderma* isolates from southern Italy by morphological and molecular studies and to ii) determine their potential as biocontrol agents against the GTD canker-causing fungi *Diplodia seriata*, *Neofusicoccum parvum* and *Eutypa lata*. A total of 16 *Trichoderma* isolates from Italy were studied at the Agriculture and Agri-food Canada research centre located in Summerland, British Columbia. Morphological characterization included studies to determine the optimum temperature for micelial growth, which showed to be between 25 and 30°C for all 16 isolates. Multi-locus phylogenetic analyses of the ITS1-5.8S-ITS2, TEF-1α and RPB2 genes revealed six species, including *T. atroviride*, *T. harzianum*, *T. koningiopsis*, *T. longibrachiatum*, *T. paraviridescens*, and *T. spirale*. The antagonistic capabilities of all *Trichoderma* isolates were screened against *D. seriata*, *N. parvum*, and *E. lata* in vitro via dual culture assay. Results revealed all 16 isolates to provide more than 50% mycelium growth inhibition for *D. seriata*, 14 isolates for *N. parvum* and 6 isolates for *E. lata*. The best performing isolates across all three species were selected to further screen in plant their potential as BCA via a detached cane assay (DCA) under controlled growth chamber conditions. Chardonnay dormant canes were pruned and inoculated with 50 µL of a 10⁶ spores/mL of each *Trichoderma* sp. Then, a 50 µL of a 10⁵ conidia/mL of *D. seriata* and *N. parvum* and an approximate 10⁵ mycelial pieces/mL of *E. lata* were used to challenge the treated pruning wounds 24h,

7d and 21d after treatment. DCA results will be presented and discussed. This study aims to provide alternative sustainable control solutions against GTD.

Effect of native and exotic ground cover on grapevine pathogens and disease severity. ANDREW RICHARDS¹, MIRANDA HART¹, JOSÉ RAMÓN ÚRBEZ-TORRES², TOM LOWERY² and PAT BOWEN². ¹*University of British Columbia Okanagan, 3333 University Way, Kelowna, BC V1V 1V7, Canada.* ²*Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Box 5000. Summerland, BC V0H 1Z0, Canada. E-mail: andrew.richards@ubc.ca*

Young vine decline (YVD) occurs when grapevines experience stunted growth, reduced yield, delayed fruiting, and root necrosis, often leading to dieback in vineyards worldwide. These symptoms are largely due to soil-borne fungal pathogens including *Ilyonectria* species which may be present in the soil or brought to the area by contaminated nursery plants. Recent restrictions in fungicide use and high density planting regimes have exacerbated the incidence of YVD in vineyards. Public demand for low-impact sustainable agriculture has urged researchers to test alternative approaches to combat soil-borne diseases. Cover crops are plants that are grown alongside main crops or during non-production seasons in order to maintain and enhance soil composition. These plants provide several well-known benefits and may serve as a line of defense against soil-borne fungal pathogens by harbouring suppressive microbes and producing volatile root exudates. We tested whether cover crop diversity and provenance affects the persistence and infectivity of YVD pathogens in agricultural soil. In a greenhouse experiment, we inoculated orchard soil with a 10⁶ conidia dilution of *Ilyonectria liriodendri* isolates (PARC60, PARC340, PARC393) prior to seeding with native and commercial cover crop mixtures. We trained soil for 3 and 4 months then replaced cover crops with *Vitis vinifera* cuttings, which were grown for 4 and 5 months. The concentration of *I. liriodendri* decreased significantly in soil samples over 3 months. We isolated the introduced pathogen from 99% of root samples, excluding fallow treatments which did not contain the inoculant. Cover crop diversity and provenance resulted in similar necrotic damage in grapevine cross sections and grapevine physiology traits. Future diversity studies should include incorporation of cover crop residues, span multiple growing seasons and assess whether cover crops are harbouring grapevine trunk disease pathogens in their roots.

Spatio-temporal distribution of Esca disease in commercial vineyards cv. Verdicchio and Chardonnay.

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Esca is a multiple fungal syndrome which can cause serious economic damages for grapevine in different viticultural areas. The aim of this study was to monitor the spatial and temporal epidemiology of esca disease incidence in three commercial vineyards (OS1, OS2, CAST) located in Marche region (Italy). During two years (2017–2018) of surveys, a consistent percentage of plants showing “leaf tiger-stripes” (ranging from 10% to 50%) and apoplectic stroke (ranging from 5% to 20%) was recorded. The data collected during the surveys were statistically elaborated by SYSTAT v.13 software, which generated maps of esca disease spread and severity. The presence of inter-row and intra-row disease gradient was determined by a regression curve, which represents the percentage of symptomatic plants in relationship with the distance from the edge of the vineyard. The data processing allowed to verify in 2017 and 2018 that i) none of the vineyards showed a gradient of esca disease among the rows; ii) there was a west-east gradient of disease in the vineyard OS1, and in the vineyard OS2 a greater concentration of disease in the center than the edges was observed. Particularly significant was the correlation recorded between the esca disease incidence and different combination of rootstocks (420A, SO4, 41B, 110R) in vineyard OS1. The obtained result represents a further set of information that can allow a better understanding of the epidemiology of esca.

***Vitis vinifera* resistance induced by *Pythium oligandrum* to control *Neofusicoccum parvum*, a pathogenic fungus involved in grapevine trunk diseases.**

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Grapevine trunk diseases (GTDs) are on the rise in vineyards all over the world. Many pathogens are involved in these diseases, including *Phaeoconiella chlamydospora*, *Phaeoacremonium minimum*, *Fomitiporia mediterranea* and *Neofusicoccum parvum*. Nowadays, few treatments are available to control these diseases. Accordingly, bio-control of pathogens implicated in GTDs was developed using microorganisms as the oomycete *Pythium oligandrum*, which naturally colonized grapevine roots in the vineyards of several countries worldwide. Recent studies showed that this oomycete induced grapevine resistance and reduced significantly *P. chlamydospora* necrosis). In this study, the ability of *P. oligandrum* to induce grapevine resistance against *N. parvum* was evaluated. Two greenhouse assays showed that the necrosis of Cabernet Sauvignon cuttings caused by *N. parvum* was reduced by 65% when *P. oligandrum* colonized the root system of young vines. The expression levels of a set of 96 genes (“NeoViGen96”chip) involved in various grapevine defense pathways were studied by real-time PCR, at the trunk level. This analysis aims to determine plant responses after inoculation by *P. oligandrum* and/or *N. parvum*, at three different sampling time points (0, 14 and 150 days after *N. parvum* inoculation). Overall, our results showed that the sampling time point has an important effect on studied gene expression levels, whatever the treatment applied. Moreover, at each sampling time point, specific grapevine responses to the different treatments (control, *P. oligandrum*, *N. parvum*, *P. oligandrum* + *N. parvum* treatments) were also differentiated. When *P. oligandrum* colonizes grapevine root systems, infection with the pathogen is associated with a more intense up-regulation of certain genes: PR protein, stilbene synthase and signaling pathway genes were over-expressed. A priming effect of plant defense system is probably induced in presence of *P. oligandrum*.

Isolation and characterisation of phytotoxins produced by the Botryosphaeriaceae and their role in grapevine trunk diseases.

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Botryosphaeria dieback (BD) is considered a serious

problem worldwide causing cankers, dieback and eventually death of vines. Foliar symptoms due to BD have been reported in some vineyards overseas. The foliar symptoms caused by grapevine trunk diseases are usually associated with the production of phytotoxic metabolites (PMs) produced by the pathogen that are translocated into the leaves. However, foliar symptoms due to BD have not been reported in Australian vineyards to date. This study investigated the production of PMs by the most virulent and widespread Botryosphaeriaceae species associated with BD in Australia. Isolates of *Diplodia mutila*, *D. seriata*, *Dothiorella vidmadera*, *Neofusicoccum australe*, *N. luteum* and *Spencermartinsia viticola* were grown in liquid media and PMs were extracted from the culture filtrates. The PMs were purified using a chromatographic method while their chemical characterization was performed by spectroscopic method, essentially 1D and 2D NMR. The purified PMs from all species showed different degrees of toxicity when tested on grapevine leaves at different concentrations. *R*-(-)-mellein and other well-known PMs were isolated from *D. mutila*, *D. seriata*, *N. australe* and *N. luteum* culture filtrates. Two novel toxic quinones (diploquinone A and B) were isolated from the culture filtrates of *D. mutila* while two novel compounds, spencertoxin and spencer acid, were isolated from the culture filtrates of *S. viticola*. Furthermore, six polyphenols were isolated from the culture filtrate of *D. vidmadera*, one being characterized as 5-hydroxy-methyl-2-isopropoxyphenol, a novel compound. The isolation and characterization of the PMs secreted by these fungi are fundamental to understanding the potential role of these PMs in the virulence and symptomatology of Botryosphaeria dieback pathogens in grapevines.

Effect of volatile compounds produced by *Trichoderma* spp. against *Phaeoacremonium minimum*. SAMUEL ÁLVAREZ-GARCÍA¹, GUZMÁN CARRO-HUERGA¹, SARA MAYO-PRIETO¹, ÁLVARO RODRÍGUEZ-GONZÁLEZ¹, SANTIAGO GUTIÉRREZ² and PEDRO ANTONIO CASQUERO¹. ¹Grupo Universitario de Investigación en Ingeniería y Agricultura Sostenible (GUIIAS), Instituto de Medio Ambiente, Recursos Naturales y Biodiversidad, Universidad de León, Avenida Portugal 41, 24071 León, España. ²Grupo Universitario de Investigación en Ingeniería y Agricultura Sostenible (GUIIAS), Área de Microbiología, Escuela de Ingeniería Agraria y Forestal, Universidad de León, Campus de Ponferrada, Avenida Astorga s/n, 24401 Ponferrada, España. E-mail: salvg@unileon.es

During the last decades, grapevine trunk diseases

(GTDs) have become a major issue for viticulture causing significant reduction on grape production and damage to the plants. Among the fungi associated with GTDs, *Phaeoacremonium minimum* plays a key role in the development of the disease. One approach to face this threat is the use of biological control agents (BCAs) against these pathogens. *Trichoderma* spp. is a widespread genera of fungi used as BCAs in agriculture. One of the control mechanism shown by *Trichoderma* is the release of active volatile compounds. The aim of this study was to evaluate *in vitro* the effect of volatile metabolites produced by three *Trichoderma* strains (T71: *T. gamsii*; T130: *T. gamsii*; T154: *T. harzianum*) against *Phaeoacremonium minimum*. The pathogen was grown for 7 days in the centre of 90mm Petri dishes with PDA prior to the addition of the BCA. A 6mm plug of *Trichoderma* was placed in the centre of PDA Petri dishes and the lids of both pathogen and BCA dishes were removed and placed together along their edge, facing each other, downwards and upwards, respectively. This was considered as day 0, and diameters of the pathogen colonies were measured in day 7, 14 and 21. The results showed a high volatile-mediated antifungal activity of all *Trichoderma* strains evaluated, with inhibition percentages of; T71: 96.87%; T130: 98.44% and T154: 100%, leading an abrupt halt in *P. minimum* growth from day 0 onwards. While controls without *Trichoderma* showed a steady growth from day 0 to day 21. The results demonstrate that *Trichoderma* volatile metabolites can play a significant role in its antagonistic activity against *P. minimum*. More assays are needed to elucidate these volatile-mediated fungal interactions.

Time-course transcriptional analysis of grapevine inoculated with the fungal pathogen *Lasiodiplodia theobromae*. WEI ZHANG¹, JIYE YAN¹, XINGHONG LI¹, QIKAI XING¹, JUNBO PENG¹ and WENSHENG ZHAO². ¹Beijing Key Laboratory for Environmental Friendly Management on Pests of North China Fruits, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China. ²State Key Laboratory of Agrobiotechnology and MOA Key Lab of Pest Monitoring and Green Management, China Agricultural University, Beijing 100193, China. E-mail: zhwei1125@163.com

Grapevine Botryosphaeria dieback, which is caused by Botryosphaeriaceae fungi, threatens the yield and quality of grapes in the past few decades. Chemical control strategies are often observed to be ineffective in controlling the disease worldwide. Increasing our understanding of the molecular resistant mechanisms of grapevine

to pathogens would facilitate the development of pathogen-tolerant grape varieties. Therefore, our study aims are to uncover the defense response of grapevine against *Lasiodiplodia theobromae*, which is the most aggressive pathogen of grapevine Botryosphaeria dieback in China, and to obtain the potential resistance associated genes in grapevine. Here, RNA sequencing analysis was used to profile the transcriptome of grapevine green shoots infected with *L. theobromae* over a time course of 4, 8 and 12 hours post inoculation. A total of 5181 genes were identified as differentially expressed genes (DEGs), and DEGs were more abundant over time. Further analysis revealed that many of these DEGs are involved in plant-pathogen interactions, hormone signal transduction, and phenylpropanoid biosynthesis pathway, suggesting that innate immunity, phytohormone signaling, and many phenylpropanoid compounds, which constitute a complex defense network in plants, are involved in the response of grapevine against *L. theobromae* infection. This study provides novel insights into the molecular mechanisms of plant-pathogen interactions that will be valuable for the genetic improvement of grapevines.

Antifungal effects of *Bacillus subtilis* and *Pantoea agglomerans* on grapevines cv. Cabernet Sauvignon and Italia against two GTD pathogens, *Neofusicoccum parvum* and *Phaeoconiella chlamydospora*. AWATEF REZGUI^{1,2}, ASMA BEN GHAYYA CHAKROUN¹, EMILIE BRUEZ^{2,3}, MAYSSA ARFAOUI^{1,2}, AMIRA YACOUB², MOKHTAR DRIDI¹, NAJLA SADFI ZOUAOUI¹, PATRICE REY^{2,3} and JESSICA VALLANCE^{2,3}. ¹Laboratoire Microorganismes et Biomolécules Actives, Faculté des Sciences de Tunis, Université de Tunis El Manar, 2092, Tunisia. ²INRA, UMR1065 Santé et Agro-écologie du Vignoble (SAVE), ISVV, 33140 Villenave d'Ornon, France. ³Université de Bordeaux, Bordeaux Sciences Agro, UMR1065 SAVE, 33140 Villenave d'Ornon, France. E-mail: amira.yacoub@inra.fr

In Tunisia, knowledge about Grapevine trunk diseases (GTDs) symptoms and the associated microflora, is still incomplete despite their ability to cause considerable damage to vineyards. In that context, the fungal microflora inhabiting the wood tissues of Tunisian GTD-foliar symptomatic vines was studied in order to identify the pathogens responsible for wood decay. Microbiological isolations and sequencing of the ITS region allowed us to identify for the first time in Tunisia three fungi previously described in the literature to be associated with GTDs, including *Lasiodiplodia pseudotheobromae*, *Neofusicoccum parvum* and *Schizophyllum commune*.

Their pathogenicity was confirmed *in planta*, and *in vivo* and *in planta* competitions between strains were noticed. The bacterial microflora colonizing the non-necrotic and necrotic wood tissues of Tunisian mature grapevines was also investigated in order to find a suitable Biological Control Agent (BCA) that can be applied under Tunisia conditions. The 19 most abundant cultivable strains, selected based on their morphology, were assigned to *Pantoea*, *Pseudomonas*, *Curtobacterium* and *Bacillus* species based on the 16S rRNA and *rpoB* genes. Microbiological and molecular screenings revealed that those 19 strains had an *in vitro* antagonistic effect against the 3 fungal pathogens previously isolated, with *B. subtilis* B6 being the most promising BCA. Finally, the efficacy of *Bacillus subtilis* B6, isolated from a Tunisian vine (cv. Italia); *Pantoea agglomerans* S5, originated from a French vine (cv. Cabernet Sauvignon); and the combination B6+S5, was investigated in controlling *N. parvum* (Tunisian strain) and *Phaeoconiella chlamydospora* (French strain) in young vines cvs. Italia and Cabernet Sauvignon. Depending on the pathogen and the cv. used, the severity of internal symptoms (necrosis length) varied. In terms of plant protection, the most efficient BCA to reduce *in planta* necrosis caused by the pathogens in both cvs was the combination of the two B6+S5 bacteria.

Construction of a grapevine yeast two-hybrid cDNA library to identify host targets of the *Lasiodiplodia theobromae* effector LtCRE1. QIKAI XING, WEI ZHANG, JUNBO PENG, YANG CAO, LINGXIAN LI, XINGHONG LI and JIYE YAN. Beijing Key Laboratory of Environment Friendly Management on Fruits Pests in North China, Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China. E-mail: qikaixing@163.com

Botryosphaeria dieback is a destructive woody plant disease caused by pathogens of the fungal family Botryosphaeriaceae. Effectors are molecules secreted by fungal pathogens that manipulate host physiological and biochemical events, thereby facilitating infection or triggering defense responses. One of the cysteine rich effectors (named CRE1) was identified from *Lasiodiplodia theobromae* based on its strong cell death-suppression activities and was proved to play critical roles in pathogen pathogenicity and host immune response. However, the biological mechanism of LtCRE1 during interaction remains to be determined. In this study, a grapevine yeast two-hybrid cDNA library was constructed to screen putative host proteins interacting with LtCRE1.

Mate & Plate™ yeast two-hybrid library method was used for constructing the grapevine cDNA library from pooled mRNA, which were extracted from plants challenged with *L. theobromae* CSS-01s isolate, and several hormones associated with environmental stress responses. The constructed library consisted of 8.8×10^6 independent clones with an average insert size of 1 kb. *LtCRE1* gene was ligated into the pGBKT7 vector (pGBKT7-LtCRE1) and was considered as the bait after evaluating the expression, auto-activation and toxicity tests in the yeast strain Y2HGGold. The yeast two-hybrid screening was performed via co-transforming the bait strain and the cDNA library. Moreover, a total of 57 candidate-interacting proteins were identified. These data suggested that the cDNA library is in high quality and suitable for identifying the interacting proteins of *L. theobromae* effectors.

Esca and training systems in the viticulture region of Charentes, France.

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The relationship between cultural practices and the development of the Grapevine Trunk Diseases (GTDs), namely Esca, has been the topic of many studies in recent years. To further examine the role of some specific training systems as aggravating factors, a study was carried out in the Charentes region (France). In 2016, 4 pairs of vineyards (cv. Ugni blanc), in which Esca was the predominant GTD, were selected and surveyed. Each pair consisted of vineyards located in similar environments and comparable age, but they varied in their training systems. For each vineyard pair one vineyard was 'espalier cordon', manually or mechanically pruned, and the other was 'espaliers Guyot-arcure', manually pruned. The prevalence of Esca was assessed

in winter and summer, based on trunk and leaf visual symptoms. Two pairs of vineyards were selected to collect leaf-asymptomatic grapevines. Vines were cut longitudinally and photographed to assess the inner necrosis surface. Half of vines were used to collect wood samples for identification and quantification of the main pathogens associated with Esca, by microbiology and/or quantitative PCR. Results were in line with those obtained in other previous experiments: vines with a low aerial volume and short cordons and trunks, such as those with Guyot-arcure (or arch-Guyot) training, were generally more affected by Esca (high incidence and necrotic surface) than those with long arms or trunks (espalier cordons). However, Esca pathogens, mostly located in the arms, were equivalent in number in both training systems. On the whole, this study confirmed the aggravating role of training systems that confine grapevines to a small aerial volume. This may encourage growers or "appellations" to change the training systems and distances between each vines.

This study was funded by the industrial chair "Gtdfree" jointly supported by the French National Agency for Research (ANR) and the Jas Hennessy Company.

Spatial dynamics of bacterial community in different organs and wood tissues of grapevines.

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The spatial dynamics of bacterial communities inhabiting different organs (i.e. leaves, berries, cordons, trunks, barks and roots) and wood tissues (i.e. non necrotic, necrotic and white rot in cordons and trunks) of 15-year-old grapevines cv. Cabernet Sauvignon having expressed or not esca foliar symptoms, were investigated through metagenomic approaches. A quantitative analysis of the different wood tissues found in cordons and trunks using image processing software did not highlight any differences between necrotic wood and white rot in symptomatic and asymptomatic vines. Bacterial cell counts revealed that each sampled organ and tissue was heavily colonized ($1,8 \times 10^9$ CFU.g⁻¹ on average).

Bacterial diversity per type of vine and organ/tissue (66 samples in total) was then analysed based on tag-encoded 454 pyrosequencing of the 16S rRNA V5-V6 region. No fewer than 80974 quality sequences were obtained, corresponding to 1203 Operational Taxonomic Units (OTUs). OTUs mostly belonged to the Proteobacteria phylum (56%) followed by Actinobacteria (18%) and Bacteroidetes (12%). Alpha diversity analysis revealed that greater diversity was detected in the woody compartments, i.e. cordons, trunk, bark and roots, when compared to the aerial parts, i.e. berries and leaves. Beta diversity analysis demonstrated that differentiation of the structure of the bacterial communities was organ- and tissue-dependent (healthy vs. necrotic), rather than between esca-symptomatic and asymptomatic plants. Three main compartments were defined and differed significantly: (i) aerial parts, i.e. leaves and berries; (ii) wood parts, i.e. cordons, trunks and barks; and (iii) roots.

Bacterial screening for the biological control of grapevine trunk diseases-associated fungi. CARMEN SANJUANA DELGADO-RAMÍREZ¹, EDELWEISS AIRAM RANGEL-MONTOYA¹, EDGARDO SEPULVEDA¹ and RUFINA HERNÁNDEZ-MARTÍNEZ¹. ¹Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Carretera Ensenada Tijuana No. 3918, Zona Playitas, 22860, Ensenada, B.C., México. E-mail: ruherman@cicese.mx

Grapevine trunk diseases (GTD) are presently considered one of the most important menaces for viticulture worldwide. They are caused by different fungal species that mostly infect the vascular system of the vine, affecting vineyard longevity and causing yield losses. Fungi associated with trunk diseases identified in Mexico include *Diplodia corticola*, *D. seriata*, *Lasiodiplodia theobromae*, *Neofusicoccum australe*, and *N. vitifusiforme*. Among them, *L. theobromae* is found to be the most aggressive. This work aimed to investigate for potential biological control agents suitable for the dry environment prevalent in Baja California. Soil samples were obtained from the rhizosphere of the shrub *Solanum hindsianum*, a plant distributed along the peninsula that is highly resistant to soil desiccation. Bacterial isolates were obtained using different culture media (Luria-Bertani, Yeast-Mannitol, ISP4, Yeast-Peptone, Potato-Dextrose Agar, and Kings B) and then evaluated, using dual plate assays against *L. theobromae*. From a total of 346 bacteria isolates, 49 showed a percentage of inhibition higher than 30%, but only nine inhibit above 60%

of the growth of *L. theobromae*. Identification, by 16S rRNA sequencing, indicates that seven isolates belong to the genus *Bacillus* and two to *Streptomyces*, among them *Bacillus* spp., *B. siamensis*, *B. subtilis*, *B. tequilensis*, *Streptomyces luteoverticillatus*, and *S. youssoufien-sis*. Further characterization showed that these strains can solubilize potassium and phosphorus and to produce indoleacetic acid or siderophores, activities that have been previously determined to be involved in plant growth promotion. To conclude, potential biological control agents were identified which might help with the management of GTD in dry environments.

Characterization of rhizosphere microbial communities associated with grapevine rootstocks and their interactions with black-foot disease. CARMEN BERLANAS¹, MÓNICA BERBEGAL², GEORGINA ELENA², MERIEM LAIDANI², JOSÉ FÉLIX CIBRIAIN³, ANA SAGÜES-SARASA³ and DAVID GRAMAJE¹. ¹Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain. ²Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain. ³EVENA/INTIA, Departamento de Desarrollo Rural, Medio Ambiente y Administración Local, Gobierno de Navarra C/ Valle de Orba 34, 31390 Olite, Spain. E-mail: carmen.berlanas@icvv.es

The soil microbiome has a great interest for its potential in improving plant nutrient utilization and suppressing soil-borne diseases. This study aimed to unravel the effects of rootstock genotype on microbial communities in the rhizosphere of grapevine. For this purpose, the rhizosphere of five grapevine rootstocks (110R, 1103P, 140Ru, 41B and 16149C) was sampled at two sampling moments (June and November) in two Spanish vineyards located in Olite, Navarra (7-year-old) and Aldeanueva del Ebro, La Rioja (25-year-old) over two years. The composition of bacterial and fungal communities was examined using high-throughput amplicon sequencing of 16S rDNA gene and ITS region. Quantitative PCR approach was used to measure the relative abundance and dynamic changes of fungal pathogens associated with black-foot disease. Host genetic control of the microbiome was evident in the rhizosphere of the mature vineyard. Microbiome composition also shifted as year of sampling, and fungal diversity varied with sampling moments. Linear discriminant analysis identified specific bacterial (i.e., *Bacillus*) and fungal

(i.e., *Glomus*) taxa associated with grapevine rootstocks. Host genotype did not predict any summary metrics of rhizosphere a and b diversities in the young vineyard. Regarding black-foot associated pathogens, a significant correlation between sequencing and quantitative PCR was observed. Results proved to be significantly influenced by rootstock genotype, year of sampling and vineyard location. The findings of this study provide evidence that rootstock genotype affects the process of microbial recruitment in the rhizosphere of grapevine, and this could be related to the plant age or the habitat (soil type and climate).

***Trichoderma atroviride* SC1 provides long-term protection from nursery to vineyard against fungal grapevine trunk pathogens.** MÓNICA BERBEGAL, ANTONIO RAMÓN-ALBALAT, MAELA LEÓN and JOSEP ARMENGOL. *Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera S/N, 46022 - Valencia, Spain. E-mail: mobermar@etsia.upv.es*

The most important objectives to be covered by a fungal grapevine trunk diseases (GTDs) integrated management program are: i) the improvement of the phytosanitary quality of the vines produced in the nursery and ii) the prevention of infections of pruning wounds in the vineyard from the moment of planting. In this research, nursery and vineyard experiments were set up to evaluate the ability of the biological control agent *Trichoderma atroviride* SC1 to reduce infections of GTDs pathogens in grapevine planting material during the propagation process. Additionally, SC1 was assessed for the long-term protection on grapevine plants in young vineyards during two growing seasons. In the 2015 growing season, cuttings of 110 R rootstock subsequently grafted with Garnacha Tintorera cultivar were treated with a suspension of formulated *T. atroviride* SC1, which was applied during the nursery propagation process. Later, in 2016 and 2017, treatments with *T. atroviride* SC1 were applied to the same plants at planting moment in two different commercial vineyards (2016) and at the first pruning (2017). Plants, treated with water at each of the previously described moments were used as controls. The effect of the treatments was compared by fungal isolation followed by molecular identification of the colonies (2015, 2016 and 2017) and by measuring undried shoot weight (2016 and 2017). Results of this study confirmed that nursery application of *T. atroviride* SC1 reduced GTDs pathogens incidence and severity on grapevine propagation material after the grafting process. Moreover, additional *T. atroviride* SC1 treatments

performed during two growing seasons in vineyards planted with these grafted plants also contributed to maintain reduced levels of GTDs infections when compared with untreated plants. *Trichoderma atroviride* SC1 can be used to manage GTDs infections in nurseries, and also to maintain an improved phytosanitary status of the plants when establishing new vineyards.

Bud necrosis associated with *Diplodia seriata* in California vineyards. JOSÉ RAMÓN ÚRBEZ-TORRES¹ and LUIS SÁNCHEZ². ¹*Agriculture and Agri-Food Canada, Summerland Research and Development Centre, Summerland, British Columbia V0H1Z0, Canada.* ²*E. & J. Gallo Winery, 600 Yosemite Blvd, Modesto, California 95354, USA. E-mail: joseramon.urbeztorres@canada.ca*

A wide range of different foliar and vascular symptoms are known to be caused by Grapevine trunk diseases (GTD) fungi, including short internodes, cupped and chlorotic leaves, poor vigor, “tiger-leaf stripes”, black measles, spur and cordon dieback, wood necrosis and streaking, and perennial cankers. In the past, few reports have also associated bud necrosis with species in the Botryosphaeriaceae family; though not much information is available on this. Primary bud necrosis (PBN) in grapevines is primarily associated with physiological causes, including excess of vigor due to high levels of irrigation and fertilization, shading, winter damage and/or spring frost. However, a different necrosis named inflorescence primordium necrosis (IPN) was observed affecting numerous vineyards in California causing significant production losses. The objective of this study was to determine if causes other than physiological were associated with IPN. In total, 135 buds were collected from 9 different vineyards affected with IPN, including Chardonnay, Pinot noir, Syrah, Cabernet sauvignon, and Merlot. Buds from basal nodes 1 to 3 were collected during mid-dormancy, dissected and the percentage of buds showing IPN recorded. Isolations were conducted from both symptomatic and asymptomatic buds on potato dextrose agar in an attempt to identify a potential biotic cause for IPN. Results showed 68% of the collected buds to have IPN. Isolations and morphological identifications revealed *Alternaria* sp. to be the most prevalent fungus isolated from IPN symptoms (60%), followed by *Diplodia seriata* (13%). Both *Alternaria* sp. and *Diplodia seriata* were not isolated from healthy buds. Identification of *D. seriata* was confirmed by sequencing the ITS1-5.8S-ITS2 gene and the TEF-1 α partial gene. This study suggests *Alternaria* sp. and the GTD fungus *D. seriata* to play a role on IPN in California vineyards. Further under-

standing on the cause of IPN will assist to develop and implement effective management strategies against the fungi involved.

Exploit biodiversity in viticultural systems to reduce pest damage and pesticide use, and increase ecosystem services provision: the BIOVINE Project.

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Organic vineyards still rely on large external inputs to control harmful organisms (i.e., pests). The BIOVINE project aims to develop natural solutions based on plant diversity to control pests and reduce pesticide dependence. The capability of plants of increasing the ecosystem resistance to pests and invasive species is a well-known ecosystem service. However, monocultures (including vineyards) do not exploit the potential of plant diversity. BIOVINE aims to develop new viticultural systems based on increased plant diversity within (e.g., cover crops) and/or around (e.g., hedges, vegetation spots) vineyards by planting selected plant species for the control of arthropods, soil-borne pests (oomycetes, fungi, nematodes), and foliar pathogens. The Project will pay special attention to fungal grapevine trunk pathogens. Candidate plants will be identified by a literature review, and the selected ones will be tested in controlled environment or small-scale experiments. The ability of the selected plants to: i) attract or repel target arthropod pests; ii) conserve/promote beneficials; iii) control soil-borne pests by means of biofumigation; iv) carry mycorrhizal fungi to the vine root system to increase plant health (growth and resistance); and v) control foliar pathogens by reducing the inoculum spread from soil, will be investigated. New viticultural systems able to exploit plant diversity will

then be designed based on results of BIOVINE activities, following a design-assessment-adjustment cycle, which will then be tested by in-vineyard experiments in France, Italy, Romania, Slovenia, Spain and Switzerland for a 2-year period. Innovative viticultural systems should represent an improved way for pest control in organic viticulture, meanwhile they should positively affect functional biodiversity and ecosystem services. New control strategies may provide financial opportunities to vine growers and lower their reliance on pesticides.

Financial support was provided by transnational funding bodies, being partners of the H2020 ERA-net project, CORE Organic Cofund, and the cofund from the European Commission.

Grapevine belowground compartments affect fungal trunk pathogens composition.

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Plant compartments of grapevine provide unique niches that drive specific microbiome associations. The microbiota colonizing the rhizosphere and the endophytic compartment (endorhizosphere) contribute to plant growth, productivity, carbon sequestration and phytoremediation. The main objective of this study was to investigate how fungal communities are enriched in different habitats inside and outside of grapevine roots, with special attention to pathogens associated with grapevine trunk diseases (GTDs). For this purpose, the spatial dynamics of the fungal communities associated with three soil-plant compartments (bulk soil, rhizosphere and endorhizosphere) in 5 young vineyards in La Rioja (Spain) were characterized by ITS high-throughput amplicon sequencing (HTAS). The fungal diversity was similar in both bulk soil and rhizosphere compartments at all taxonomic levels. A significant reduction in species richness was found in the endorhizosphere relative to bulk soil and rhizosphere, with distinct community structure especially at lower-order taxonomic analysis.

HTAS analysis showed a significantly higher abundance of fungi belonging to the genera *Cadophora* and *Ilyonectria* at rhizosphere and endorhizosphere compartments as compared with bulk soil. *Phaeoacremonium* spp. were significantly more abundant in the rhizosphere than in the other compartments, with very low fungal abundance in the endorhizosphere. Roots of asymptomatic vines were found to be a microbial niche that is inhabited by GTD fungi, which opens up new perspectives in the study of the endophytic role of these pathogens on grapevines. Results obtained in this study provide helpful information to better understand how grapevine shapes its fungal microbiome and the implications for vineyard management and productivity.

Epidemiology of Diatrypaceae spp. in California vineyards. JOSÉ RAMÓN ÚRBEZ-TORRES¹, CARMEN GISPERT² and FLORENT P. TROUILLAS³. ¹*Agriculture and Agri-Food Canada, Summerland Research and Development Centre, Summerland, British Columbia V0H1Z0, Canada.* ²*University of California Cooperative Extension, Riverside County, Indio, California 92201, USA.* ³*Department of Plant Pathology, University of California, Davis and Kearney Agricultural Research and Extension Centre, Parlier, California 93648, USA. E-mail: joseramon.urbeztorres@canada.ca*

The seasonal abundance of Diatrypaceae spp. spores was studied in California vineyards by using Burkard

volumetric spore traps and glass microscope slides covered with petroleum jelly placed on grapevine cordons. Burkard spore traps were located from October to April during the 1999-2000 and 2000-2001 seasons in vineyards at Santa Helena (Napa Co.), Healdsburg (Sonoma Co.), and Lodi (San Joaquin Co.). Petroleum jelly slides were placed from September 2006 to May 2009 in the arid grape-growing region of Coachella Valley. Results from these studies showed Diatrypaceae spores to be released after a rain event from October to April at Santa Helena, Healdsburg and Lodi vineyards. However, the largest amount of spores trapped after a rain event were primarily recorded during the months of November and January. Results from Burkard volumetric spore traps showed releasing of Diatrypaceae spores to continue at least for a period of 20 hours after the end of the rain event. Petroleum jelly spore traps showed Diatrypaceae spores to be released from October to March in the vineyard located in Coachella Valley. Interestingly, spore released was detected the first two season with no rain event. Further analyses showed overhead sprinkling irrigation used in this vineyard to correlated with spore released during that period. Epidemiological studies are critical to understand the seasonal spore release of GTD spores, which can be used to target pruning wound protection and thus, improve control of these diseases. This work suggests that overhead sprinkling irrigation is sufficient to initiate spore release of Diatrypaceae spp. in vineyards located in arid grape-growing regions where rain event are limited.