Phytopathologia Mediterranea (2017)
DOI: 10.14601/Phytopathol_Mediterr-21865

ABSTRACTS

Abstracts of oral and poster presentations given at the 10th International Workshop on Grapevine Trunk Diseases, Reims, France, 4–7 July 2017

The 10th International Workshop on Grapevine Trunk diseases was held in Reims, France, on July 4–7 2017. This workshop was co-organized with the COST Action FA1303 entitled "Sustainable control of grapevine trunk diseases" and supported by the International Organization of Vine and Wine (OIV). The meeting was attended by 240 participants from 29 countries and 155 papers were presented either as oral (63) or poster (92) presentations in four sessions: Pathogen characterization, Detection and epidemiology, Microbial ecology, Host-pathogen and fungus-fungus competitive interactions and Disease management. A field tour in the champagne vineyard was co-organized by the Comité Interprofessionnel du Vin de Champagne (CIVC).

Delegates were presented with an overview of the Champagne region focussing on "terroir", varietal creation and grapevine diseases, especially GTDs. The tour concluded with a visit to Mercier cellar with champagne tasting.

The workshop is the 10th organized by the International Council on Grapevine Trunk Diseases (www.icgtd.org) and the 2nd one organised by the members of the COST Action FA1303 (www.managtd.eu). The next 11th IWGTD will be held in British Colombia Canada in 2019.

Pathogen identification and characterization

Characterization and pathogenicity of *Phaeoacremonium* species associated with Petri disease and esca of grapevine in Spain. C. BERLANAS[1], J. PECENKA[2], B. LÓPEZ-M.[1], J. ARMENGOL[3] and D. GRAMAJE[1]. [1]*Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas, Universidad de la Rioja, Gobierno de La Rioja, Ctra. LO-20 Salida 13, 26071 Logroño, Spain.* [2]*Mendel University in Brno, Faculty of Horticulture, Mendeleum - Institute of Genetics CZ-69144 Lednice, Valtická 334, Czech Republic.* [3]*Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain. E-mail: david.gramaje@icvv.es*

Phaeoacremonium species have been associated with disease symptoms of a number of woody hosts worldwide, especially with grapevine trunk diseases such as Petri disease and esca. Over the last 20 years, 29 species of this genus have been isolated from affected grapevines. However, the role of some species as causal agents of grapevine dieback as well as canker-causing agents is stills unknown. In this study, we surveyed vineyards of *Vitis vinifera* 'Tempranillo' in La Rioja and Navarra regions in Spain (2015–2016). Wood samples from grapevines showing general decline, dieback and "tiger-stripe" foliar symptoms yielded 40 fungal isolates identified as *Phaeoacremonium* spp. These isolates were characterized based on the effect of temperature on mycelial growth, comparisons of DNA sequence data of β- tubulin and actin and on the basis of their MSP-PCR profiles. Additionally, 32 isolates belonging to ten *Phaeoacremonium* species collected from 2003 to 2015 in different regions of Spain were retrieved from the

www.fupress.com/pm
Firenze University Press
ISSN (print): 0031-9465
ISSN (online): 1593-2095

culture collection of the IAM-UPV (Spain) and included in this study. Phenotypic and molecular studies identified five *Phaeoacremonium* species associated with grapevine decline: *P. fraxynopennsylvanicum*, *P. krajdenii*, *P. minimum*, *P. parasiticum* and *P. sicilianum*. *P. minimum* was the most frequently isolated species (65% of the isolates) followed by *P. sicilianum* (25%). A total of 11 isolates representing all *Phaeoacremonium* species were used in three different pathogenicity studies. All species caused black discolouration in the xylem vessels of green shoots and potted grapevine rootstock five months after inoculations, being *P. fraxynopennsylvanicum* and *P. minimum* the most virulent species. Seedlings rated 2 months after inoculation showed that *Phaeoacremonium* spp. caused low vascular discoloration and symptom expression. *P. fraxynopennsylvanicum* and *P. scolyti* were the most virulent species, with 31.25% of the inoculated plants showing symptoms of chlorotic leaves, severe defoliation and wilting.

An improved medium for estimating black-foot disease pathogens populations in naturally infested soils and their relation to production systems and soil properties. C. BERLANAS, B. LÓPEZ-MANZANARES and D. GRAMAJE. *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, 26071 Logroño, Spain E-mail: david.gramaje@icvv.es*

Early, specific, and accurate detection of black-foot pathogens in soil is essential to prevent the infection of grapevine planting material by these pathogens in field nurseries and also during the first years after planting. In this study, we comparatively assessed the accuracy and efficiency of three modified culture media protocols published in the literature for the detection of viable inoculum of soilborne pathogens phylogenetically related to black-foot genera based on plating dry soil samples: a modification of Rose Bengal Agar (MRBA), Glucose-Faba Bean Rose Bengal Agar (GFBRBA) and Glucose-Faba Bean Agar (GFBA). Firstly, we compared their efficiency for growing selected black-foot pathogens and the recovery of *Dactylonectria torresensis* from sterile and non-sterile soil. GFBRBA was selected for further studies based on the ability of most of the black-foot species to produce a brown or yellow pigment on the agar and the good performance obtained in the recovery of *D. torresensis* from both sterile and non-sterile soil samples. Secondly, we assessed the usefulness of the GFBRBA medium for estimating black-foot disease pathogens populations in eight naturally infested soils (nursery field, nursery field in rotation, young and mature vineyards). All fields were positive for the presence of black-foot pathogens. *D. torresensis* was the most frequently isolated species from all soils tested. In general, established vineyards and nursery fields had higher inoculum density of *D. torresensis* than nursery fields in rotation. Other black-foot species isolated from soil were *D. alcacerensis* (young vineyard and field nursery) and *Ilyonectria liriodendri* (field nursery). We also examined how shifts in the abundance and composition of black-foot pathogens correspond to changes in specific soil properties. Our results showed a positive relationship between calcium carbonate and the CFUs level of black-foot pathogens in soil. Further research is needed to elucidate the interactions of primary and secondary macronutrients, micronutrients and other physicochemical properties, with disease in enhancing or minimizing black-foot disease incidence in grapevine.

Are stone fruit trees alternative hosts of grapevine trunk diseases in Germany? S. BIEN, U. BRAUN[2] and U. DAMM[1]**.** *Senckenberg Museum of Natural History Görlitz, PF 300 154, 02806 Görlitz, Germany.* [2]*Luther-Universität Halle-Wittenberg, Neuwerk 21, 06108 Halle (Saale), Germany***.** *E-mail: steffen.bien@senckenberg.de*

Germany belongs to the important producers of sweet cherry, sour cherry and plum (*Prunus* spp.) worldwide. Surveys on fungi associated with wood necroses, dieback and decline symptoms of stone fruit trees and grapevine in South Africa report similar pathogen communities. As part of the German Barcoding of Life project, the fungal diversity associated with wood necroses of commercially grown *Prunus* trees from the most important fruit producer regions in Germany was investigated. First results that are based on morphological and ITS sequence data of more than 600 isolates comprise at least 60 genera of Ascomycota, 12 genera of Basidiomycota and two genera of Zygomycota. The dominating ascomycete genera include *Aposphaeria*, *Collophora*

and *Calosphaeria*. So far, our study revealed several grapevine trunk disease pathogens on stone fruit trees in Germany, for example *Cytospora* spp., *Diaporthe* spp., *Eutypa lata* and *Phaeoacremonium* spp., while Phaeomoniellaceae is lacking. Based on results from South Africa, a wider occurrence of members of Botryosphaeriaceae was expected, however only *Diplodia seriata* has been found.

Development of molecular tools for the detection and quantification of Eutypa and Botryosphaeria dieback pathogen inoculum in Australian vineyards. R. BILLONES-BAAIJENS[1], J.R. URBEZ-TORRES[2], M. AYRES[3], M.R. SOSNOWSKI[3,4] and S. SAVOCCHIA[1]. [1]*National Wine and Grape Industry Centre, School of Agricultural and Wine Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga NSW 2678, Australia. Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Box 5000. Summerland, BC V0H 1Z0, Canada.* [3]*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001.* [4]*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 caused by several Diatrypaceae and Botryosphaeriaceae species, respectively and are considered important grapevine trunk diseases worldwide. Their spores (ascospores and/or conidia) are primarily dispersed by rain splash and wind and infect susceptible pruning wounds leading to cankers, dieback and eventually death of vines. The objective of this study was to develop molecular tools to detect and quantify Diatrypaceae and Botryosphaeriaceae spores from the environment. These tools are essential for investigating spore dispersal patterns, thus, high risk infection periods of ED and BD pathogens in Australian vineyards. Four DNA extraction protocols were evaluated and one was found suitable for extracting DNA from artificially-inoculated tapes and Burkard spore trap tapes collected from the vineyards. Two quantitative PCR (qPCR) protocols using two sets of multi-species primers were further developed to detect and quantify Diatrypaceae and Botryosphaeriaceae spores from a single environmental sample. Specificity tests showed that the two multi-species primers were able to amplify the DNA of their corresponding target Diatrypaceae and Botryosphaeriaceae species (nine each) while none of the 20 non-target species were amplified. The two qPCR methods were suitable for amplifying purified DNA, synthetic DNA fragments (gBlocks®) and mixed DNA from spore trap tapes. The Diatrypaceae primers that amplify a fragment of the rRNA gene had a limit of detection (LOD) of ~20 fg of purified DNA which is equivalent to less than one spore. The LOD for Botryosphaeriaceae primers, that amplify a fragment of the β-tubulin gene, was ~300 fg of purified DNA which is equivalent to seven spores. The qPCR methods developed in this study were shown to be rapid and sensitive in detecting ED and BD pathogens from environmental samples and are currently being used to analyse spore trap samples from different viticulture regions in Australia.

Wood-rotting basidiomycetes associated with grapevine trunk diseases in North America. A. BROWN[1], D.P. LAWRENCE [1] and K. BAUMGARTNER[2] .[1]*Department of Plant Pathology, University of California, Davis, CA 95616, USA.* [2]*United States Department of Agriculture-Agricultural Research Service, Davis, CA USA, 95616. E-mail: abibrown@ucdavis.edu*

The grapevine trunk disease Esca impacts vineyards in all major grape-growing regions of the world. The commonly associated causal pathogens in North America are the ascomycetes *Phaeomoniella chlamydospora* and *Phaeoacremonium* species. Basidiomycetes thought to exacerbate the debilitating effects of Esca are reported from Australia, Europe, New Zealand, South Africa, and South America as species of *Auricularia*, *Fomitiporia*, *Inonotus*, *Phellinus*, *Stereum*, and *Trametes*. With only two reports of basidiomycetes causing white rot of Esca-symptomatic vines in North America, more work is needed. The first report identified *Phellinus igniarius* (Chiarappa, 1959) based on morphological characters. Pathogenicity was confirmed, but the identity of this isolate remains tentative, as *Phellinus igniarius* is now thought to not occur in North America. The second report identified *Fomitiporia polymorpha* (Binder and Fischer 2004) from a single sample in California. A recent survey of 16 vineyards with white-rot symptoms in Texas and California from 2014-2017 revealed the presence of three genera: *Coprinellus*, *Fomitiporia*, and *Tropicoporus*. These species, identified through genet-

ic barcoding, have not been reported on grapevines, demonstrating new host-pathogen relationships. A majority of wood samples yielding these basidiomycetes in culture (150 of 164) were collected from Esca-symptomatic vines. This high frequency of basidiomycetes in North American vineyards suggests that such fungi may be as commonly associated with Esca as in European vineyards. Further studies should investigate novel and improved management options for these newly-reported pathogens.

A novel species of *Thelonectria* associated with young grapevines and rootstocks in Italy. M.L. RAIMONDO, F. LOPS, F. CIBELLI and A. CARLUCCI. *Department of the Science of Agriculture, Food and Environment, University of Foggia, Via Napoli 25, Foggia 71122, Italy. E-mail: antonia.carlucci@unifg.it*

Young grapevine plants with decline and wood necrosis symptoms were collected from vineyards and nurseries in the Apulia and Molise regions, Italy, from 2013 to 2015. Isolations of fungi were performed from 45 diseased grapevine plants, and the cultures were identified using morphological and molecular methods. Several species commonly associated with Petri disease, Botryosphaeria dieback, and black foot disease were isolated. Thirty-four strains belonging to *Thelonectria* genus were isolated from grapevine roots and rootstocks along with other fungi associated with black foot disease, namely 83 isolates of *Dactylonectria torresensis* and 65 isolates of *Ilyonectria liriodendri*. A new *Thelonectria* species is described here as *T. blackeriella* based on morphological characters and multigenic analysis using sequence data for five loci (large subunit RNA, internal transcribed spacers, β-tubulin, actin, RNA polymerase II subunit 1). Pathogenicity tests, carried out with representative strains of each of the three, showed that these species can cause black streaking in the wood of 1-year-old grapevine rootstock shoots. The identification of *T. blackeriella, D. torresensis* and *I. liriodendri* from young grapevine plants and rooted rootstock highlights the importance of black foot disease in Italy, which has previously been overlooked.

Botryosphaeria strains diversity in vine stocks. M. COARER. *Institut Français de la Vigne et du Vin, Château de la Frémoire, 44120 Vertou, France. E-mail*: *morvan.coarer@vignevin.com*

An UP-PCR method for infraspecific characterization of *Neofusicoccum luteum* has been described by Billones-Bajens *et al.* in 2011. We tested this method on other Botryosphaeriaceae species, mainly *N. parvum* and *Diplodia seriata*, and applied other RAPD primers (OPERON D03, B15, A2) that have been previously used for *Botrytis cinerea* strains delineation. These methods have been tested on more than 300 strains of Botryosphaeriaceae stored in IFV's "Vine and Wine Microorganisms" BRC. If L15 and B15 primers allowed a good discrimination of *D. seriata* strains, both primers were irrelevant to characterize *N. parvum* strains at a subspecies level. To achieve this aim, it has been necessary to use OPERON A2 primer. After application of this method to differentiate fungal strains from two AOP Muscadet plots, these results have been obtained: several different strains may be present in a single trunk (average number of strains = 2.95/stock); wood and bark didn't share common strains; for the same vine stock, every shoot have its own strains (average number of strains = 1.92/shoot) and in the same row, different stocks didn't share common strains.

Real-time PCR assay for detection and quantification of *Cadophora luteo-olivacea* during the nursery propagation process. R. COBOS[1], S. GONZÁLEZ-GARCÍA[2], J.M. ÁLVAREZ-PÉREZ[2], M. ÁNGEL OLEGO[2], A. IBÁÑEZ[2], A. DIEZ-GALÁN[1], J. ENRIQUE GARZÓN-JIMENO[2] and J.J. RUBIO-COQUE[2]. [1] *RGA bioinvestigación S.L. Instituto de Recursos Naturales. Av. Portugal, 42, 24071 León, Spain.* [2]*Instituto de Investigación de la Viña y el Vino, University of León, Av. Portugal, 42, 24071 León, Spain. E-mail: rebeca.cobos@unileon.es*

During decades *Cadophora luteo-olivacea* had been considered as a minor pathogen associated with Petri disease of grapevine. It has been isolated from both vines displaying Petri or Esca disease symptoms and from asymptomatic vines. In the last years, *C. luteo-olivacea* has frequently been isolated from a range of nursery grapevines and soil samples. *C. luteo-olivacea* is a relatively slow wood colonizer, so the early detection of the pathogen may prevent the spread of the diseased material. The aim of this study was to design a species specific real-time PCR assay to quantify *C. luteo-olivacea* in wood, soil and water samples. New primers were designed and tested

against DNA from *C. luteo-olivacea* and other pathogens associated with trunk disease. No amplification was observed with DNA from isolates other than *C. luteo-olivacea* indicating the assay is species specific. The assay detection limit was 0.25 pg of DNA with a reaction efficiency of 90%. The real-time PCR described was used to detect *C. luteo-olivacea* during the propagation process in a Spanish nursery and the results suggest that soil is the most important inoculum source. The system described could be used to detect *C. luteo-olivacea* in nurseries to determine the health status of the plant material and for detection in soil.

Botryosphaeriaceae species associated with grapevine in France: distribution, phylogeny and virulence. G. COMONT[1], V. MAYET[1], A. NIVAULT[1], C. COPPIN[1], A. BELLÉE and M.-F. CORIO-COSTET[1]. [1]*INRA, UMR Santé et Agroécologie du Vignoble (1065), ISVV, Labex Cote, CS 20032, 33882 Villenave d'Ornon, France. E-mail: Gwenaelle.comont@inra.fr*

Many fungi in the Botryosphaeriaceae are well-known pathogens causing dieback on various hosts worldwide, including grapevine. More than 600 Botryosphaeriaceae isolates were collected from both asymptomatic and symptomatic vines in French vineyards. Isolates' identification was based on phenotypic characters and phylogenetic analyses of the internal transcribed spacers region (ITS), and partial translation elongation factor (EF1-alpha), large subunit ribosomal RNA (28S), and β-tubulin gene regions. Seven species were identified, including *Botryosphaeria dothidea, Diplodia mutila, Diplodia intermedia, Diplodia seriata, Dothiorella viticola, Neofusicoccum parvum, and Lasiodiplodia viticola.* Three species, *D. intermedia, Do. viticola, L. viticola* and were described for the first time in the French vineyards (Comont *et al.*, 2016). *Diplodia seriata* was the most abundant species representing 80% of all isolates and was isolated inform the bark, and both healthy and necrotic wood. The number of species isolated in symptomatic plants was higher than in asymptomatic plants. Multi-locus phylogenetic analyses showed genetic diversity within *D. mutila, D. seriata* and *N. parvum* isolates. Fourteen haplotypes were identified for the 7 species. Haplotypes' pathogenicity was tested on rooted cuttings and the length of cankers and necrosis was measured after 3 months after inoculation showing a large variability depending on species. *L. viticola* and *N.* parvum showed the largest canker and necrosis lengths, while *D. mutila, D. seriata* and *D. intermedia,* showed the shortest. At 28°C on Petri dishes, *L. viticola and N. parvum* isolates exhibited the highest growth rate, followed by *D. mutila, D. seriata* and *B. dothidea* isolates. *Do. viticola* and *D. intermedia* had the slowest growth rate. *Lasiodiplodia viticola* and *N parvum* were the most virulent species and showed high capability to grow at 28°C, while *Do. viticola* isolates were the least virulent.

Identification of *Neofusicoccum parvum* extracellular proteins captured by As(III), a potent GTDs fungicide. M.-L. GODDARD[1,2], F. LEDIG[1], A. GOLFIER[1], M. GELLON[2], S. FARINE[2], C. BERTSCH[2] and C. TARNUS[1]. [1]*Laboratoire de Chimie Organique et Bioorganique, Université de Haute-Alsace, 3bis rue Alfred Werner, 68093 Mulhouse Cedex, France.* [2]*Laboratoire Vigne, Biotechnologies et Environnement, Université de Haute-Alsace, 33 rue de Herrlisheim, 68008 Colmar Cedex, France. E-mail: mary-lorene.goddard@uha.fr*

Sodium arsenite has been used during decades to treat grapevine against various pathogens and has particularly proved its efficacy towards GTDs fungi. This fungicide was prohibited in the 2000s, without any substitute for winegrowers. In the program CASDAR V1301 (2013-2016), we investigated the mode of action of sodium arsenite on vineyards. Simultaneously with this large collaborative study, we have undertaken, at the Haute-Alsace University, the identification of fungal proteins which production is modulated by arsenite (As(III)) as well as those which are directly targeted through a specific binding. We firstly focused on the identification of *Neofusicoccum parvum* extracellular proteins that interact with As(III). We have synthesized and developed an arsenic-based chromatography and arsenic-based fluorescent probe. The fluorescent probe can be directly used on electrophoresis gel in order to highlight trapped fungal proteins identified by mass spectrometry analysis and MASCOT database searches. Targets already identified by this way will be presented.

Characterisation of Diatrypaceae species from grapevine in South Africa. P. MOYO[1], L. MOSTERT[1]

and F. HALLEEN[1,2]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa.* [2]*Plant Protection Division, ARC Infruitec-Nietvoorbji, Private Bag X5026, Stellenbosch, 7599, South Africa. E-mail: halleenf@arc.agric.za*

For many years, Diatrypaceae species were considered to be saprophytic although several are serious pathogens on economically important crops. On grapevines, *Eutypa lata* is the most commonly known Diatrypaceae pathogen causing Eutypa dieback. Recent studies on grapevines in Californian and Australian vineyards have, however, revealed an extensive diversity of species of Diatrypaceae, of which several have been found to be pathogenic on grapevines. These findings have raised questions as to whether the newly discovered Diatrypaceae species are a threat to the sustainability of the grapevine industry and what the role of these species is in the development of Eutypa dieback. Little information is available concerning the diversity of Diatrypaceae species associated with declining grapevines in South Africa. The aim of this study was therefore, to characterise Diatrypaceae species occurring in diseased grapevines in South Africa. Isolations were carried out from wedge-shaped cankers typical of Eutypa dieback, dying spurs on grapevines as well as from fruiting bodies (perithecia). Diatrypaceae species were characterised based on their morphology and were compared in phylogenetic analyses, based on ITS and ß-tubulin gene regions, to DNA sequence data available. Seven species namely *Cryptovalsa ampelina*, *Cryptovalsa rabenhorstii*, *Eutypa consobrina*, *Eutypa lata*, *Eutypella citricola*, *Eutypella microtheca* and *Eutypa cremea* sp. nov. were identified. *Eutypa lata* and *Cryptovalsa ampelina* were, however, found to be the dominant species on samples with wedge-shaped necrosis and dying spurs, respectively. All seven species were virulent when inoculated on grapevine tissues. All species, except *C. ampelina* and *E. lata*, are first reports on grapevine in South Africa. Evidence emerging from this study suggests that several species of Diatrypaceae may be involved in the development of dieback and symptoms originally thought to be caused by *Eutypa lata* in South Africa.

***Diaporthe nebulae* sp. nov. and other *Diaporthe* species associated with Phomopsis dieback in South African grapevines.** P. LESUTHU[1], P. MOYO[2], L. MOSTERT[2], C. SPIES[2] and F. HALLEEN[1,2]. [1]*Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599.* [2]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602. E-mail: halleenf@arc.agric.za*

Phomopsis cane and leaf spot and Phomopsis dieback are important grapevine diseases caused by *Diaporthe ampelina* (*Phomopsis viticola*) and other *Diaporthe* species. A previous study identified fifteen *Diaporthe* species in South African vineyards of which *D. ampelina* and *D. amygdali* caused the most severe lesions on green grapevine shoots. The study was conducted more than 10 years ago and ever since then several new species have been identified on grapevines worldwide and the prominence of *D. ampelina* as trunk disease pathogen associated with Phomopsis dieback has been established. The aim of this study was to identify *Diaporthe* species associated with Phomopsis dieback in South African grapevines. Isolations were made from dormant rootstock propagation material, dormant grafted nursery vines and dying spurs of field-grown vines. Cultures identified as *Diaporthe* based on cultural and morphological features were identified to species level by sequencing the internal transcribed spacer regions (ITS1, 5.8S rRNA gene, and ITS2) and for a representative sub-sample of isolates, the partial β-tubulin region. Phylogenetic analysis of the combined ITS and β-tubulin data revealed a total of nine *Diaporthe* species associated with grapevines in South Africa, three of which are reported on this host in South Africa for the first time, namely *Diaporthe serafiniae, D. novem* and *D. cynaroidis*. A new species, described as *Diaporthe nebulae*, was also revealed which is closely related to *D. anacardii*. Pathogenicity studies conducted on dormant detached grapevine shoots indicated that, of the species tested, *D. ampelina*, *D. novem* and *Diaporthe nebulae* were the most pathogenic. The fact that these species were present in propagation material as well as dying spurs of established vines suggests that a comprehensive management strategy will need to be developed in future to address these infections.

Sporadic occurrence of the grapevine trunk disease pathogen *Diplodia mutila* in the Tokaj Wine Region, Hungary. C. KOVÁCS[1, 2], P. BALLING[3], Z. BIHARI[3], F. FONTAINE[4] and E. SÁNDOR[1]. [1]*University*

of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Food Science, Böszörményi út 138., H-4032 Debrecen, Hungary. [2]National Agricultural Research and Innovation Centre, Fruitculture Research Institute (NARIC-FRI), Újfehértó, Vadastag 2., H-4244 Újfehértó, Hungary. [3]Research Institute for Viticulture and Oenology, Könyves Kálmán u. 54., H-3915 Tarcal, Hungary, [4]Université de Reims Champagne-Ardenne, Unité de Recherche de Vigne et Vin de Champagne EA4707, Laboratoire Stress, Défenses et Reproduction des Plantes, Bât. 18, BP 13039, 51687 Reims cedex 2, France. E-mail: k.csilla20@gmail.com

Black dead arm (BD) was first described by Lehoczky (1974) in mature Hungarian vineyards. The disease was characterised with sporadic occurrence in several grapevine-growing districts, including the Tokaj Wine Regions. The causal agent was identified as *Diplodia mutila*, but its pathogenic status was uncertain. A survey was carried out to confirm the occurrence of *D. mutila* in five vineyards in the Tokaj Wine Region located in north-eastern Hungary between 2013 and 2015. The plantations had different ages (between 10- and 21-year-old in 2013) and cultivars ("Hárslevelű", "Furmint" and "Zéta"). BD was detected only one year (2013) in the Tokaj Wine Region with less than 1% occurrence in the monitored vineyards. Dead parts of the plants with BD symptoms were removed and used for laboratory analysis to identify the pathogens. Fungi were isolated from woody parts of the seven sampled grapevines expressing BD symptoms. Based on the morphological characters, *D. mutila* was identified from all grapevines in 2013. To confirm its identity, DNA was extracted from the pure colonies, and rDNA region containing ITS1 and ITS2 were amplified from all *D. mutila* samples. The sequences of the amplified rDNA region were deposited in GenBank (Accession numbers: KU377231, -32, 377242, 377245, 377250, 377263, KU377212). Interestingly, the expression of BD was not detected visually following the removal of the dead plant parts and *D. mutila* was not isolated from the cordon of the seven plants with BD symptoms in 2013.

Phylogenetic characterization of grapevine trunk pathogens isolated from vineyards in southern Brazil. J. TASCHETO BERLATTO[1], M.A. KURTZ ALMANÇA[2], B. GABRIELE LOESER[1], F. VIEIRA TORMENTE[1] and F. ROSSI CAVALCANTI[1]. *[1]Embrapa Grape and Wine, Plant Pathology Laboratory – II, Livramento 515, Juventude da Enologia, Bento Gonçalves-RS, CEP 95700-252, Brazil. [2]Rio Grande do Sul Federal Institute of Education, Science and Technology, Bento Gonçalves-RS, CEP 95700-206. E-mail: marcus.almanca@bento.ifrs.edu.br*

This study deals with the characterization of new strains of causal agents of grapevine trunk diseases (GTD) isolated from diseased vines found in southern Brazil's vineyards surveyed between 2013 and 2016. In all, 30 isolates of fungal trunk pathogens (*Phaeomoniella chlamydospora*, *Phaeoacremonium* sp., *Fusarium* sp., *Ilyionectria macrodidyma*, *Neofusicoccum parvum* and *Botryosphaeria dothidea*) were morphologically characterized and compared through the DNA sequence data of the nuclear ribosomal DNA-internal transcribed spacer (ITS1-2) region. The sequence alignments were assayed for most parsimonious trees obtained from the ITS sequence data and 450 replications bootstrap, and they were compared to each other and with Genbank ITS1-2 sequences from GTD's pathogens from other countries. Along the pathogenicity tests with some reference isolates to confirm Koch postulates, PCR-RFLP (CAPS) assays with CfoI and HaeIII were performed in order to characterize a restriction band patterns that may be used to support the quick diagnosis of those pathogens.

TrunkDiseaseID.org: A molecular database for trunk pathogen diagnostics. D.P. LAWRENCE[1], R. TRAVADON[1], M. NITA[2] and K. BAUMGARTNER[3]. *[1]Department of Plant Pathology, University of California, Davis, CA 95616. [2]Virginia Polytechnic Institute and State University, AHS Jr. Agricultural Research and Extension Center, Winchester, VA 22602. [3]United States Department of Agriculture - Agricultural Research Service, Davis, CA 95616. E-mail: dlawrence@ucdavis.edu*

The grapevine trunk-disease complex limits vineyard productivity and longevity around the globe. Trunk diseases have traditionally been distinguished based on causal agents and etiologies (e.g., Botryosphaeria-, Eutypa-, and Phomopsis diebacks, and Esca). However, mixed infections are frequent in vineyards and they confound accurate diagnosis. Diverse fungal assemblages of trunk pathogens and

other wood-colonizing fungi span four classes in the *Pezizomycotina* (Ascomycota) and 10 genera in the *Hymenochaetales* (Basidiomycota). Species identification based on morphology is largely untenable because of overlap in colony characteristics or spore dimensions, or lack of sporulation in culture. When based on DNA sequencing, searches of uncurated, public molecular databases can lead to misidentifications. We introduce the new molecular database TrunkDiseaseID.org, populated with accurate rDNA ITS sequences from over 250 isolates (pathogens and saprobes). Secondary molecular barcodes (e.g., TEF1-alpha and beta-tubulin) are also included for delineating closely related species because the ITS barcode is inadequate for some members of the Botryosphaeriaceae, *Diatrypaceae*, and *Hypocreales*. Currently, no such comprehensive, curated database exists. In addition to ITS and secondary barcode sequences, this database provides a scientific reference, host, origin, and ecological status for each isolate. Accurate species-level identification and ecological categorization will help practitioners (growers, cooperative extension farm advisors, pest-control advisers) to make informed management decisions.

Identification of fungal pathogens associated with grapevine trunk diseases in Hungary S. LENGYEL, Z. KARÁCSONY, Á. JUHÁSZ and K.Z. VÁCZY. *Eszterházy Károly University, Food and Wine Research Institute, 3300 Eger, Eszterházy tér 1. Hungary. E-mail: lengyel.szabina@uni-eszterhazy.hu*

Grapevine trunk diseases (GTDs) are widespread in vineyards causing serious damages and large economic losses in wine producing countries. However, in Hungary, there is no comprehensive study about the causal agents of these diseases, such as Botryosphaeria dieback, Esca, and Eutypa dieback. The objective of this study was to isolate and identify fungal species from symptomatic grapevines, in order to learn more about these pathogens associated with GTDs in Hungary. Samples of grapevine trunks exhibiting the symptoms of GTDs, such as longitudinal lesions, cankers and diebacks, were collected from Eger, Neszmély, Pécs, and Szekszárd Hungarian wine regions in 2015. More than 100 symptomatic trunk samples were included in this work and approximately 700 fungal strains were isolated from them. The isolates were identified by comparing their ITS (internal transcribed spacer), EF (transcription elongation factor) and BT (β-tubulin) sequences with those retrieved from GenBank. Among the isolated strains, *Eutypa lata*, *Fomitiporia mediterranea*, *Phaeoacremonium minimum*, *Phaeomoniella chlamydospora*, as well as some species belonging to *Diaporthe* and the Botryosphaeriaceae were identified as well-known GTDs-related fungal pathogens. Pathogenicity tests were performed by inoculating young potted grapevines with fifteen representative isolates. The tested fungal strains were re-isolated from the infected grapevines and identified by multiple sequence analysis, as described above. Further studies will be conducted to monitor Hungarian vineyards and reveal the population structure of GTDs associated fungal pathogens.

Characterization of new mycoviruses identified by NGS from *Botryospheriaceae* species involved in grapevine trunk diseases. A. MARAIS[1], A. NIVAULT[2], C. FAURE[1], S. THEIL[1], G. COMONT[2], T. CANDRESSE[1] and M.-F. CORIO-COSTET[2]. *[1]UMR 1332 Biologie du Fruit et Pathologie, INRA, Univ. Bordeaux, CS20032, 33882 Villenave d'Ornon Cedex, France. [2]UMR 1065 Santé et Agroécologie du Vignoble, CS20032, 33882 Villenave d'Ornon Cedex, France INRA, Bordeaux Sciences Agro, CS20032, 33882 Villenave d'Ornon Cedex, France. E-mail: armelle.marais-colombel@inra.fr*

Botryosphaeria dieback is known to be one of the main diseases associated with grapevine trunk decay. At least, 21 fungal species in the family *Botryospheriaceae* have been described to be involved in the disease. In order to better understand the life-history traits of these phytopathogenic fungi, especially their virulence, the presence of mycoviruses was investigated. Double stranded (ds) RNAs were purified from five fungal species (*Neofusicoccum luteum*, *N. parvum*, *Lasiodiplodia viticola*, *Diplodia mutila*, and *D. seriata*) collected in vineyards. Extracted dsRNAs were then submitted to a random, whole genome amplification and analyzed by Next Generation Sequencing (NGS). After trimming and cleaning steps, the reads were assembled into contigs and annotated by Blastn and Blastx comparisons with GenBank using a 10^{-3} e-value cut-off. Interestingly, all mycoviruses detected in the analyzed samples are novel and had not been described before. The *N. parvum* isolate analyzed as well as one of the two *D.*

mutila isolates were found to be free of RNA viruses. In contrast, the two isolates of *N. luteum* were found to be infected by several new viruses belonging to the genera *Mitovirus* and *Totivirus*, as well as unclassified viruses (single stranded positive- or negative-strand RNA viruses, and dsRNA viruses). A new partitivirus species was also characterized from *L. viticola*, *D. mutila* and *D. seriata* isolates, in addition to a new endornavirus in *D. mutila*. Completion of the genomic sequence of some of these new viruses is currently underway, as well as the determination of their distribution among a collection of *Botryosphaeriaceae*. Further investigations are being implemented to evaluate their role in terms of virulence of their fungal hosts.

Quantitative assessment of grapevine wood colonization by fungal pathogens for association genetics studies. C. MOISY[1], G. BERGER[2], T. FLUTRE[2], L. BIDEL[2], J.-P. PEROS[2] and L. LE CUNFF[1]. *[1]Institut Français de la Vigne et du Vin, UMT Géno-Vigne, F-34060 Montpellier, France. [2]INRA, UMR AGAP, F-34060 Montpellier, France. E-mail: cedric.moisy@supagro.inra.fr*

Grapevine trunk diseases (GTD) are severe diseases affecting grapevines worldwide. They dramatically shorten the longevity of vineyards and endanger their sustainability because the causal pathogens attack the long-lasting organs, inducing the death of vines on the shorter or longer term. Foliar symptoms, induced by one or more toxic metabolites produced by fungi are frequently used to detect and monitor infected plants, but they can vary drastically from one year to another. They are also difficult to reproduce under controlled conditions. One possibility to assess fungal development in inoculated cuttings without measuring external symptoms is by the use of PCR methods. Our first objective was therefore to develop a method based on qRT-PCR for phenotyping resistance of grapevines to GTD, reflecting the real fungal colonization in the wood of the infected plant. This method should ideally be accurate, fungus-specific, and repeatable on a large number of individuals. By comparing high-aggressive and low-aggressive isolates, we aimed at establishing if there was a link between aggressiveness, foliar symptom expression, and wood colonization, in order to define the proper strategy for phenotyping GTD susceptibility. Using this approach on both a self-fertilized Savagnin cultivar population and a diversity panel of cultivated grapevines, we have highlighted differences among cultivars for their susceptibility to fungal colonization. Our second objective is now to evaluate the genetic part of tolerance to fungal colonization and to identify, by association genetics, the regions of the grapevine genome that might be responsible for variation of this susceptibility among the genotypes tested. Finally, we have monitored the development of pathogens in the wood using non-destructive imaging approaches, opening new perspectives for detection, study and monitoring of GTD. Altogether, these results could possibly lead to better knowledge of plant-pathogen interactions, to the development of new tools for monitoring GTD, and new genetic markers to improve and speed-up breeding and selection.

Development of a method to biopsy grapevines in New Zealand vineyards for microbial content. D. C. MUNDY and B. R. VANGA. *The New Zealand Institute for Plant & Food Research Limited, Marlborough Wine Research Centre, PO Box 845, Blenheim 7240, New Zealand.E-mail: dion.mundy@plantandfood.co.nz*

New Zealand Winegrowers are funding a research programme investigating the impacts of different vineyard management practices on multiple measures of biodiversity in the vineyard. One of the objectives is to measure microbial communities within individual vines over time. This has led to the development of a nondestructive method for collecting wood samples by biopsy for use in Next Generation Sequencing (NGS). Samples were collected from Sauvignon blanc and Pinot noir vines using a sterilized 4-mm drill bit after the bark was removed with a knife. The hole in the vine was sealed using linseed wood putty to prevent infection of the trunk following the procedure. The tissue from the drill cutting was placed in 4-mL cryogenic tubes and snap frozen in liquid nitrogen. The sample tubes were returned to the laboratory in liquid nitrogen and stored at -80°C. The tissue was homogenized using an eight-well bead beater (Mini-BeadBeater 8, BioSpec Products, Inc., Bartlesville, USA). DNA was isolated using a high-throughput DNA extraction procedure using a modified cetyltrimethylammonium bromide (CTAB)/β-mercaptoethanol meth-

od. DNA was quantified using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Polymerase chain reaction (PCR) was performed to amplify the microbial DNA using internal transcribed spacer (ITS) primers to identify the presence of fungal species, and bacterial 16S primers to identify the bacterial species. The purified PCR products were sent to New Zealand Genomics Limited (NZGL), Auckland for NGS. Bioinformatics and data processing allowed the assignment of Operational Taxonomic Units. Over time, these snapshot samples of microbial communities will be used to investigate how management of vines influences both pathogen and non-pathogen DNA within the vine. This method also provides the opportunity to study possible biocontrol agents and to investigate microorganisms which may be indicators of good vine health.

Grapevine trunk pathogens detected in symptomatic young vineyards from the Castilla-La Mancha region of Spain. R.M. MUÑOZ[1], V.M. TOLOSA[1], M.L. LERMA[1], P. CASTILLO[1] and J. ARMENGOL[2]. [1]*Servicio de Diagnóstico y Asistencia Fitosanitaria (SEDAF), Instituto Técnico Agronómico Provincial de Albacete (ITAP), Parque Empresarial Campollano, 2ª Avenida, 61, 02007 Albacete, Spain.* [2]*Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain. E-mail: rmg.itap@dipualba.es*

Castilla-La Mancha region harbors the largest vine-growing area in the world, with more than 440,000 ha. In this study, 197 samples from symptomatic young vineyards (0–15 years old) located in this region were analyzed in the period 2009–2015. Affected vines showed decline symptoms and reduced growth. The grapevine trunk pathogens detected were *Botryosphaeria dothidea*, *Cylindrocarpon*–like anamorphs, *Diplodia seriata*, *Fomitiporia mediterranea*, *Lasiodiplodia theobromae*, *Neofusicoccum luteum*, *N. parvum*, *Phaeoacremonium minimum*, *Pm. iranianum* and *Phaeomoniella chlamydospora*. *Cylindrocarpon*–like asexual morphs (black-foot disease pathogens) were by far the most frequently isolated fungi, detected in 85% of samples, followed by *Pa. chlamydospora*, *D. seriata* and *Pm. aleophilum*, which were found in 26, 21 and 18% of the samples, respectively. The other fungi detected were present in less than 3% of samples. *Cylindrocarpon*–like anamorphs were isolated more frequently in roots (78%), whereas *Pa. chlamydospora* was more frequently isolated in the lower part of the rootstock (27%) and in the scion (26%). *D. seriata* was detected mainly in the graft union (45%) and in the area just below it (24%), whereas *Pm. aleophilum* was isolated from the lower part of the rootstock (39%) and in the area just below graft union (25%). The molecular identification of 22 *Cylindrocarpon*–like isolates obtained in 2014 and 2015 was performed by sequence homology of a fragment of the histone H3 gene using the H3F/H3R primer pair. The most frequently detected species was *Dactylonectria torresensis* (32% of the isolates), followed by *Ilyonectria liriodendri* (27%) and *D. novozelandica* (23%); the species *D. alcacerensis* and *D. macrodidyma* were the less frequent (9%).

Life-traits history of Botryosphaeriaceae spp. found in French vineyard: aggressiveness, fungicide sensitivity, growth rates and mycovirus. A. NIVAULT[1], G. COMONT[1], A. MARAIS[2], M.-C. DUFOUR[1] and M.F. CORIO-COSTET[1]. [1]*INRA, UMR Santé et Agroécologie du Vignoble (1065), ISVV, Labex Cote, CS, 33882 Villenave d'Ornon, France.* [2]*INRA, UMR Biologie du Fruit et Pathologie, Virologie, 33883 Villenave d'Ornon, France. E-mail: marie-france.corio-costet@inra.fr*

Grapevine Trunk Diseases (GTD) are responsible for vineyards decline, and grapevine yield losses. Amongst the presence of several pathogens, the Botryosphaeriaceae family, comprises many species and are found worldwide upon many different hosts including grapevine, causing Botryosphaeria dieback. Fourteen species (65 strains); *Botryosphaeria dothidea*, *Diplodia intermedia*, *D. mutila*, *D. pinea*, *D. rosulata*, *D. seriata*, *Lasiodiplodia lignicola*, *L. parva*, *L. pseudotheobromae*, *L. viticola*, *Neofusicoccum luteum*, *N. parvum*, *N. ribis* and *Spencermartinsia viticola* belonging to 19 different genotypes, were characterized. *In planta* cankers and necrosis upon Cabernet Sauvignon cuttings under greenhouse conditions and the optimal growth temperature of the isolates (mycelium growth measured at 15°C, 22°C, 25°C, 28°C, 33°C and 36°C) were carried out. *Neofusicoccum parvum* and *Lasiodiplodia* spp. had the highest optimal temperature growth and were the most aggressive with the longest necrosis registered. In addition, the determination of the presence or absence

of mycoviruses, were also studied and different DsRNA were identified. Moreover, the behaviour of the isolates towards nine fungicides with different mode of action (respiration, sterol biosynthesis, succinate dehydrogenase, tubuline inhibtors and multi-site), was tested at different concentrations of fungicides, by measuring the daily mycelium growth. The EC_{50} and the CMI concentrations were determined. Some isolates were found as resistant to some fungicides, suggesting a non-intentional effect of fungicides on these populations. A global analysis will help out to understand whether all these life traits are correlated to the difference of aggressiveness in these species and isolates.

First report of *Lasiodiplodia gilanensis* associated with grapevine in Mexico. C. ORDÓÑEZ-VALENCIA[1], C. VALENZUELA-SOLANO[2] and R. HERNÁNDEZ-MARTÍNEZ[1]. [1]*Departamento de Microbiología, Centro de Investigación y de Educación Superior de Ensenada, Baja California, México.* [2]*Sitio Experimental Costa de Ensenada. INIFAP. Calle del Puerto Núm. 375-23 Fracc. Playa Ensenada. Ensenada, B. C. 22880. E-mail: ruhernan@cicese.mx*

The Botryosphaeriaceae family has a cosmopolitan distribution and a wide range of plant hosts, with several species recognized as important pathogens of grapevines. Members of this family cause cankers and internal infection of the wood, which leads to grapevine decline and dieback. The aim of this study was to characterize Botryosphaeriaceae spp. associated to grapevine dieback. Samples were obtained from grapevines growing at Valle de Guadalupe (Ensenada, Mexico) showing Botryosphaeria dieback symptoms. Pure isolates were grown on potato dextrose agar (PDA) and malt extract agar (MEA) medium. To induce sporulation, isolates were transferred to Vogel´s minimal medium with sterilized toothpicks on the agar surface and incubated under white light in a 1h light-dark regime. The isolates were characterized and subsequently identified. Based upon morphology and EF-1α nucleotide sequences, two isolates: MXCS01 and MX50 were identified as *Lasiodiplodia gilanensis*. Fungal colonies on PDA are smoke-grey to olivaceous-grey with abundant aerial mycelia reaching the lid of the Petri plate. The mycelia on MEA have slower growth and smoke-gray color. Conidia, formed in pycnidia, initially are aseptate, hyaline, ellipsoid to ovoid, with granular content. When pigmented, shape is ellipsoid to ovoid, with a single septation and longitudinal striations. The average size of the conidia was 15.6±1.1 × 7.6±0.52 (mean ± SD) μm. To our knowledge, this is the first report of *L. gilanensis* in grapevine in Mexico.

Characterization of Botryosphaeriaceae isolates in gravepine in Spain. C. PINTOS VARELA, V. REDONDO FERNÁNDEZ, O. AGUÍN CASAL, D. COSTAS IMBERNÓN and P. MANSILLA VÁZQUEZ. *Estación Fitopatolóxica Areeiro. Deputación Pontevedra. Subida á Carballeira s/n, 36153, Pontevedra, Spain. E-mail: vanesa.redondo@depo.es*

Several Botryosphaeriaceae species are known to be pathogens in grapevine worldwide. In a recent review, Larignon (2016) cited a total of 46 species within this family associated with grapevine dieback symptoms. From 2014 to 2017, grapevine plants from nurseries and dieback (declined) plants from different vineyards located in Spain with cankers and/or black vascular necrosis were processed in our laboratory. A total of 145 Botryosphaeriaceae isolates were identified based on morphological and molecular techniques. Molecular characterization was performed by sequencing and phylogenetic analysis of the ITS region combined with other loci (LSU, *tef1-α*, *tub*2 and *rpb*2) in cases when it was necessary. Based on molecular and morphological analysis from 139 isolates, 7 species were identified, including *Botryosphaeria dothidea, Diplodia mutila, D. seriata, L. theobromae, Neofusicoccum australe, N. luteum* and *N. parvum*. Nevertheless the results obtained from the phylogenetic analysis of the studied regions did not allow a conclusive identification of 6 isolates belonging to 3 different species (representative isolates of each species coded EFA 436, EFA 437 and EFA 440 respectively). The combined analysis of the *tef*1-α, *tub*2 and ITS regions grouped EFA 436 in a clade with isolates of *N. algeriense* whilst EFA 437 formed a separate group close to isolates of *N. cryptoaustrale*. Morphological characterization of both isolates disagrees with their original descriptions. The *rpb*2 sequences data analysis of EFA 440 resulted in the inclusion of this isolate in a well-supported clade with *L. mediterranea, L. missouriana* and *L. viticola* suggesting the possible identification of our isolate as *L. mediterranea.* Moreover, morphological results could agree with the described for this spe-

cies although our isolate produced slightly smaller conidia. The results reflect the critical importance of using multiple gene genealogies to identify species and to characterize diversity within the Botryosphaeriaceae.

Gravepine Trunk Diseases detected in nurseries. C. PINTOS VARELA, V. REDONDO FERNÁNDEZ, O. AGUÍN CASAL, D. COSTAS IMBERNÓN and P. MANSILLA VÁZQUEZ. *Estación Fitopatolóxica Areeiro. Deputación Pontevedra. Subida á Carballeira s/n, 36153, Pontevedra, Spain. E-mail: vanesa.redondo@depo.es*

Grapevine trunk diseases (GTD) are a worldwide concern and it is known that plant infection is frequently produced during the nursery process. Between 2015-2017, grapevine plants from 3 different nurseries located two from Spain and one from France were collected: 40 *Vitis vinifera* cv. Albariño one year potted plants from nursery 1, 22 *V. vinifera* cv. Albariño grafted plants without potting from nursery 2 and 88 *V. vinifera* cv. Savagnin half year potted plants from nursery 3. All grafted vines, with plant passport, presented external good conditions when they were bought. In laboratory, each plant was cut in 4 sections: variety, graft-union, rootstock and roots. All the sections were surface sterilized and small pieces of the tissues were cultured under sterile conditions. Species were identified based on morphological features and DNA analysis using ITS, *tub2 and tef1* gene regions. All the three nurseries presented a very high incidence of GTD ranged between 81%-100%, in fact, we could identify up to 5 different GTD species in the same plant. The GTD fungi detected included at least 7 Botryosphaeriaceae species, 6 *Cylindrocarpon*-like species, 8 *Phomopsis* species and a 3 species causing Petri disease. Black foot disease is the main disease found in nurseries 1 and 2 whereas Botryosphaeriaceae species were the GTD fungi more prevalent in nursery 3. In addition, other pathogen fungi not included in that complex were identified, such as *Fusarium, Pestalotiopsis, Truncatella or Rhizoctonia*. Despite that nursery policies are trying to reduce the presence of harmful organisms at the lowest possible level, not only GTD fungi are still present, but our results suggest the current situation is getting worse.

Characterization of *Cylindrocarpon*-like isolates associated with black-foot disease. C. VARELA, V. REDONDO FERNÁNDEZ, O. AGUÍN CASAL, D. COSTAS IMBERNÓN and P. MANSILLA VÁZQUEZ. *Estación Fitopatolóxica Areeiro. Deputación Pontevedra. Subida á Carballeira s/n, 36153, Pontevedra, Spain. E-mail: vanesa.redondo@depo.es*

Black foot disease caused by *Cylindrocarpon*-like anamorphs is considered as one of the most important grapevine trunk diseases (GTD) being responsible for the decay and death of young vine plants worldwide. From 2014 to 2017 decayed grapevine plants from different vineyards and nurseries were analyzed in our laboratory. Symptomatic plants presented drying and dying of shoots, short internodes, abnormal development of roots with parallel growth to the soil surface, necrotic root crowns, brown to black wood of rootstocks, internal necrosis extending from the bark to the pith in diseased parts of the plants and wood necroses. Most *Cylindrocarpon*-like fungi were isolated from the roots and the basal area of the rootstock (83%) although some isolates were taken from the graft union (6%) or even from the variety (5%). In addition, these pathogens, typical of young vines, were also present in some adult vines (10%). Species were identified based on DNA phylogenetic inference supported by morphological characterization. The phylogenies of representative isolates were evaluated based on DNA sequence data of five loci (LSU, ITS, *tef1*-α, *tub*2 and HIS). A total of 120 isolates were identified during these years in ten different species: *Cylindrocladiella parva, Dactylonectria alcacerensis, D. hordeicola* (EFA 442), *D. macrodidyma, D. pauciseptata, D. torresensis, Ilyonectria capensis* (EFA 443), *I. cyclaminicola* (EFA 444), *I. liriodendri and I. robusta.* The incidence of *D. torresensis* was the highest followed by *I. liriodendri* in analyzed plants. Regarding morphological features, both EFA 442 and EFA 443 disagree with their original descriptions. In addition, EFA 444 was lost and molecular characterization could not be done. More studies must be performed in order to confirm the identity of *D. hordeicola, I. capensis and I. cyclaminicola* to report their presence for first time in grapevine.

Study of *Lasidiodiplodia pseudotheobromae*, *Neofusicoccum parvum* and *Schizophyllum commune*,

three pathogenic fungi associated with Grapevine Trunk Diseases in the Northern region of Tunisia. A. REZGUI[1,2], J. VALLANCE[2,3], A. BEN GHNAYA-CHAKROUN[1], E. BRUEZ[2], N. SADFI-ZOUAOUI[1] and P. REY[2,3]. [1]*Laboratoire Microorganismes et Biomolécules Actives, Faculté des Sciences de Tunis, Université de Tunis El Manar, 2092 Tunisia.* [2]*INRA, UMR 1065 Santé et Agroécologie du Vignoble, ISVV, F-33882 Villenave d'Ornon, France.* [3]*Université de Bordeaux, Bordeaux Sciences Agro, UMR 1065 SAVE, F-33175 Gradignan, France. E-mail: foufarezgui07@gmail.com*

Vineyards worldwide are affected by Grapevine Trunk Diseases (GTDs) that lead to vine decline and loss in productivity. Until now, GTDs have been poorly studied in Tunisia regarding disease incidence and the pathogenic fungi involved. To investigate this point, a survey was conducted in 10 vineyards in the north of Tunisia in order to monitor GTDs, in particular Esca, on four cultivars used to produce table grapes, namely "Muscat d'Italie", "Red Globe", "Rich Baba Sam" and "Victoria". Incidence of GTDs ranged from 0.03% to 6%; and only plants from cultivar "Muscat d'Italie" showed the typical Esca-foliar symptoms. Investigation on the fungal communities inhabiting the necrotic wood tissues led to the ITS-DNA sequencing identification of three pathogenic species known to be involved in GTDs: *Lasiodiplodia pseudotheobromae*, *Neofusicoccum parvum* and *Schizophyllum commune*. The isolated strains were studied for their (i) *in vitro* mycelial growth depending on the temperature, (ii) *in vitro* competition abilities and (iii) pathogenicity on "Muscat d'Italie" young vines. This study represents the first approach to characterize GTD fungi in grapevines used to produce table grapes in Tunisian vineyards.

Comparative metagenomic analysis of fungal and bacteria communities associated with development process of grapevine trunk diseases (Piura, Peru) and fungal identification by MALDI TOF/TOF mass spectrometry shotgun proteomic. M. SAUCEDO-BAZALAR[1,2,3], C. SANTOS[3], P. MASÍAS[3], P. DUARTE[4], G. LEÓN[4], V. CEDEÑO[3] and E. MIALHE[3]. [1]*Molecular Biotechnology Master Program, Universidad Nacional de Tumbes, Peru.* [2]*CIENCIACTIVA – CONCYTEC, Peru.* [3]*INCA'BIOTEC S.A.C., Filipinas 212, Tumbes, Peru.* [4]*ECOSAC AGRICOLA S.A.C., Piura, Peru. E-mail: mjsaucedob@gmail.com*

Grapevine Trunk Diseases (GTDs) are serious problems for wine and table grape industries worldwide. GTDs have been associated with a large list of pathogenic fungi considered to be responsible for necrosis in the grapevine vascular system. However, the necrotic processes in woody tissues remain poorly understood, with recent research suggesting the involvement of some bacteria in the pathogenesis whereas other bacteria could prevent the necrosis. Peru is the third largest world exporter of table grapes, being the Region of Piura one of the major grape-production Peruvian areas. GTDs have been increasingly detected in Peruvian vineyards through clinical signs and classical mycological analysis. In the present study, a metagenomic approach has been applied to characterize fungal and bacterial communities inside the caulosphere of both healthy and sick grapevine plants. Bacteria and fungi have been isolated and molecularly identified based on partial sequencing of rDNA. Moreover fungal isolates were characterized and identified through proteomic MALDI TOF/TOF mass spectrometry. The analysis of bacterial communities revealed 512 bacterial OTUs with *Proteus*, *Bacillus*, *Staphylococcus* and *Enterococcus* more represented in healthy plants whereas *Pseudomonas* and *Curtobacterium* were markers in sick plants. Fungal diversity revealed 433 fungal OTUs, being *Aspergillus* and *Cladosporium* present in healthy plants whereas *Peniophora*, *Lasiodiplodia*, *Alternaria* and *Fusarium* were present in sick plants. MALDI TOF/TOF mass spectrometry shotgun proteomic has been successfully used for identification of *Lasiodiplodia theobromae*, *L. brasiliensis*, *Botryosphaeria parva* and *Phaeoacremonium* sp. A *Bacillus* sp. strain, isolated from internal bark, is currently successfully used *in vitro* as a native antagonist of fungi related to GTDs in Piura.

Incidence and pathogenicity of *Campylocarpon* species associated with black foot disease of table grape in the main Brazilian exporting region. M.A. SILVA[1], K. C. CORREIA[2], M.A.G. BARBOSA[3], M.P.S. CÂMARA[1], M. LEÓN[4], J. ARMENGOL[4] and S.J. MICHEREFF[1]. [1]*Universidade Federal Rural de Pernambuco, Departamento de Agronomia, 52171-900 Recife, PE, Brazil.* [2]*Universidade Federal do Cariri, Centro de Ciências Agrárias e da Biodiversidade, 63133-610 Crato, CE, Brazil;* [3]*Embrapa Semiárido, Laboratório de Fitopatologia, 56302-970 Petrolina, PE, Brazil.* [4]*Universitat Politècnica*

de València, Instituto Agroforestal Mediterráneo, 46022 Valencia, Spain. E-mail: sami.michereff@ufrpe.br

The São Francisco Valley, located in Northeastern Brazil, is responsible for 98% of the table grape production intended for export. In the last years, the occurrence of black foot disease was reported in the region. The objectives of this study were to (a) identify species of *Campylocarpon* associated with black foot disease of table grape in the São Francisco Valley, (b) investigate the distribution of the species and (c) evaluate their pathogenicity and aggressiveness in excised green shoots of table grapes. Thirty-eight *Campylocarpon* spp. isolates were obtained from table grape plants showing black foot disease symptoms in 12 vineyards from five localities (Casa Nova, Curaçá, Juazeiro, Lagoa Grande and Petrolina) in the São Francisco Valley. Fungal identifications were made using a combination of morphological characters together with a phylogenetic analysis based on internal transcribed spacer (ITS) region of rDNA and portions of the actin (ACT), translation elongation factor 1-α (EF1-α), histone 3 (H3), large subunit 28S nrRNA (LSU) and RNA polymerase II largest subunit (RPB1) genes. Three species of *Campylocarpon* (*C.*) were identified: *C. fasciculare*, *C. pseudofasciculare* and *C. semiaridus* sp. nov. The last species grew at temperatures ranging from 15 °C to 35 °C, with optimum at 30.8 °C. The first two species had been reported in table grapes in Northeastern Brazil, but not in the São Francisco Valley. *Campylocarpon pseudofasciculare* was the most prevalent species (27 isolates), followed by *C. fasciculare* (7 isolates) and *C. semiaridus* (4 isolates). The distribution of *Campylocarpum* species differ among the localities producing table grapes in the São Francisco. Only in Petrolina, all *Campylocarpon* species were found. All species of *Campylocarpon* were pathogenic on detached shoots of table grape, but there were no differences in aggressiveness among the species. These pathogenicity results should be confirmed with table grape vines in the vineyard.

Incidence and pathogenicity of *Phaeoacremonium* species associated with Petri disease of table grape in Northeastern Brazil. M.A. SILVA[1], K.C. CORREIA[2], M.A.G. BARBOSA[3], M.P.S. CÂMARA[1], D. GRAMAJE [4] and S.J. MICHEREFF[1]. *[1]Universidade Federal Rural de Pernambuco, Departamento de Agronomia, 52171-900 Recife, PE, Brazil. [2]Universidade Federal do Cariri, Centro de Ciências Agrárias e da Biodiversidade, 63133-610 Crato, CE, Brazil; [3]Embrapa Semiárido, Laboratório de Fitopatologia, 56302-970 Petrolina, PE, Brazil. [4]Instituto de Ciencias de la Vid y del Vino, Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, 26071 Logroño, La Rioja, Spain. E-mail: sami.michereff@ufrpe.br*

Table grape is an important fresh fruit exported by Brazil, and the Northeastern region is responsible for 99% of Brazilian exports. A wide range of diseases impact on grapevine production and grapevine trunk diseases (GTDs) are known to occur wherever grapes are grown. Petri disease is one of the most destructive GTDs worldwide. The objectives of this study were (a) to identify and characterize species of *Phaeoacremonium* associated with Petri disease of table grapes in three regions in the Northeastern Brazil, (b) to investigate the distribution of the species in these regions and (c) to evaluate their pathogenicity and aggressiveness in excised green shoots of table grapes. Twenty-two isolates of *Phaeoacremonium* were obtained from table grape plants of ages ranging from 6 months to 10-years old showing Petri disease symptoms in 12 vineyards located in the São Francisco, Siriji and Baixo Jaguaribe Valleys (Northeastern Brazil). Fungal identifications were made using a combination of morphology together with a phylogenetic analysis based on portions of the β-tubulin (TUB2) and actin (ACT) genes. Three species of *Phaeoacremonium* (*P.*) were identified: *P. minimum, P. nordesticola* sp. nov. and *P. parasiticum*. *Phaeoacremonium nordesticola* grew at temperatures ranging from 10 to 37°C, with optimum at 29.7°C. *Phaeoacremonium minimum* and *P. parasiticum* had previously been reported in grapevine in Brazil. *Phaeoacremonium minimum* was the most prevalent species (15 isolates), followed by *P. nordesticola* (4 isolates) and *P. parasiticum* (3 isolates). The three *Phaeoacremonium* species were found only in São Francisco Valley. *Phaeoacremonium minimum* and *P. parasiticum* were found in Siriji Valley. A single isolate of *P. nordesticola* was obtained in Baixo Jaguaribe Valley. All species of *Phaeoacremonium* were pathogenic on detached shoots of table grape, with *P. minimum* being the most aggressive and *P. nordesticola* less aggressive.

Sampling method of young plant tissues in grapevine nurseries for GTDs. M. SINEUX[1] and O. ZE-

KRI[2]. [1]*Mercier Novatech Le Champ des Noëls, Le Gué de Velluire, France* [2]*Mercier Frères S.A.R.L, 16 Rue de la Chaignée, Vix, France. E-mail: marion.sineux@mercier-groupe.com*

Grapevine trunk diseases (GTDs) are currently considered as the most destructive diseases of vineyards worldwide and are of rapidly growing concern in all wine producing countries. The pathogens responsible for these diseases attack the long-lasting organs, causing the death of vines on shorter or longer term, but also reduce the quality and quantity of grapes and wine production. The life cycle and epidemiology of those fungi are well known, but the diseases are complex and their symptoms usually take several years to develop. A healthy vine is fundamental to the successful beginning and sustainability of all grape vineyards. That starts in the grapevine nurseries, which are the first point in the production chain. To produce "healthy/clean" plants, nurseries need a tool to control the quality of the wood before grafting and along the production process, to ensure the sanitary state of the grafted plant. Even though some accurate diagnostic tools are available for GTDs pathogens no reliable sampling method can be used along the nursery process. The aim of our work is to develop an efficient sampling method on young plant tissues. We used qPCR (TaqMan) to detect and quantify five pathogens (*P. minimum*, *P. chlamydospora*, *D. seriata*, *E. lata* and *N. parvum*). Entire plants (30 cm) were cut in several fragments. After DNA extraction, each fragment was analyzed for the five pathogens and the quantity of pathogen DNA was determined. The purpose was to see if the distribution of the pathogens was heterogeneous inside an entire young plant. The results of our study showed variabilities that allow to understand better the distribution of the fungi inside a young plant. This is the first step for nursery to choose the segment representing best its sanitary state.

***Phaeoacremonium* species diversity and host-range in the Western Cape Province of South Africa.** C. SPIES[1,2], P. MOYO[2], F. HALLEEN[1,2] and L. MOSTERT[2]. [1]*Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa.* [2]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa. E-mail: SpiesC@arc.agric.za*

The genus *Phaeoacremonium* currently includes 48 species of which most are generally associated with wood diseases of plants, eg. Petri and esca diseases of grapevines, and dieback and cankers on other fruit trees and woody hosts. Although several *Phaeoacremonium* species are known only from grapevine this is more likely a reflection of limited sampling rather than limited host range. Species like *Phaeoacremonium minimum* and *Phaeoacremonium parasiticum* are known from more than 10 hosts each, and most *Phaeoacremonium* species that are known from more than one country are also known from more than one host. In South Africa (SA) 19 *Phaeoacremonium* species have been reported from grapevines, fruit trees and arthropods. Elsewhere, some of these species have also been associated with hosts such as olive, quince and willow that frequently occur in the Western Cape Province. This study investigated the diversity of *Phaeoacremonium* species on various woody hosts in this region. Representatives from previously reported *Phaeoacremonium* species in SA were included in an actin/beta-tubulin phylogeny along with 156 previously unreported isolates from 29 hosts in SA. The 156 isolates comprised 31 species including thirteen new species and three known species not previously reported in South Africa. Previous South African records of *P. alvesii* were re-identified as *P. italicum*. In South Africa grapevine is the host from which most *Phaeoacremonium* species (20 spp.) are known. Species with the widest host ranges in South Africa include *P. scolyti* (20 hosts), *P. minimum* (18 hosts) and *P. parasiticum* (16 hosts). A total of 36 *Phaeoacremonium* species are known in South Africa, more than double reported for other countries. Extensive and broad host sampling, climatological variation across different regions, and the high biodiversity of indigenous flora in the Western Cape Province are possible reasons for the high number of *Phaeoacremonium* species in SA.

Investigating the role of *Fusarium* spp. in the young vine decline complex. J.R. ÚRBEZ-TORRES, J. BOULÉ and D.T. O'GORMAN**.** *Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Box 5000. Summerland, BC V0H 1Z0, Canada. E-mail: joseramon.urbeztorres@agr.gc.ca*

Petri disease and black foot are responsible for the decline observed in newly established vineyards world-

wide known as young vine decline complex (YVD). Contrary to other crops, studies on the role that *Fusarium* spp. play on grapevine health are lacking. In a recent study conducted in British Columbia (BC) to investigate the health status of grapevine nursery propagated material, a high number of *Fusarium* colonies were constantly isolated from both necrotic roots and tissue at the basal end of the rootstock. Accordingly, the objectives of this study were to characterize by means of morphological and molecular studies the different *Fusarium* spp. isolated from symptomatic tissue and to complete Koch's postulates to determine whether or not *Fusarium* spp. were responsible for the symptoms observed. Five *Fusarium* spp., including *Fusarium* sp., *F. oxysporum*, *F. proliferatum*, *F. ramigenum*, and *F. solani* were identified from symptomatic nursery stock. Koch's postulates were conducted in one year-old dormant rooted rootstocks (Riparia Gloire, Schwarzmann, 3309C, 101-14, SO4) and treatments included: 1) plant as is, 2) trimming of roots, 3) cut of the basal end of the rootstock and a combination of 2+3. Plants were inoculated by submerging them in a 10^5 conidia mL^{-1} spore suspension for 24 h and then planted in a greenhouse. Negative controls included inoculation with autoclaved distilled water and positive controls were individually inoculated with *Dactylonectria pauciseptata*, *Ilyonectria liriodendri* and *I. macrodidyma*. Results showed that *Fusarium* spp. were capable to cause necrosis in roots and rootstocks similar to those observed by black foot fungi. However, virulence varied depending on the *Fusarium* spp., rootstock inoculated and treatment. Overall, the 2+3 treatment showed significant reduction in pruning weight and dry root weight when inoculated with *Fusarium* spp. This study shows for the first time the role that *Fusarium* spp. could have on grapevine health.

Validation of the Young Vine Decline DNA-macroarray to determine the health status of grapevine nursery propagation material in British Columbia. J.R. ÚRBEZ-TORRES and D.T. O'GORMAN. *Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Box 5000. Summerland, BC V0H 1Z0, Canada. E-mail: joseramon.urbeztorres@agr.gc.ca*

Young vine decline (YVD) is a complex disease caused by fungal species in the genera *Cadophora*, *Campylocarpon*, *"Cylindrocarpon"*, *Dactylonectria*, *Phaeomoniella*, and *Phaeoacremonium* and affect newly established vineyards. Because a long lifespan for vines is essential for economic success in vineyards considering the large investments in vineyard establishment and maintenance, YVD can cause substantial untenable economic losses to grape-growers. YVD fungi are known to occur in nursery propagated material and thus, detection prior to planting is critical to assure longevity of newly established vineyards. Our laboratory recently developed a molecular diagnostic tool based on the reverse dot-blot hybridization capable to simultaneously detect and identified 61 fungal species including 34 YVD pathogens known as YVD-DNA-macroarray (Úrbez-Torres *et al.* 2015). The objectives of this study were to validate the YVD-DNA-macroarray to evaluate the health status of grapevine propagated material planted in British Columbia and to compare this technique against currently used fungal pathogen detection tools. Ready to plant material was provided by several nurseries from different countries and included different rootstock-scion combinations and self-rooted plants. For each plant, total DNA was obtained from roots, rootstock basal end, graft-union, and scion and processed with the YVD-DNA-macroarray. The YVD-DNA-macroarray successfully detected and identified several YVD fungi and showed *Phaeomoniella chlamydospora* to be the most prevalent pathogen followed by *Cadophora luteo-olivacea*, *Phaeoacremonium* spp., and *Ilyonectria* spp. Among all different plant parts, YVD pathogens were detected primarily at the basal end of the rootstock. The DNA-macroarray was shown to be an accurate, sensitive and a much faster detection tool than other techniques used such as standard PCR or traditional plating.

Molecular diagnostic assays for evaluating the impact of hot water treatment on grapevine trunk pathogens. E. VAN ZIJLL DE JONG[1], R. O'NEILL[1], C. PROBST[2], J. ALLWOOD[2], S. HICKLING[1], Y. WRUCK[1], Á. VIDIELLA[1,3], N. HOSKINS[3,4] and G. THORPE[3]. [1]*Linnaeus, PO Box 1199, Gisborne 4040, New Zealand.* [2]*Landcare Research, Private Bag 92170, Auckland Mail Centre, Auckland 1142, New Zealand.*[3]*Riversun Nursery Ltd, PO Box 1199, Gisborne 4040, New Zealand.* [4]*Vine Manager, 3 Millard Avenue, Masterton, New Zealand. E-mail: eline@linnaeus.co.nz*

Previous studies to evaluate the effectiveness of hot water treatment for the eradication of grapevine trunk pathogens on propagated material from nurseries have been hampered by the lack of quantitative diagnostic tools. This has limited the ability to detect the range of fungal species causing disease in grapevine. Molecular diagnostic assays based on real time (RT)-PCR technology were developed for the specific detection of fungal pathogens attributed to Botryosphaeria dieback, Eutypa dieback, black foot disease, and Petri disease in grapevines in New Zealand. These assays quantitatively detected the target pathogens to at least nanogram levels in grapevine tissues. Field trials were established in 2014 and 2015 in Marlborough, New Zealand, to evaluate the efficacy of hot water treatments for control of grapevine trunk pathogens in Sauvignon Blanc vines. The vines were exposed pre-grafting and/or pre-dispatch to hot water treatments at 48 or 50°C. Application of the molecular diagnostic assays to monitor grapevine trunk pathogens and establish the efficacy of hot water treatments in the field will be presented.

An inter-laboratory comparison of DNA barcoding protocols for identifying fungi involved in grapevine trunk disease. J. WOODHALL[1], K. PERKINS[2], P. REIS[3], C. REGO[3], A. AVES[4], A. EICHMEIER[5], G. ROMANAZZI[6], C. KOVACS[7], E. KARAFFA[7], K. Z. VÁCZY[8], L. MUGNAI[9], A. CARLUCCI[10], R. MUÑOZ[11], D. GRAMAJE[12], J. ARMENGOL[13], C. PINTO[14] and F. FONTAINE[14]. *[1]University of Idaho, Parma, USA. [2]Fera Science Ltd, York, UK. [3]University of Lisbon, Portugal. [4]University of Aveiro, Portugal .[5]Mendel University in Brno, Czech Republic. [6]Marche Polytechnic University, Italy. [7]University of Debrecen, Hungary. [8]Eszterházy Károly University Hungary. [9]University of Florence, Italy. [10]University of Foggia, Italy. [11]Técnico Agronómico Provincial de Albacete, Spain. [12]Instituto de Ciencias de la Vid y del Vino, Spain. [13]Polytechnic University of Valencia, Spain. [14]University of Reims Champagne-Ardenne, France. E-mail: jwoodhall@uidaho.edu*

Many species of fungi are present in the grapevine trunk disease (GTD) complex. Accurate identification and characterisation of the causal agent or agents is essential for both successful diagnosis of trunk diseases and research into their epidemiology and control. Since identification based on morphological observations can be ambiguous due to similarity in colony characteristics many researchers rely on DNA sequencing based approaches to identify the fungal organism present. For fungi, the ribosomal DNA internal transcribed spacer (ITS) region has been adopted as the primary DNA barcode marker. However, despite its widespread use there has been relativity few studies comparing its use in routine fungal identification between laboratories. In this study, the utility of the ITS region as a means of accurately identifying GTD fungi was assessed in an international inter-laboratory comparison comprising of researchers participating in the EU cost action on GTD (manaGTD). Twelve samples of fungal DNA associated with GTD fungi were distributed to researchers in 14 laboratories. The researchers were asked to use their own DNA barcoding protocols to identify the GTD species using phylogenetic markers for the ITS, histone and elongation factor regions. The results and the relative robustness of DNA barcoding approaches for GTD fungi are discussed.

Epidemiology

Development of a real-time PCR protocol to quantify the airborne inoculum of *Phaeomoniella chlamydospora*. M. BERBEGAL, S. ARIZMENDI, S. CATALÁ, V. MORANT and J. ARMENGOL. *Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain. E-mail: mobermar@etsia.upv.es*

Knowledge of pathogen inoculum dynamics in the field and its relationship with the environmental conditions is essential for disease management. One of the methods used for monitoring the airborne inoculum of plant pathogenic fungi in the field is to take samples of the airborne inoculum impacted on traps and subsequently quantified by observation under the microscope. The objective of this research was to develop and validate protocols for DNA extraction, detection and quantification of *Phaeomoniella chlamydospora* using real-time PCR. Conidia of known concentrations obtained *in vitro* and from field samples were used in this study. A stock solution with high concentration of conidia was initially prepared from *P. chlamydospora* grown *in vitro* and serial dilutions of 1:10 and 1:5 were further made. Known quantities of each dilution were inoculated on tapes to simulate the Burkard spore-trapping system and used for DNA

extractions. For field samples, two slides with tapes were installed in a 9-year old vineyard in Albacete and in a 20-year old vineyard in Onteniente (Valencia province). Preliminary tests showed nested-PCR was necessary to detect low concentrations of DNA. In the first round of PCR reaction, a fragment of the rDNA region was amplified using a combination of a universal primer and a species-specific primer for *P. chlamydospora*. The resulting PCR products were used as template for the subsequent real-time PCR using species-specific primers for *P. chlamydospora*. The protocol was shown to be specific and sensitive. Several field samples were tested positive to *P. chlamydospora* especially those collected in the older vineyard. This study showed that molecular techniques are highly sensitive for the detection and quantification of *P. chlamydospora* conidia compared to conventional techniques like microscopy . The use of these molecular tools in combination with spore samplers could help in developing GTD prediction methods and optimize disease management.

Grafting process and rooting nursery soils are critical propagation stages where fungal trunk pathogens can infect planting material in grapevine nurseries. C. BERLANAS[1], Á. KUN[2] and D. GRAMAJE[1]. [1]*Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas, Universidad de la Rioja, Gobierno de La Rioja, Ctra. LO-20 Salida 13, 26071 Logroño, Spain,* [2]*Government Office of Baranya County, Plant Protection and Soil Conservation Directorate, H-7634 Kodó dűlő 1, Pécs, Hungary. E-mail: david.gramaje@icvv.es*

A survey was carried out covering all the stages of the propagation process in three grapevine nurseries in northern Spain: rootstock mother plants, pregrafting hydration tanks, scissors used for cutting buds, grafting machines, water used to promote root development and grafted plants after one growing season in nursery fields. Samples from hydration tanks, scissors, grafting machines and water during the callusing stage were analyzed using two different techniques: nested PCR and fungal isolation by culturing on semi-selective medium. Rootstock cuttings and grafted plants were sampled to isolate the fungal pathogens by culturing on malt extract agar with streptomycin sulphate. *Cadophora* spp. and *Phaeoacremonium* spp. were detected at all of these stages, with the exception of hydration tanks, and more importantly they were viable since they were detected by isolating on culturing medium. Other detected fungal trunk pathogens were: *Eutypa lata* (rootstock mother plants), Botryosphaeriaceae spp. and *Dactylonectria torresensis* (grafted plants). The average percentage of fungal trunk pathogens detection in the three grapevine nurseries varied among the propagation stages, being 9.1% in rootstock mother plants, 7.2% in scissors, no detection in hydration tanks, 22.5% in grafting machines, 2.8% in the callusing stage and 60% of fungal detection in grafted plants prior to dispatch. In these grafted plants, 49.2% of infection was caused by *Dactylonectria torresensis* at the base of the rootstock and roots, while 10.4% and 40.3% of infection was caused by *Cadophora luteo-olivacea* and *Phaeocremonium* spp. in rootstock wood and the graft union, respectively. Our results demonstrated that infections caused by fungal trunk pathogens in grapevine planting material increase markedly after one growing season in nursery fields. Management strategies should then focus in reducing the inoculum level of soilborne pathogens in grapevine nursery soils.

Prevalence, damage and potential yield loss of vineyards cv. Cabernet Sauvignon in two regions of Chile. C. TORRES[1], R. CAMPS[1], B.A. LATORRE[2] and X. BESOAIN[1]. [1]*Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, Casilla 4-D, Quillota, Chile.* [2]*Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Casilla 306-22, Santiago, Chile. E-mail: ximena.besoain@pucv.cl*

Grapevine is one of the most important fruit crop cultivated in Chile for wine and fresh fruit production. A total of 137,592 ha are planted with wine grapes with Cabernet Sauvignon as the main cultivar. Recent studies have reported the importance of Bot Canker affecting grapevines in Chile. Therefore, the objective of this work was to assess the incidence and severity of Bot Canker disease and its impact on yield in the two most important Cabernet Sauvignon-producing regions in Chile. It also aimsto identify the main Botryosphaeriaceae species involved in this disease. The incidence and severity were assessed in 10 vineyards from the O'Higgins Region (n=8 blocks) and in the Maule Region (n=6 blocks) for a total of 14 blocks

at 100 vines per block. Yield loss was estimated by comparing the yield of 10 healthy plants to the yield of affected plants of each block. The mean disease incidence was 79.9 % and 95.4% for the O'Higgins and Maule region, respectivelywhile, the mean damage index was 34.3% and 44.9% for the O'Higgins and the Maule Region, respectively. Yield loss was estimated to an average of 4.52 ton/ha for the O'Higgins Region and 6.20 ton/ha for the Maule Region. The main species frequently isolated from these two regions were*Diplodia seriata* (692%), *D. mutila* (12.8%) and 10.2% for *Neofusicoccum parvum* (10.2%).

Climate and GTD symptoms. Z. BIHARI, P. BALLING, S. ÉLES and G. ZSIGRAI**.** *Research Institute for Viticulture and Oenology, Tokaj, Hungary***,** *Könyves K. u. 54. 3015 Tarcal, Hungary***.** *E-mail: biharitokaj@gmail.com*

Grapevine Trunk Diseases (GTDs) are widespread throughout Europe and affected producers can suffer signficant financial losses. In the Tokaj wine region, Hungary, the loss of income per year attributed to GTDs was estimated to be approximately €3 million. . The visible symptoms' number is changing every year in the vineyards while somes vines with the GTD pathogens do not show any obvious symptoms. Therefore, the number of vines showing symptoms does notnecessarily replect the actual number of infected vines present in the vineyard. Furthermore, it was further observed that some vines exhibiting symptoms in one year may appear "non-symptomatic" the following year. This indicates that the symptom expression is the field may be related to the environmental impacts on the vine's physiological condition. To investigate the relationship between climate and GTD symptoms, a study was carried out in five vineyards in Tokaj, Hungary. These vineyards were using different training systems (Guyot, mid-high cordon) and different variety of vines were used in the vineyards (Furmint, Hárslevelű, Zéta). A total of 8013 vines were were monitored for four years (2013 – 2016)and the symptomatic vines from each vineyard were recorded every year. Temperature and the precipitation- were measured for and the relationship between symptom development and meteorological data were anlaysed. Overall results showed that that greater number of vines expressing GTD symptoms were observed after a mild and rainy winter.

The evaluation of the incidence of Grapevine Trunk Diseases in the Hungarian winegrowing regions. N. BURGHARDT, B. TEMPFLI and K.Z. VÁCZY. *Eszterházy Károly University, Food and Wine Research Institute, 3300 Eger, Eszterházy tér 1. Hungary. E-mail: burghardt.natasa@uni-eszterhazy.hu*

From the beginning of 2000 a significant increase of Grapevine Trunk Diseases (GTDs) prevalence has been observed in the Hungarian vineyards, which jeopardise grapevine productivity and sustainability. The aim of this study was to monitor and evaluate the incidence of five significant winegrowing regions in Hungary (Neszmély, Eger, Pécs, Szekszárd and Villány). The field evaluations have been carried out between 2015 and 2016. At least three vineyards have been selected in each region considering different varieties (Cabernet Sauvignon, Chardonnay and Kékfrankos). The main criteria to select the vineyards was the age of the vines (12-18 years old). Where it was possible, two different training systems (cordon and umbrella) per variety have also been studied. About 500 vines per vineyard were evaluated during the field surveys, the investigated parameters included the missing vines, the number of replacements, vines showing the symptoms (apoplexy, foliar, shoot and berry) and the number of apparently healthy vines. The study considered GTDs without discriminating each specific disease, e.g. Esca or Botryosphaeria dieback. In both years, the surveys have been carried out between the end of July and August, considering the usual appearance and development of GTDs symptoms. The recorded data of the evaluation have been summarized, the incidence of the infected vines have been defined, a comparison of the two years investigated was prepared, and then the results were evaluated using statistical methods. This study aimed to finally answer the question: can a definite relationship between the incidence of GTDs and the different factors – such as the winegrowing region, the variety, the training system, the rootstock, and the age of vines – be hypothesized? Currently, this is the first large scale study in Hungary regarding the investigation of the prevalence of Grape Trunk Disease infection.

Influence of rainfall and temperature on the expression of leaf symptoms in vines affected by grapevine leaf stripe disease (esca complex). F.

CALZARANO[1], P. DI FRANCESCANTONIO[2] and S. DI MARCO[3]. [1]*Universita degli Studi di Teramo, Facoltà di BioScienze e Tecnologie Agro-Alimentari ed Ambientali, Via R. Balzarini 1, 64100 Teramo, Italy.* [2]*Self-employed engineer.* [3]*Istituto di Biometeorologia (IBIMET), CNR, Via Gobetti 101, 40129 Bologna, Italy. E-mail: fcalzarano@unite.it*

The dynamics of the leaf symptom expression on vines affected by grapevine leaf stripe disease (GLSD) have not yet been fully elucidated to date. In recent years, several studies investigated the mechanisms involved in the expression of the leaf symptom. This study investigated influence of climatic conditions in leaf symptom expression . Daily rainfall and temperature data for 21 consecutive years(1994 to 2014) from two vineyards were analysed to assessed tp assess the influence of climatic conditions on foliar symptom expression. These vineyards are located in Abruzzo, in central Italy and vines in both vineyards were 15 years-old in the first year of investigation. For each vineyard and year, rainfall and temperature data collected for each single month or different combinations of months were correlated with the incidence and severity of foliar symptoms which were assessed in September, where maximum symptom expression was usually observed. Rainfall in April to July showed some correlation with the foliar symptom expression, appearing July the most important month for the correlation between rainfall (ranging from 0 to 174.2 mm) and symptom. The correlation assessed in the 1994–2007 period was not longer noticed when the age of those same vineyards increased (2008-2014). Symptomatic vines showing high symptom severity died over the years with a mortality incidence of 22% in 2003 and 20% in 2005, at Giulianova and Controguerra, respectively. However, the mortality decreased significantly from 2006 in both vineyards. In 2008–2014, when a reduced correlation between rainfall and symptom was observed, a decrease of severity and incidence of leaf symptom expression was noticed in both vineyards. The temperature would not seem to have effect on the foliar symptom expression.

Susceptibility assessment of different grapevine cultivars to esca disease complex in Southern Italy. F. LOPS, M. L. RAIMONDO, F. CIBELLI and A. CARLUCCI. *Department of the Science of Agriculture, Food and Environment, University of Foggia, Via Napoli 25, Foggia 71122, Italy. E-mail: antonia.carlucci@unifg.it*

A field survey was carried out for five years on 32 different grapevine wine varieties (17 with black berries and 15 with white berries) in South Italy to assess their susceptibility to esca disease complex. All grapevine varieties grafted on rootstock 1103 Paulsen were transplanted on February 1992. The survey was performed from June 2008 until September 2012. Approximately 90% of the vines for each cultivar showed Esca foliar symptoms including apoplexy at different severity scale. Incidence and severity indices were calculated for each variety in order to evaluate the degree of susceptibility by an empiric gravity scale. The data were subjected to homogeneity and normality tests before performing one-way ANOVA analysis. In general, the results showed that the black varieties were less susceptible compared to the white varieties, although the incidence and severity indices varied depending on the year. Overall results showed that four white varieties and eight black varieties were found to be less susceptible to esca disease.

Building a conceptual model of GTDs. M. CLAVERIE. *Institut Français de la Vigne et du Vin, Institut Rhodanien, 84100 Orange, France. E-mail: marion.claverie@vignevin.fr*

A conceptual model (CmA) is a problem solving tool based on the integration of both knowledge and assumptions into a graphical frame. It implies a systemic approach and is frequently used to describe complex or multi-factorial situations. A CmA on GTDs has been built in order to explain why and how foliar symptoms emerge and ultimately culminate in plant death. The model describes the succession of events taking place from the planting of a grafted plant up to the expression of foliar symptoms on an adult vine. Our CmA focuses on the contamination and colonization phases, vine defense and plant-pathogens interactions, using notions based on the forestry literature (forest pathology, particularly tree decays, and ecophysiology). It also proposes hypotheses for apoplexy, symptoms fluctuation and other commonly assumed observations on GTDs. Finally, this sequence is integrated into the natural environment of the vine including soil, climate, genotype

and cultural practices, in order to point out the many contributing factors that can modulate the emergence of foliar symptoms. The interesting points in building such a model is to represent the complexity of the syndrome and its multi-factorial dimension altogether on a single graph. Furthermore, since it focused on both the host-pathogen interactions and vine defense functions, the model automatically takes into consideration the vine physiology: assimilate production, vegetative growth, reserve mobilization, grape growth and maturation, thus bringing together agronomy and pathology. The perspectives for what this model can be potentially used for identifying gaps of knowledge on GTDsand help design a low-symptom vineyard management system.

Evidence of foliar symptom association with decline of the vine. M. CLAVERIE, N. JOUANDOU[1], O. JACQUET[2] and D. RICHY[3]. *[1] Institut Français de la Vigne et du Vin, Institut Rhodanien, 84100 Orange, France. [2] Chambre d'agriculture de Vaucluse, Institut Rhodanien, 84100 Orange, France. [3]Chambre d'agriculture des Bouches du Rhône, 22 Avenue Henri Pontier, 13626 Aix en Provence Cedex 1, France. E-mail: marion.claverie@vignevin.com*

Foliar symptom expression, mortality due to apoplexy, yield losses and grape quality alterations are the most commonly described drawbacks for GTDs affected vines. However, intra vine mortality and vigor alterations are scarcely mentioned. A survey on GTDs symptoms (esca/Botryophaeria dieback), vine growth and grape production has been conducted on two vineyard plots (Grenache N and Cabernet sauvignon) in South Eastern France. The monitoring was carried out for 2 years on the Cabernet sauvignon plot and for 3 years on the Grenache one. Assessment was done on the spurs of the two cordons of each spur-pruned vine. In 2014, both asymptomatic and GTDs symptomatic vines and spurs were observed while incidence of winter mortality, spring growth delay, total pruning and grape weight were also recorded. The survey results showed correlations between current year symptom and mortality of the concerned spurs but incidences vary between years, vineyard and type of foliar symptom. Severe and defoliating symptoms induced more spur death than the mild ones. Foliar symptoms reduced both the spring vegetative growth and the grape yield of the following year suggesting perturbations in the reserve replenishment and floral initiation in the buds. No further increase in foliar symptom has been observed on vines when monitoring was extended for another 2 years . Based on these observations, it suggests that foliar symptoms observed at year n can alter grapevine functioning both on that current and n+1 years but has no obvious impact on the n+2 year in the absence of recurring foliar symptom in n+1 year. Overall, our survey showed that the yield loss due to GTDs impact ranged between 15% and 25%.

Spatial and temporal variation of active fungal communities on grapevine propagating material after hot-water treatment. A. EICHMEIER[1], D. GRAMAJE[2], M. LEÓN[3], S. CATALÀ[3], J. PEČENKA[1], E. PEŇÁZOVÁ[1], M. BARÁNEK[1] and J. ARMENGOL[3]. *[1]Mendel University in Brno, Faculty of Horticulture, Mendeleum - Institute of Genetics, Valtická 334, 69144 Lednice, Czech Republic.; [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, 26071 Logroño, Spain; [3]Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain. E-mail: ales.eichmeier@mendelu.cz*

Grapevine propagating material is particularly vulnerable to infections caused by fungal trunk disease (TD) pathogens in nurseries since many wounds and cuts are made during the propagation process. Hot-water treatment (HWT) is the only currently recognized means of controlling these internal infections. The objective of this study was to examine the effect of HWT on the total active mycoflora, including fungal TD pathogens. For this purpose, two experiments were simultaneously carried out in Spain and the Czech Republic. Dormant grafted plants were hot-water treated at 50 or 53ºC for 30 min. In April 2015, HWTed vines were planted immediately in vineyards, following standard cultural practices in each country. At the end of the growing season (October 2015), plants were uprooted and evaluated. Fungal communities were analyzed at two periods: i) immediately after HWT; and ii) after one growing season for a total of 480 grafted plants. For each plant, tissue samples from the root and graft union were analyzed by classical isolation and fungal identification, and high throughput metatranscriptomic sequenc-

ing of the ITS2 region. The results revealed significant differences in the detection levels of both methods. The metatranscriptomic sequencing increased the resolution of the fungal community analysis and revealed a highly diverse and complex mycoflora of grapevine wood compared to the classical approach. In classical isolation approach, the total active mycoflora, including fungal TD pathogens, was reduced immediately after HWT. However, the number of fungal colonies increased after one growing season. The results demonstrate that the HWT process does not completely sterilize the internal wood of grapevine propagating materials. This represents the first study on active fungal communities on grapevine grafted plants using metatranscriptomics approach in combination with classical isolation. Furthermore, it provides important and practical useful insights on the dynamics of the active fungal communities of HWTed plants.

Grapevine trunk diseases in European and Mediterranean vineyards: a survey for assessing their distribution and the associated cultural factors. L. GUÉRIN-DUBRANA[1,2], B. BENEDETTI[3], D. MORAIS[1,2] and L. MUGNAI[3]. [1]*Université de Bordeaux, ISVV, UMR1065 Santé et Agroécologie du Vignoble, Bordeaux Sciences Agro, F-33175 Gradignan, France.* [2]*INRA, ISVV, UMR1065, F-33140 Villenave d Ornon, France.* [3]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DiSPAA)- Sez. Patologia vegetale ed Entomologia, University of Florence, 50144 Florence, Italy. E-mail: Lucia.Guerin-Dubrana@inra.fr*

A large survey based on a reporting questionnaire of qualitative information was carried out in 22 European and some Mediterranean countries. The aim of the survey was to gain an overview on the main fungal grapevine trunk diseases (GTDs) in Europe. Information from 105 viticulture regions, representing almost four million hectares was recorded. Vintage and table wine represent the main production followed by nursery and table grape production. A large diversity of scion cultivars are grown with 13 cultivars representing 50% of the total reports (Riesling Italico, Chardonnay, Cabernet Sauvignon, Merlot being the main ones). The 5 most reported rootstocks were SO4, 110R, K5BB, 41B and 1103P, which represent alone about 50% of the reports. Despite the diversity of grapevine training, Guyot and cordon remain the most widespread methods, with grapevines mostly hand-pruned. However, the use of pneumatic pruning shears and mechanical pruning is becoming increasingly more common in some countries. Six syndromes were proposed in the questionnaire for mature vineyards: apoplexy, Grapevine Leaf Stripe Disease, *Eutypa* dieback, dead cordon, Botryosphaeria dieback and cankers, *Phomopsis* cane and leaf spot. Apoplexy and GLSD were reported to be the most frequent and increasing syndromes within almost all countries, except in Israel and the UK (not present or not recorded). Dead cordon and *Phomopsis* cane and leaf spot were mentioned as occurring in a large number of regions but not frequently. Within a country, the profile of GTDs varied according to the region, pedo-climatic, and production conditions. GTDs on young vines were not so frequent, but remain a subject of concern in some of the European countries with large nursery production. This overview on spread and relevance of GTDs and related aspects may be a useful starting point for policy makers and for shared studies on contributing factors to the diseases increasing incidence.

Spore production by Botryosphaeriaceae species from grapevine lesioned tissue under natural conditions in New Zealand vineyards. A. SHAFI[1], H. RIDGWAY[1], M. JASPERS[1] and E. JONES[1]. [1]*Department of Ecology, Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln, Canterbury, New Zealand. E-mail: Eirian.Jones@lincoln.ac.nz*

Botryosphaeriaceae species are important pathogens of grapevines worldwide. This study investigated Botryosphaeriaceae sporulation from naturally infected Sauvignon blanc canes and shoots in two vineyards in Marlborough, New Zealand. Lesions characteristic of Botryosphaeriaceae infection were tagged on grapevine canes and shoots (24 /vineyard) and observed during or soon after each rainfall event. Pycnidia were observed to ooze conidia on all tagged lesioned tissue soon after rainfall events on five occasions between Sept 2014 and April 2015. Microscopic observations of ooze samples revealed conidia characteristic of *Neofusicoccum* and *Diplodia* species in 66.7% and 50% of samples from Vineyards A and B, respectively. Visible oozing of cirrhi was observed with a minimum of 7 mm of rain, but less rainfall could have released fewer conidia in

minute cirrhi not obvious to the naked eye. Plating of conidial ooze samples resulted in colonies characteristic of Botryosphaeriaceae species which were identified using amplified ribosomal DNA restriction analysis and DNA sequencing of the internally transcribed spacer region of the rRNA. A total of 69 isolates morphologically identified as Botryosphaeriaceae species were recovered from the conidial ooze across both vineyards and assessment times, 40 and 29 isolates from Vineyards A and B, respectively. Of these, the frequency of recovered species was *D. mutila* (39.1%), *N. australe* (30.4%), *N. parvum* (14.5%), *N. luteum* (5.8%), *D. seriata* (7.2%) and *N. ribis* (3.0%). All of these species have been previously reported as grapevine pathgens in New Zealand. At Vineyard A, the predominant species was *D. mutila* (47.5%), followed by *N. australe* (25.0%) and *N. parvum* (12.5%) whilst in Vineyard B, the predominant species was *N. australe* (38.0%) followed by *D. mutila* (27.7%) and *N. parvum* (17.2%). This results of the study will provide information on the risk period with regards to inoculum production under vineyard conditions.

Soil and topology were the most important abiotic factors influencing GTDs incidence in the Tokaj Wine Region, Hungary. C. KOVÁCS[1,2], P. BALLING[3], Z. BIHARI[3], A. NAGY[4] and E. SÁNDOR[1]. [1] *University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Food Science, Böszörményi út 138., H-4032 Debrecen, Hungary.* [2] *National Agricultural Research and Innovation Centre, Fruitculture Research Institute (NARIC-FRI), Újfehértó, Vadastag 2, H-4244 Újfehértó, Hungary.* [3] *Research Institute for Viticulture and Oenology, Könyves Kálmán u. 54., H-3915 Tarcal, Hungary,* [4] *University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Plant Protection, Böszörményi út 138., H-4032 Debrecen, Hungary. E-mail*: *karaffa@agr.unideb.hu*

Grapevine Trunk Diseases (GTD) are of great importance worldwide, including Hungary, a central European country with long history in wine production. It is a complex disease with unique characteristics where infection can be latent without visible disease symptoms for years. However, the factors affecting the expression of the disease symptoms are still unclear. Five vineyards within 15 km radius were studied for three consecutive years (2013 – 2015). The incidence of GTD infection was determined every year for each vineyard. Two vineyards were planted on cambisols, while three on slope sediment from luvisols soil. There was Guyot training system in one vineyard, and mid-high cordon in the other four. The rootstock *Teleki –Kober* 125AA was used only in one vineyard for the variety Zéta (*Vitis vinifera* L.), while all the other vineyards used Teleki 5C with Furmint and Hárslevelű (*V. vinifera* L.) varieties. The direction of the rows on the slopes varied between vineyards with one vineyard being terraced, while the others were on steep slopes. Data from one vineyard was divided into two parts: the upper part of the vineyard with 5–8% slope, and a lower, flat part at the foot of the hill. There was significant difference in the disease incidence of GTD at the different vineyards, ranging from 0.17 and 42.11%. Topology and soil type appeared to be major abiotic factors affecting incidence of GTD symptoms. Disease incidence was also positively correlated with the age of the vineyards and was found to be the primary biotic factor affecting disease incidence.

Grapevine Trunk Disease Incidence and Control Practices in Rhineland-Palatinate, South West of Germany. T. KELLERER, C. MESCA, J. EDER and A. KORTEKAMP. *State Education and Research Center DLR Rheinpfalz, Institute for Plant Protection, Breitenweg 71, 67435 Neustadt an der Weinstraße, Germany. E-mail*: *tabitha.kellerer@dlr.rlp.de*

Field observations carried out in the years 2015 and 2016 in vineyards in Rhineland-Palatinate (southwest Germany) revealed an increase in Grapevine Trunk Disease (GTD) incidence, especially for the predominant variety Riesling. Within vineyards, GTD distribution patterns did not indicate a main route of infections, neither naturally nor due to pruning or canopy management. However, a markedly increased disease incidence (apoplectic vines) emphasizes the impact of environmental conditions, such as the change from cool and rainy to warm and dry weather conditions as observed in the last two years. To develop control measures to manage GTDs, the European project WINETWORK (Horizon 2020, agreement N°652601) was initiated. Within the scope of this project, interviews were carried out with winegrowers and nursery men in different winegrowing areas to understand their knowledge with regards

to GTDs and to identify new and innovative practices. The overall results showed most winegrowers in Germany were not aware of the GTD complex, even though they know "Esca" and the two forms of the disease, the apoplectic and the chronic form. The most popular practices used by German winegrowers to control GTDs are the trunk renewal, replacing of the entire plant, minimal pruning, wound protection using balms and waxes, disinfection of pruning tools, and the disposal of infected trunks (e.g. burning, heat- composting). One new method identified was the use of copper nails that were placed in the scion part of the trunk to lower the progress of wood rot in infected plants by a slow but constant release of copper. Preliminary results indicate the upward and downward spread of copper as seen by discoloured wood in the surrounding area of each nail. Furthermore, there are indications that the spread of white rot was restricted when reaching the copper enriched wood.

Susceptibility of grape pruning wounds to grapevine trunk diseases and effectiveness of a new BASF wound protectant. A. KÜHN[1], A. ZAPPATA[2], R.E. GOLD[1], R. ZITO[1] and A. KORTEKAMP[3]. *[1]BASF SE, Agricultural Center, Speyerer Strasse 2, 67117 Limburgerhof, Germany, [2]BASF Italia S.p.A., C306-14, Via Marconato 8, 20811 Cesano Maderno, Italy, [3]DLR Rheinpfalz, Phytomedizin für den Weinbau, Breitenweg 71, 67435 Neustadt. E-mail: annett.kuehn@basf.com*

Knowledge about the infection pathway and infection timeframe is one of the basic pre-requisites to develop effective approaches against the infestation of vines with Grape Trunk Disease (GTD) pathogens. The main entrance points for most GTD pathogens are the wounds, which are inevitably set during pruning. Field trials were carried out to observe the change in susceptibility of grape pruning wounds to infections with GTD-fungi over 3 months, beginning at pruning. A further objective was to test the effectiveness of the BASF wound protectant Tessior® under these conditions, following its application directly after pruning. All wounds (protected and unprotected) were inoculated with spore suspensions of either *Phaeomoniella chlamydospora* or *Diplodia* spp. The inoculation was made either 1 day, 1 month or 3 months after pruning or at all three timings. After 4-6 months incubation time in the field, the shoots below inoculated pruning wounds were harvested. In the laboratory, re-isolation of inoculated pathogens was assessed visually using the 3-slice method. Frequency of infested shoots and intensity of infection were determined. The trials were conducted in Germany, Greece and Spain in 2015 and 2016. The susceptibility of pruning wounds to infections was highest directly after pruning (58-100% infested shoots) and decreased significantly over time in all trials. Inoculation of unprotected wounds 3 months after pruning resulted in a 25-90% lower frequency of attacked shoots compared to fresh wounds. The expected additional effect of multiple infections on infestation level was minimal. These trends were observed in all trials. The BASF wound protectant decreased the infection of grape wood significantly during the whole period of 3 months between application and inoculation, demonstrating its long-lasting activity during the susceptible phase of wounds.

Remote sensing to detect Grapevine leaf stripe symptoms (Esca complex) in grapevine leaves. A. HEEMANN JUNGES[1], M. ANDRÉ KURTZ ALMANÇA[2], J.R. DUCATI[3] and C. SCALVI LAMPUGNANI[1]. *[1]Agricultural and Livestock Research Foundation (Fepagro), Centro de Pesquisa Carlos Gayer, Veranópolis, Rio Grande do Sul, Brasil.[2]Federal Institute of Rio Grande do Sul (IFRS), Bento Gonçalves, Rio Grande do Sul, Brazil. [3]Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil. E-mail: marcus.almanca@bento.ifrs.edu.br*

Research about vegetative spectral reflectance is important to a better understanding of the physical, physiological and chemical process in plants due to diseases. Furthermore remote sensors can be useful tools for non-invasive disease detection and diagnosis. In Brazil, one of the obstacles to production is due to grapevine trunk diseases caused by Esca complex. The aim of this study was to characterize the spectral features of vine leaves by ground spectroradiometry, to detect changes due to the occurrence of grapevine leaf stripe disease (GLSD, one of the diseases within Esca complex) in two stages of the vine cycle: January (pre harvest) and May (post-harvest). For this purpose, grapevine leaves were collected in a 'Merlot' vineyard located in Veranópolis, Brazil. The reflectance measurements were performed using a spectroradiometer in the wavelength

range 350 to 2500 nm. The variables examined were asymptomatic leaves and leaves with initial and severe symptoms of the GLSD. Average normalized spectral profiles were obtained for each date and for each type of foliar symptom to describe the spectral behavior and to establish the relationship between symptomatic and asymptomatic leaves. The results indicated that the spectral reflectance of leaves with GLSD symptoms were distinct from the asymptomatic leaves. There were increase of reflectance at wavelengths of visible light, especially near 700 nm, due to the sensitivity of chlorophyll concentrations to metabolic disturbance. The results are in accordance with Fontaine *et al.* (2016) and Di Gennaro *et al.* (2016) that indicated that GLSD pathogens affected the photosynthetic rate that demonstrated the potential use of vegetation index to discriminate symptomatic and asymptomatic plants in vineyard. The occurrence of spectral changes - in leaves with GLSD symptoms, especially in leaves with initial symptoms, as slightly yellowish that are not easily to detect by human eye, confirm that the remote sensing technologies can be useful to quantitative and qualitative analysis of spatial distribution of GLSD symptomatic plants.

Effect of sodium arsenite on the life cycles of pathogenic agents involved in wood grapevine diseases. P. LARIGNON. *Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, 7 avenue Cazeaux, F30230, Rodilhan, France. E-mail: philippe.larignon@vignevin.com*

Wood grapevine diseases (esca disease, Botryosphaeria dieback) were controlled in France by the use of sodium arsenite. An understanding of its mode of action will enable to find substitute products. Our aim was to determine the effect of arsenite on the life cycle of different pathogens and on their main microflora. Experiments were made in a vineyard close to Nîmes, cultivar Merlot on SO4 rootstock, planted in 1974 and cordon trained. Nine percent of the vines showed characteristics symptoms of esca and Botryosphaeria dieback in 2015. Twenty-two days after pruning made 20 January 2016, sodium arsenite (1250 G/L) was applied with a sprayer on the upper part of the plant (arm, spur, canes) to the point of run-off. Each week, thirty spurs were collected randomly until 2 May 2016. Other samples (spurs and shoots) were also taken at phenological stages of inflorescences clearly visible, flowering, bunch closure and veraison, to follow the colonization of pathogens in the shoots. Comparative studies of treated and untreated plants showed that the microflora is modified in the woody tissues and bark subjacent to pruning wounds. *Diplodia seriata*, *Diaporthe* spp. and *Epicoccum* sp. were rarely isolated from bark and wood tissues of spurs and shoots in treated plants between the date of the treatment and veraison, while *Cladosporium* sp. and *Pullularia* sp. were frequently isolated. *Phaeomoniella chlamydospora* was less detected from woody tissues in the spurs compared to the control. This study also showed that *D. seriata* was more often isolated from woody tissues and bark of diseased shoots than apparently healthy shoots. In conclusion, a high reduction of sources of inoculum due to the treatment with sodium arsenite can be hypothesized. This study suggests that annual contaminations could be responsible for herbaceous symptoms. Our future studies need to further validate this hypothesis.

Influence of rainfall on development of esca disease. N. LATINOVIĆ and J. LATINOVIĆ. *University of Montenegro, Biotechnical Faculty, Mihaila Lalića 15, 81000 Podgorica, Montenegro. E-mail: nlatin@ac.me*

Grapevine is a very important crop in Montenegro. Esca disease is widespread and appears in vineyards older than 5 years with the number of infected vines correlated to grapevine age. The highest percentage of infected vines recorded so far was 66% in a 29-year-old vineyard. In order to detect the spread of esca, three plots (cultivar Vranac) in the vineyard (22 ha) at the Biotechnical Faculty in Podgorica were monitored for symptoms in July of 2009, 2015 and 2016. The first plot was established in 2004 with a total of 4900 vines. In 2009 three vines (0.1%) were recorded with disease symptoms, in 2015 the number of symptomatic vines was 39 (0.8%), increasing to 187 (3.8%) in 2016. The second plot was established in 2005 with 2000 vines. In 2009 the number of vines with esca symptoms was 5 (0.2%), in 2015 it was 12 (0.6%), increasing to 38 (1.9%) in 2016. The third plot was set up in 2007 with 5000 vines. No vines were recorded with symptoms in 2009, 30 vines (0.6%) were symptomatic in 2015 and 61 (1.6%) in 2016. Results indicated an increase of infected vines in correlation

to vineyard age. However, the number has increased exponentially, especially between 2015 and 2016. Therefore, besides age, this sudden increase could be attributed to the rainfall and its distribution recorded during the growing season from the beginning of vegetation (early-April) to the assessment of symptoms (mid-July) in 2016 compared with 2015. In 2015 and 2016, total rainfall in April was 66.2 mm and 72.6 mm, in May was 32.8 mm and 215.4 mm, in June was 28.6 mm and 137.6 mm, and in the first half of July was 0.2 mm and 62.8 mm, respectively. In 2016, rainfall was unusual in Podgorica where dry weather normally prevails in May-July.

Spatiotemporal modelling of Esca complex grapevine disease at vineyard scale. S. LI[1,2], Y. CAO[1,2], D. MORAIS[1,2], S. BASTIEN[1,2], V. BONNARDOT[3], A. GEGOUT-PETIT[4] and L. GUÉRIN-DUBRANA[1,2]. [1]*Université de Bordeaux, ISVV, UMR1065 Santé et Agroécologie du Vignoble, Bordeaux Sciences Agro, F-33175 Gradignan, France.* [2]*INRA, ISVV, UMR1065, F-33140 Villenave d' Ornon, France.* [3]*LETG-Rennes, UMR6554 CNRS, Université Rennes 2, F-35043 Rennes, France.* [4]*Institut Elie Cartan, Université de Lorraine, F-54506 Vandoeuvre-lès-Nancy, France. E-mail: lucia.guerin@agro-bordeaux.fr*

Esca complex is a disease that represents one of the major threats to viticulture around the world. To better understand the underlying process of the spread of esca, in particular surveying expression of foliar symptoms (Grapevine Leaf Stripe Disease and apoplectic foliar symptoms) and the environmental risk factors associated with this disease, we carried out quantitative analyses of its spatiotemporal development at vineyard scale. In the first step, non-parametric statistical tests, based on join count statistics were developed in order to assess the capacity of the disease to spread within vineyards of the Bordeaux region. Data from over 8 years of annual records, containing between 1,200 and 2,300 contiguous Cabernet Sauvignon vines from 15 mature vineyards were used. Among vineyards, a large range of spatial patterns, from random to strongly structured, associated with various prevalence, were found. In the vineyards with strongly aggregated patterns, there was no significant increase in the size of the clusters over time. These results, associated with those from the analysis of the closed spatial dependence between previously and newly symptomatic vines, suggested a limited potential for secondary local spread from neighboring symptomatic vines. Consequently, in the second step, to test the effect of the environment in the disease spread, we applied spatiotemporal logistic regression models which explicitly accounted for both local environmental covariates and spatiotemporal correlation. The Bayesian inferences of these models were performed by using the INLA (Inverse Nested Laplace Approximation) approach. The models were assessed using the spatiotemporal data from three vineyards showing a strongly structured spatial pattern, and including covariates, such as climatic indicators and physiological plant indicators. The results showed the effect of both environmental factors and physiological indicators on the occurrence of esca complex symptoms. The research opened perspectives for risk prediction and recommendations of specific control strategies to prevent the spread of esca.

Detection and quantification of black foot pathogens in grapevine nursery soil, rotation crops and weeds in South Africa. S. DENVER LANGENHOVEN[1], F. HALLEEN[1,2] and L. MOSTERT[1]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602.* [2]*Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599. E-mail*: *lmost@sun.ac.za*

Black-foot disease is important in the grapevine nurseries of South Africa. To determine the role of rotation crop on black foot pathogens in nurseries a three year survey of plants and soil was conducted at five nurseries between 2013 and 2015. In 2013 and 2015 grapevines were sampled (10 per nursery), while in 2014 rotation crops and weeds were sampled. The rotation crops sampled included Triticale, lupins, canola, white mustard and forage radish. Ten soil samples per nursery, to the depth of 60 cm, were also collected. Isolations were made from the roots and basal ends of the rootstocks, rotation crops and weeds. In 2013, the predominant fungal pathogens isolated from grapevine roots and crowns were *Dactylonectria macrodidyma* (22 vines) and *Campylocarpon fasciculare* (six vines). In 2015, *Campylocarpon pseudofasciculare* (20 vines) was the predominant fungal pathogen followed by *Campylocarpon fasciculare* (11 vines). *Dactylonectria macrodidyma* (three plants), *D.*

novozelandica (two plants) and *D. pauciseptata* (two plants) were obtained from Triticale roots. *Dactylonectria macrodidyma* was found once from the weed, Corn spurry. No black foot pathogens were isolated from the roots of the rotation crops: lupins, canola, white mustard and forage radish. Quantitative real-time PCR (qPCR) assays developed for the genus '*Cylindrocarpon*' was used to detect and quantify *Dactylonectria* and *Ilyonectria* spp. from rhizosphere soil. Black foot pathogens were detected in all soil samples from the five nurseries, across the three years. The mean DNA concentration for the five nurseries over three years were: 11.32 pg.μL^{-1}, 2.65 pg.μL^{-1}, 4.54 pg.μL^{-1}, 3.03 pg.μL^{-1} and 0.32 pg.μL^{-1}. The mean DNA concentration for soil samples up to 30 cm was 5.34 pg.μL^{-1}, and between 30 and 60 cm was 3.41 pg.μL^{-1}. Apart from confirming the presence of black foot pathogens in nursery soils this is also the first report of *D. pauciseptata* and *D. alcacerensis* in South African grapevine nurseries.

Detection and quantification of Diatrypaceae and Botryopshaeriaceae inoculum in Australian vineyards. R. BILLONES-BAAIJENS[1], S. SAVOCCHIA[1], M. AYRES[2] and M.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: ssavocchia@csu.edu.au*

Eutypa dieback (ED) and Botryosphaeria dieback (BD) are major grapevine trunk diseases (GTD), causing significant yield reduction and threatening the sustainability of Australian vineyards. The spores of GTD pathogens that infect primarily via grapevine pruning wounds are generally dispersed by rain splash and travel distances depending on wind speed. However, there is limited data on the climatic conditions required for spore release by GTD pathogens in different climatic regions of Australia. This 3-year study investigated the spore dispersal patterns of GTD pathogens using Burkard spore traps, which were deployed from 2013 in South Australia (Barossa Valley and Coonawarra) and New South Wales (Hunter Valley and Griffith). Spore trap tapes were collected and replaced monthly at each site and analysed using two quantitative PCR protocols (qPCR) to detect spores of Diatrypaceae and Botryopshaeriaceae. Preliminary data analysed to date showed spores of ED and BD pathogens were released sporadically at different times of the year with the highest number of spores detected in late winter and early spring when temperatures were relatively mild and rainfall was high. However, a high number of spores were also trapped in Griffith and Hunter Valley over summer. The spore release was generally observed during or immediately after the occurrence of rain with as little as 0.2 mm of rain resulting in spore release of either pathogen but not all rain events resulted in spore release. In current research, six spore traps have been deployed across Australian wine regions, and will be monitored for a further 3 years. Data will be analysed to determine the critical times of the year when GTD pathogen spores are abundant in vineyards of the different climatic regions will be elucidated. This will assist growers in making decisions on optimal timing of pruning and wound treatment.

Response of four Portuguese grapevine cultivars to infection by *Phaeomoniella chlamydospora*. J. SOFIA[1,2], M. MOTA[2], M.T. GONÇALVES[1] and C. REGO[2]. [1]*Centre for Functional Ecology, Department of Life Sciences, University of Coimbra Coimbra. Portugal.* [2] *LEAF - Linking Landscape, Environment, Agriculture and Food, School of Agriculture, University of Lisbon, Lisbon, Portugal. E-mail: jorge.sofia@student.uc.pt*

Little is known on the response of Portuguese Dão wine appellation's most common grapevine cultivars to the causal agents of Esca and Petri disease, despite the high incidence of both diseases in the region and consequent economic losses. *Phaeomoniella chlamydospora* has been considered one of the major causal agents of these diseases in that region. In this study, the response of the four most propagated Dão grapevine cultivars – Aragonez (Tempranillo), Jaen (Menciá), Touriga Nacional and Alfrocheiro – was evaluated regarding infection by *Pa. chlamydospora*. Infections were performed during the pruning seasons of 2012, 2013 and 2015 on freshly pruned shoots of standing vines. For each cultivar, four groups of vines were formed. Each one of these groups was infected with one of three different fungal isolates from different Portuguese origins (south, centre and

northern Portugal) and one with a control solution. Nine months after inoculation, infected shoots were collected and analysed for the presence and dimension of internal visible necrosis. For each shoot, three cross sections of debarked wood (from middle lesion, lower border of the lesion and one cm below lesion border) were analysed for evaluation of *Pa. chlamydospora* eventual recovery. *Pa. chlamydospora* was consistently recovered from infected shoots, but never from control plants. All three isolates were able to infect and produce lesions on inoculated spurs. Recovery of *Pa. chlamydospora* and lesions length varied according to trial years' weather data, isolates' virulence and cultivars' sensitivity. Longest lesions were recorded in 2013. Average temperature, was never a limiting factor for pathogen infection and xylem colonization. Differences in virulence among the isolates were found. Isolates age did not influence its virulence. No relation between Dão native isolates and virulence was observed. Low values of recovery of *Pa. chlamydospora* were obtained from symptomless wood, indicating that a pruning control strategy of leaving longer spurs, might prevent trunk colonization by *Pa. chlamydospora*. Cultivars Alfrocheiro and Touriga Nacional proved to be the most sensitive to infection by *Pa. chlamydospora*, while cv. Jaen appeared as less sensitive.

Does infection by *Phaeomoniella chlamydospora* alters musts? A curiosity trial on three Portuguese grapevine cultivars. J. SOFIA[1,2], M. MALFEITO-FERREIRA[2], M. T. GONÇALVES[1] and C. REGO[2]. [1]*Centre for Functional Ecology (CFE), Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456, Coimbra, Portugal.* [2]*Linking Landscape, Environment, Agriculture and Food Research Center (LEAF), Instituto Superior de Agronomia (ISA), University of Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. E-mail: jorge.sofia@student.uc.pt*

Esca is a widespread disease in the Portuguese Dão wine appellation. Esca manifestation can be divided into apoplectic or chronic, where some grapevines can die suddenly or while some just show typical symptoms like tiger striped leaves, foliage decline, black measles and delayed fruit maturation. Grapevines with chronic symptoms are known to decline and eventually die, sometimes some years later. These vines can remain in the vineyards as their grapes can not be harvested as they will not reach full maturation. In this study, the effect of infection by three different *Phaeomoniella chlamydospora* Portuguese isolates was evaluated on the production of three Dão grapevine cultivars (Jaen, Aragonez/Tempranillo and Alfrocheiro). Inoculations were performed during 2013 pruning season on freshly pruned canes of Guyot trained standing vines. During the subsequent harvest, all clusters produced from the one year old canes of the infected spurs, were harvested. Number of clusters, clusters weight, must volume, pH and probable alcohol content of the obtained musts per spur were recorded. Statistical analysis showed the total clusters weight and must volume of grapes from infected spurs were not significantly different from those of the control plants. However, probable alcohol content was lower on musts obtained from infected plants than on the control plants ones. These preliminary data was based only on one year trial but these results suggest a possible influence of *Pa. chlamydospora* infection on grape ripening, emulating the weak maturation observed on grapes obtained from grapevines affected with grapevine leaf stripe disease in Esca complex.

Hiding in the bushes: woody plants near vineyards harbour grapevine trunk pathogens. C. SPIES[1,2], P. MOYO[2], M. BESTER[1,2], P. LESUTHU[1], I. DU PLESSIS[1,2], W. VAN JAARSVELD[2], L. MOSTERT[2] and F. HALLEEN[1,2]. [1]*Plant Protection Division, ARC Infruitec-Nietboorbij, Private Bag X5026, Stellenbosch, 7599, South Africa.* [2]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa. E-mail: spiesc@arc.agric.za*

Grapevine trunk diseases such as esca, Eutypa dieback, Botryosphaeria canker, Petri disease and Phomopsis dieback are caused by fungi from the Botryosphaeriaceae, Diaporthaceae, Diatrypaceae, Hymenochaetaceae, Phaeomoniellaceae, Togniniaceae, and some other members of the Ascomycota and Basidiomycota. In South Africa, 78 species from these fungal groups have been associated with symptomatic grapevine wood. Some grapevine trunk pathogens are known to have wide host ranges, eg. *Eutypa lata* is known globally from 65 plant host genera (Farr and Rossman). This raises the question of whether crops and other plants near vineyards can harbour grapevine trunk pathogens and serve as inoculum

sources of these fungi. This question is being investigated through an ongoing survey of woody hosts growing near vineyards in the Western Cape. To date isolations have been made from almost 1000 samples of wounds and symptomatic wood from more than 35 hosts in the Western Cape. Twenty-five of these hosts are represented by more than 10 samples each. A total of 46 species known to occur on grapevines in South Africa were also identified from these 25 hosts. Several grapevine trunk pathogens exhibit broad host-ranges, eg. *Eutypa lata*, *Neofusicoccum australe*, *Phaeoacremonium minimum*, and *Phaeoacremonium scolyti* were recovered from more than 14 hosts each. Other species appear to be host-specific (eg. *Diaporthe ampelina*) or have very limited host ranges (eg. *Phaeomoniella chlamydospora* was only recovered from cherry and plum in addition to grapevines). Hosts from which 15 or more different GTD pathogens were recovered include apple, fig, guava, loquat, Peruvian pepper, persimmon, quince, and rose. The relative importance of individual host species as potential inoculum sources, the movement of inoculum between these hosts and vineyards, and cross-pathogenicity of fungal isolates from different hosts require further investigation.

Trunk and scaffold canker diseases of almond: The Californian experience. F. TROUILLAS, L. HOLLAND, M. NOURI, M. CRESPO PALOMO and N. MORRIS. *University of California, Davis, Department of Plant Pathology and Kearney Agricultural Research and Extension Center, 9240 South Riverbend Avenue, Parlier, CA 93648. E-mail: flotrouillas@ucanr.edu*

In 2016, the California almond industry accounted for about 5.9 billion dollars in farm value and the total almond acreage was estimated at 1.11 million acres. Trunk and scaffold canker diseases (TSCD) of almond can cause significant yield and tree losses within orchards, while also reducing orchard life spans. TSCD of almond have become a major concern in recent years in California as they can affect young, recently planted orchards, and usually become prevalent as orchards get older. Symptoms of TSCD include discoloration of vascular tissues, wood necrosis and extensive gumming. Dieback of scaffold branches can occur and eventually the whole tree may die. During the years 2015 and 2016, surveys were conducted throughout California to determine the main pathogens associated with TSCD of almond. A total of 292 fungal isolates were isolated from cankers and identified for this study. Results revealed a broad diversity of fungi associated with cankers including Botryosphaeriaceae spp., *Ceratocystis variospora*, *Eutypa lata*, *Cytospora* spp., *Collophora* spp., *Phomopsis*/*Diaporthe* spp. and Basidiomycete spp. Botryosphaeriaceae spp. accounted for the largest group of pathogens with *Spencermartinsia viticola, Neofusicoccum vitifusiforme, Neoscytalidium dimidiatum, Diplodia mutila* being reported for the first time in California almond. The fungus *Eutypa lata* was detected mainly from cankers collected in the Northern part of the state. *Collophora hispanica* and *C. paarla* constituted first reports in California almond. Ceratocystis cankers were common in orchards that suffered bark injuries caused by mechanical shakers. Field observations suggested that pruning wounds made for primary and secondary scaffold selections serve as the main sites for infection with canker pathogens. Pathogenicity tests are underway to determine the most aggressive canker pathogens of almond. Fungicides, pastes and sealants as well as biocontrol agents are being screened to determine the most effective products for pruning wound protection and management of TSCD.

Grapevine trunk diseases epidemiological studies in British Columbia: implementation of droplet digital™ PCR. J.R. ÚRBEZ-TORRES, M. WALKER, J. BOULÉ and D.T. O'GORMAN. *Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Box 5000. Summerland, BC V0H 1Z0, Canada. E-mail: joseramon.urbeztorres@agr.gc.ca*

Botryosphaeria dieback (BD) and Eutypa dieback (ED) are the most widespread and prevalent grapevine trunk diseases (GTD) in British Columbia (BC). Fungi associated with BD and ED infect grapevines primarily through pruning wounds. Spores (ascospores or conidia) reside in fruiting bodies (perithecia or pycnidia) embedded in the bark of cankered spurs, cordons, and/or trunks and are airborne under favourable climatic conditions. Accordingly, understanding the environmental factors that favour spore release and hence, knowledge of the high risk infection periods throughout the growing season are critical for the development of effective

control strategies. The main objective of this study was to detect and quantify BD and ED inoculum in BC vineyards by implementation of droplet digital™ PCR (ddPCR™, Bio-Rad Laboratories, Inc.), a recently developed technology that provides absolute quantification of target DNA or RNA molecules. Five cyclone samplers for airborne particles (Burkard Manufacturing Co. Ltd.) were deployed in five different vineyards along the Okanagan Valley, the largest grape-growing region in BC. Samples were collected weakly since April/May of 2014. Total genomic DNA was extracted using the DNeasy PowerSoil Kit (MO BIO Laboratories) with some modifications and quantified in the ddPCR™. BD and ED inoculum were quantified simultaneously in the same sample by using multi-species primers in a multiplex reaction. To date, preliminary results show spores of BD and ED to be released intermittently throughout the growing season (March/April to October). Overall, the first release of inoculum was detected at the end of winter beginning of spring when average temperatures were above freezing and correlated, though not always, with rainfall. Significant numbers of spores were also trapped during the summer months. Results of this study will assist to develop more effective control strategies by optimizing chemical and/or biological agent applications as well as identifying the best time for pruning in BC.

Study of the contamination kinetics of young plants by fungi responsible for grapevine trunk diseases, from plant material produced free from pathogens. O. YOBREGAT[1], P. LARIGNON[2], B. MILLE[1], P. BLOY[3], D. CARCENAC[1], P. SACCHARIN[1], F. DIAS[1] and S. CHARLOT[4]. *[1]Institut Français de la Vigne et du Vin, Pôle Sud-Ouest, 81310 Lisle sur Tarn, France. [2]IFV Pôle Rhône-Méditerranée, 30230 Rodilhan, France. [3]IFV Pôle National Matériel Végétal, 30240 Le Grau du Roi, France. [4]Domaine Expérimental Viticole Tarnais, 81 600 Gaillac, France. E-mail: olivier.yobregat@vignevin.com*

The role of the many fungal species involved in grapevine trunk diseases has been the subject of numerous studies over the past 20 years. Observations of symptoms in plots sometimes less than 10 years old raised questions among vinegrowers and scientists about the involvement of plant material in these early attacks. Studies carried out in several countries examined the contamination of young grafted plants just issued from nurseries and, more broadly, the role of plant material in the spread of pathogens. Numerous fungi have been shown to spread throughout the nursery process. However, no study made possible to link the presence of this primary inoculum and the subsequent symptoms observed. In the same way, the questions about the kinetics of post-plantation contamination by the external environment have not been or very little addressed. From a plot implanted in 2013 comparing Sauvignon blanc on rootstock 110 Richter, obtained by herbaceous grafting and containing no pathogenic fungus, with the same material produced by ligneous grafting, the recontamination of the plants was measured. In the post-planting season (2014–2015), trunk sampling was carried out on both types of material, and a sampling program was planned, implementing annual microbiological analysis. Immediately after planting, the first measurements show colonization of the plants by pathogens, notably Botryosphaeriaceae species, resulting after two years in a rapid convergence of the health status of the two studied lots. The fungi are found both on the surface (bark) and inside the woody tissues. These results may call into question the interest of extensive disinfection in the nurseries, and argue for a decisive role for the external environment in contamination. This study should be continued and other points explored, in particular concerning the origin of the inoculum, its ways of penetration and the occurrence of the first symptoms.

Plant-pathogen interactions

Influence of carbohydrate on the biosynthesis of laccases and the temporal production of secondary metabolites by grapevine isolates of *Neofusicoccum parvum*e. A. BOULANGER[1] G. SCHMITT[1], O. CRAGUE[1], C. JOYEUX[1], C. REGO[2], P. LARIGNON[3], F. FONTAINE[4] and E. ABOU-MANSOUR[5]. *[1]Laboratoire de Chimie Organique et Bioorganique EA-4566, Université de Haute-Alsace, IRJBD 3 bis rue Alfred Werner, 68093 Mulhouse Cedex, France. [2]Centro de Investigação em Agronomia, Alimentos, Ambiente e Paisagem (LEAF), Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. [3]Institut Français de la Vigne et du Vin Pôle Rhône-Méditerranée, France, Domaine de Donadille, 30230 Rodilhan, France. [4]SFR Condorcet, Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire*

Stress, Défenses et Reproduction des Plantes, BP 1039, 51687 Reims Cedex 2, France. [5]*Département de Biologie, Université de Fribourg, 10 rue du Musée 10, Fribourg, Suisse. E-mail: eliane.abou-mansour@unifr.ch; anna.boulanger@uha.fr*

Neofusicoccum parvum (Botryosphaeriaceae, Ascomycetes) is a plant pathogenic fungus, widespread on woody hosts and associated with botryosphaeria dieback of grapevine. *N. parvum* was reported to produce laccases and several bioactive secondary metabolites, including compounds related to the dihydroisocoumarins and toluquinols families such as (*R*)-mellein and (+)terremutin. In this poster, we examine the influence of carbohydrate source and culture time period**,** on the production of laccases and the biosynthesis of secondary metabolites**,** in order to understand their regulation. Two strains of *N. parvum* (Bourgogne 2S-16 and Bt67) and four different concentrations (10, 20, 50, 100 g L^{-1}) of a monosaccharide (glucose) or a disaccharide (sucrose) as substrates were used in the experiments over a 17 day period. Both strains were able to grow and produce laccases and secondary metabolites on the tested carbohydrate sources. A change in the metabolite profile was recorded especially for Bt67 at 50 and 100g L^{-1} of sucrose suggesting that a high carbohydrate concentration plays a significant role in the regulation of their biosynthesis.

Cloning and expression of necrosis and ethylene-inducing proteins (NEPs) from *Neofusicoccum parvum*. F. NAZAR POUR[1], R. COBOS[2], J.-J. RUBIO-COQUE[2], A. ALVES[1], A.C. ESTEVES[1] and A.S. DUARTE[1]. [1]*Departament of Biology, CESAM, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal.*[2]*Instituto de Investigación de la Viña y el Vino, University of León. Av. Portugal, 42, 24071 León, Spain. E-mail: artur.alves@ua.pt*

Neofusicoccum parvum, one of the causal agents of Botryosphaeria dieback of grapevine, is known as an aggressive fungus infecting a wide range of hosts. *N. parvum* virulence has been related to the ability of this fungus to colonize woody tissue combined with the production of several phytotoxins and also the expression of extracellular proteins with phytotoxic properties. However, a complete understanding of the pathogenicity mechanism is still lacking. Analysis of the *N. parvum* genome led to the identification of several putative genes encoding necrosis and ethylene-inducing proteins (NEPs). These have a conserved GHRHDWE motif and have been found in a diversity of microorganisms with the ability to induce necrosis and trigger defense responses in their hosts. Consequently, analysis of these genes and their proteins can help in understanding their role in *N. parvum* pathogenicity. In order to attain our goal, 4 of the 6 NEP genes were successfully cloned from *N. parvum* as a His tag fusion protein and expressed in *E. coli* BL21 (DE3). Subsequently, the recombinant proteins were purified by affinity chromatography. In the future, pure recombinant proteins will be characterized according to their phytotoxic effect on grapevine thus providing additional insight into the pathogenicity mechanism of *N. parvum*.

Screening of grapevine rootstock germplasm for resistance to *Cadophora luteo-olivacea*, *Neofusicoccum parvum* and *Phaeomoniella chlamydospora*. M. BERBEGAL[1], C. BERLANAS[2], D. GRAMAJE[2], G. MUÑOZ-ORGANERO[3], F. CABELLO[3], M. SIEBERHAGEN[4], M. NOCENTINI[5] and J. ARMENGOL[1]. [1]*Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain.* [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, 26071 Logroño, Spain.* [3]*Instituto Madrileño de Investigación y Desarrollo Rural Agrario y Alimentario (IMIDRA), Alcalá de Henares, Madrid 28800, Spain.* [4]*University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa.* [5]*Università degli Studi di Firenze, DISPAA, Piazzale delle Cascine, 28, 50144 Firenze, Italy. E-mail: jarmengo@eaf.upv.es*

Grapevine rootstocks contribute to control of disease related problems in vineyards, being critical for long-term sustainability. Rootstock materials are exposed to infections by fungal trunk disease pathogens, however, they can harbour different levels of tolerance to Petri disease or Botryosphaeria dieback pathogens. The objective of this study was to examine the response of grapevine rootstocks to inoculation with *Cadophora luteo-olivacea*, *Neofusicoccum parvum* and *Phaeomoniella chlamydospora* in two independent experiments. In experiment 1, a simple hydroponic culture method for the inoculation of 48

rootstocks with *P. chlamydospora* spore suspensions in a growth chamber at 20 °C was developed. This allowed testing of a high number of material in a small space. Nine cuttings per treatment were used and the experiment was repeated. Forty-five days after inoculation, all rootstocks showed different degrees of leaf chlorosis and wilting, and *P. chlamydospora* was isolated from 80% of the inoculated rootstocks. In experiment 2, dormant cuttings of 22 rootstocks were cut into uniform lengths containing four buds. Potted rootstock cuttings were then wounded between the two upper internodes with a cork borer and inoculated with 5 mm mycelium PDA plug of *C. luteo-olivacea* or *N. parvum*. Negative controls were inoculated with uncolonized agar plugs. Twelve cuttings per treatment were used and the experiment was repeated. Inoculated cuttings were immediately placed in a greenhouse at 25 °C. Ten (*C. luteo-olivacea* inoculated cuttings) and 6 (*N. parvum* inoculated cuttings) months after inoculation, rootstock cuttings were collected and inspected for lesion development. All rootstocks were susceptible to infection by *C. luteo-olivacea* and *N. parvum*, but the severity of internal wood discolouration varied significantly, suggesting possible disease tolerance in some accessions. These assays are being repeated and will confirm that some rootstocks are less susceptible to colonization by fungal trunk pathogens.

Imaging methods to evaluate rootstock colonization by a GTD related pathogen and its control in grapevine nursery process. E. BATTISTON[1-3], S. COMPANT[2], F. FONTAINE[3], S. DI MARCO[4] and L. MUGNAI[1]. [1]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DiSPAA) - Sez. Patologia vegetale ed Entomologia, Università degli Studi di Firenze, P.le delle Cascine 28, 50144 Firenze, Italy;* [2]*AIT Austrian Institute of Technology GmbH, Center for Health & Bioresources, Bioresources Unit, 3430 Tulln, Austria;* [3]*URVVC EA 4707, Laboratoire Stress, Défenses et Reproduction des Plantes, Université de Reims Champagne-Ardenne, BP 1039, Reims (Cedex 2) 51687, France;* [4]*Istituto di Biometeorologia (IBIMET), CNR, Via Gobetti 101, 40129 Bologna, Italy. E-mail: enrico.battiston@unifi.it*

The application of imaging methods, already applied to study plant-microbe interactions, could represent a crucial tool to understand the effect of experimental treatments on Grapevine Trunk Disease (GTD) related pathogens and on the tissues they colonized. Trials were carried out to assess the efficacy of treatments based on 2 copper salts and an innovative co-adjutant applied to control the wood colonization by a GTD related pathogen on propagating material. The distribution and the effect of the treatments in rootstock that had been inoculated with a *Phaeoacremonium minimum::gfp7* or with the wild-type strain, compared to non-inoculated control rootstocks were studied. Treatments were applied during the hydration water process of rootstocks (*Vitis Berlandieri* x *Vitis riparia* cv. K5BB) and scions (*Vitis vinifera* L, cv. Chardonnay). After callusing, grafted vines were grown under greenhouse conditions. Grafted vines were inoculated with an agar plug containing the pathogen by drilling a hole in the rootstock. Plants were then harvested fifteen weeks post-inoculation and woody tissues close to the inoculation site were observed using a Confocal Laser Scanning Microscopy (CSLM) and an Environmental Electron Scanning Microscope (ESEM). Results were presented according to (i) the *P. minimum* strain, wild-type strain and *P. minimum::gfp7*, (ii) the analyzed tissues in the inoculation site, i.e. cavity, pith border and xylem plus parenchyma, (iii) and the applied treatment. The imaging methods revealed distinctly the fungal presence and colonization within and around the inoculation site in the rootstocks, treated with experimental substances and untreated. By processing the images with Image J software 1.47v it was possible to estimate the area of *P. minimum::gfp7* colonization confirming the large quantity of pathogen present in the untreated but inoculated control rootstocks and in uninoculated control rootstocks treated by the pure delivery system. Treatments based on copper limited the pathogen colonization to different extents. The correlation between the observed *P. minimum::gfp7* at the CLSM and any morphological response induced by the treatments was verified by observing the non-inoculated plant material by the ESEM.

Developing a delivery system for the control of plant diseases: from leaf pathogen control to grapevine trunk diseases control in the nursery. E. BATTISTON[1-3], L. MUGNAI[1], M. NOCENTINI[1], T. CINELLI[1], F. OSTI[2], F. FONTAINE[3] and S. DI MARCO[2]. [1]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DiSPAA) - Sez. Patologia vegetale ed Entomologia, Università degli Studi di Firenze,*

P.le delle Cascine 28, 50144 Firenze, Italy. [2]*URVVC EA 4707, Laboratoire Stress, Défenses et Reproduction des Plantes, Université de Reims Champagne-Ardenne, BP 1039, 51687 Reims Cedex 2, France;* [3]*Istituto di Biometeorologia (IBIMET), CNR, Via Gobetti 101, 40129 Bologna, Italy. E-mail: enrico.battiston@unifi.it*

Grapevine trunk disease management has several critical aspects, and one of the main ones is the lack of suitable products with low toxicity but good efficacy. This indicated a need to investigate the applicability of micro-structured inorganic crystals based on a specific apatitic phase of calcium phosphate (carbonate hydroxyapatite, CHA) in plant protection, already in use in the medical field. To this aim, several formulations were prepared, functionalizing CHA with two different copper salts (tribasic sulphate and sulphate pentahydrate) and uploading afterwards zinc sulphate and a commercial essential oil product. The formulations were applied successfully in downy mildew control trials on grapevine to investigate their drug-delivery proprieties in enhancing the efficacy of bioactive substances. The most stable formulations were obtained with copper tribasic sulphate and zinc sulphate. The essential oil seems to only bind with CHA by physical interactions. The formulations were also tested *in vitro* to evaluate the possible application in GTD pathogens control in the nursery. Growth inhibition tests were carried out on *Phaeomoniella chlamydospora, Phaeoacremonium minimum* and *Neofusicoccum parvum*, and also on *Botrytis cinerea,* given the great relevance of this pathogen in the nursery. *In vitro* tests showed CHA stimulated the growth of all the fungi assessed, and the most efficient formulation was based on CHA and copper sulphate, followed by the CHA and copper tribasic sulphate based formulation. The formulations were applied in the nursery during the hydration of the propagating material, a proportion of which was afterwards artificially inoculated with *P. chlamydospora.* None of the treatments showed phytotoxic effect on plant growth and yield. Leaves where sampled from the plants for transcriptomic analysis (RT-PCR) in order to evaluate their effect on the plant defense response. Furthermore data were obtained on reisolating *P. chlamydospora* from the inoculated propagation material. The pathogen reisolation confirmed the *in vitro* test results, revealing the non-toxic effect of the pure CHA and positive pathogen control enhanced by the formulation CHA-copper sulphate pentahydrate.

Variation amongst 'Tempranillo' clones in susceptibility to *Neofusicoccum parvum*. C. BERLANAS[1], A. SONGY[2], C. CLÉMENT[2], F. FONTAINE[2] and D. GRAMAJE[1]. [1]*Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas, Universidad de la Rioja, Gobierno de La Rioja, Ctra. LO-20 Salida 13, 26071 Logroño, Spain.* [2]*SFR Condorcet, Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire Stress, Défenses et Reproduction des Plantes, BP 1039, 51687 Reims Cedex 2, France. E-mail: david.gramaje@icvv.es*

Botryosphaeria dieback is caused by a number of Botryosphaeriaceae spp. belonging to six genera that impairs vineyard productivity and longevity worldwide. Among them, *Neofusicoccum parvum* has been shown to be one of the most virulent species associated with this disease. The aim of this study was to identify sources of resistance to *N. parvum* among the 'Tempranillo' germplasm collection of the ICVV in Spain. For this purpose, dormant grapevine cuttings of 61 'Tempranillo' clones were cut into uniform lengths containing four buds. Potted cuttings were then wounded between the two upper internodes with a cork borer and inoculated with a 5-mm mycelium agar plug of *N. parvum* strain BV-056. Negative controls were inoculated with uncolonized agar plugs. Twelve cuttings per treatment were used and the experiment was repeated. Five months after inoculation, cuttings were collected and lesion length measured. In addition, to investigate the induction of defense gene expression in grapevines, leaves from control and inoculated plants were studied at 2 and 5 months after inoculation. The severity of internal wood symptoms caused by *N. parvum* varied considerably amongst the 'Tempranillo' clones. No foliar symptoms were observed during the experiment. The most susceptible clone (234) and the most tolerant one (110) were selected for real-time RT PCR analysis of relative gene expression. The relative expression patterns in leaves compared to the control treatment were different in both 'Tempranillo' clones five months after inoculation for most of the targeted genes. In addition, the patterns were also different two months after inoculation for *SOD* (over-expressed in clone 110 and down-regulated in clone 234) and *Gluc* (over-expressed in clone 234 and weakly down-regulated in clone 110). This detached cutting assay is being repeated and will confirm that some 'Tempranillo' clones are less susceptible to colonization by *N. parvum*.

Levels of phytoalexins in leaves of vines affected by GLSD at different degree of severity. F. CALZARANO[1], V. D'AGOSTINO[1], F. OSTI[2] and S. DI MARCO[2]. [1]*Università degli Studi di Teramo, Facoltà di BioScienze e Tecnologie Agro-Alimentari ed Ambientali, Via R. Balzarini 1, 64100 Teramo, Italy.* [2]*Istituto di Biometeorologia (IBIMET), CNR, Via Gobetti 101, 40129 Bologna, Italy. E-mail: fcalzarano@unite.it*

The foliar symptom expression in grapevine leaf stripe disease (GLSD) causes quantitative and qualitative yield losses correlated with the severity of the symptoms. A preliminary investigation of symptom formation dynamics assessing the amount of resveratrol in leaves collected at different symptoms severity and phenological growth stages showed a higher presence of *trans*-resveratrol in symptomatic leaves. The present study extended the investigations to the dynamics of other phytoalexins (including *trans*-resveratrol), recorded in leaves of grapevines in two vineyards in the Abruzzo region of central Italy. The levels of substances were observed in leaves collected from symptomatic, asymptomatic and healthy vines. For symptomatic leaves, four categories depending on the extent of symptoms were considered: 1) 5% chlorosis; 2) 20% chlorosis; 3) 40% chlorosis + necrosis, 4) 65% chlorosis + necrosis. The leaves were collected at the "berries beginning to touch," "berries developing color" and "berries ripe for harvest" stages. For each phytoalexin, results showed a pattern similar to what was preliminarily observed for *trans*-resveratrol, with a progressive increase in phytoalexin levels, increasing associated with the increased severity of leaf symptoms. It was particularly evident at the "berries beginning to touch" and "berries ripe for harvest" stages. A laboratory assay was also performed: petioles of healthy leaves were immersed in culture filtrates of *Phaeomoniella chlamydospora* (Pch), before and after immersion in different concentrations of *trans*-resveratrol or pterostilbene, to evaluate possible effects on the leaf lamina. The addition of *trans*-resveratrol and pterostilbene did not influence the effect of Pch filtrates. Overall, our results seemed to exclude the involvement of phytoalexins in the alteration in the formation of the leaf lamina that lead to the foliar symptoms.

Discovery of virulence factors of grapevine trunk pathogens by whole-genome sequencing, comparative genomics, and community-wide metatranscriptomics. D. CANTU[1], A. MORALES-CRUZ[1], M. MASSONNET[1], R. FIGUEROA-BALDERAS[1], D. P. LAWRENCE[2], R. TRAVADON[2], P.E. ROLSHAUSEN[3] and K. BAUMGARTNER[4]. [1]*Department of Viticulture and Enology, University of California Davis, One Shields Ave, Davis CA 95616, USA.* [2]*Department of Plant Pathology, University of California Davis, One Shields Ave, Davis CA 95616, USA.* [3]*Department of Botany & Plant Sciences, University of California Riverside, 900 University Ave, Riverside CA 92521, USA. E-mail: dacantu@ucdavis.edu*

We have sequenced and assembled whole genomes of the most common grapevine trunk pathogens (GTPs), namely *Eutypa lata, Phaeoacremonium minimum, Phaeomoniella chlamydospora, Diaporthe ampelina, Diplodia seriata, Neofusicoccum parvum,* and other 16 Botryosphaeriaceae species. Using single molecule sequencing technology we reconstructed (telomere-to-telomere) the complete DNA sequences of all chromosomes of *E. lata, N. parvum* and *P. minimum*. The availability of complete genomes annotated focusing on specific families of virulence factors, such as plant cell wall-degrading functions and gene clusters involved in secondary metabolism, has provided a first comprehensive view on the virulence potential of these pathogens. Comparative analysis of gene family evolution pointed to specific patterns of gene family expansion with lineage-specific evolution of distinct mechanisms of virulence, such as specific cell wall oxidative functions and secondary metabolic pathways in *N. parvum*, *Dia. ampelina*, and *E. lata*. RNA sequencing was also performed to improve gene model calls in the genomes and determine expression patterns in function of growth substrates and stages of infection. Co-expression network analysis of transcriptome data revealed that physically clustered genes coding for virulence functions are induced depending on the substrate or stage of plant infection. Co-expressed gene clusters were significantly enriched not only in genes associated with secondary metabolism, but also in those associated with cell wall degradation, suggesting that dynamic co-regulation of transcriptional networks contributes to multiple aspects of GTP virulence modulation. The availability of annotated genomes for the most relevant GTPs also provided the unprecedented opportunity to conduct community-level transcriptomics (i.e., metatranscriptomics) and

monitor simultaneously the virulence activities of multiple GTPs in planta. Using controlled inoculation as well as naturally-infected field samples expressing a variety of trunk disease symptoms, we show that our approach provides quantitative assessments of species composition as well as genome-wide transcriptional profiling of potential virulence factors for all co-infecting trunk pathogens.

Study of molecular mechanisms during the interaction between three grapevine cultivars and *Eutypa lata*. C. CHLOE[1-2], G. MAPPA[1], C. VRIET[1], S. LA CAMERA[1], G. FERRARI[2] and P. COUTOS THEVENOT [1]. [1]*Université de Poitiers, Unité de recherche Ecologie et Biologie des Interaction, équipe sucres et échanges végétaux-environnement, Bât. B31, 3 rue Jacques Fort, TSA 51106, 86073 Poitiers Cedex 9, France.* [2]*BNIC - Station Viticole, 69 rue de Bellefonds, BP 90018, 16101 Cognac Cedex, France. E-mail: chloe.cardot@univ-poitiers.fr*

Eutypa lata is the causal agent of Eutypa dieback or Eutypiosis, a severe and widespread grapevine trunk disease for which all grapevine cultivars are more or less susceptible. With the aim to identify molecular markers of tolerance to *E. lata*, we analyzed the physiological responses of three cultivars with contrasting level of susceptibility to this vascular pathogen: Merlot (tolerant), Cabernet Sauvignon (susceptible) and Ugni Blanc (highly susceptible). The level of susceptibility in these three cultivars was confirmed by recording foliar symptom development one year post infection in inoculated potted plants growing under controlled conditions in the greenhouse. In order to enable a rapid and efficient monitoring of the molecular dialog involved in grapevine response to infection with the wood pathogen *E. lata*, we developed a new *in vitro* simplified system of interaction using a cell culture insert (Millicell, Millipore®). In this system, foliar discs are physically separated from *E. lata* mycelium plugs by a semi-permeable membrane that allows molecular communication and plant responses to elicitors secreted by the pathogen. Following a co-culture period of three days in this *in vitro* system, we analyzed the transcriptional and biochemical responses of each of the three cultivars foliar discs. The expression profiles of a set of grapevine genes of interest, including some defense, Pathogenesis-Related (PR), sugar transporters and carbon metabolism genes, were analyzed by Real Time-quantitative Polymerase Chain Reaction (RT-qPCR). This analysis was completed with quantification of invertase activities and a measure of the accumulation of stilbenes, *trans*-resveratrol and ε-viniferin, three natural phytoalexins known to be involved in plant defense. Overall, our results bring new knowledge on the transcriptional and biochemical mechanisms involved in *Vitis vinifera* - *Eutypa lata* interaction.

Life traits of four Botryosphaeriaceae species and molecular responses of different grapevine cultivars or hybrids. A. BELLÉE[1], G. COMONT[1], A. NIVAULT[1], E. ABOU -MANSOUR[2], C. COPPIN[1], M. C. DUFOUR[1] and M.F. CORIO-COSTET[1]. [1]*INRA, UMR Santé et Agroécologie du Vignoble (1065), ISVV, Labex Cote, CS, 33882 Villenave d'Ornon, France;* [2]*Plant Biology Department, University of Fribourg, 3 rue Albert Gockel, 1700 Fribourg, Switzerland. E-mail: marie-france.corio-costet@inra.fr*

Botryosphaeriaceae is a fungal family comprising many species involved in Botryosphaeria dieback, a grapevine trunk disease of importance worldwide. Currently, the interactions between Botryosphaeriaceae species and various grapevine cultivars are poorly understood and few data are available. This study investigated various life traits of five isolates belonging to four species of Botryosphaeriaceae found in French vineyards (*Diplodia mutila*, *Diplodia seriata*, *Lasiodiplodia viticola* and *Neofusicoccum parvum*). The two species, *N. parvum* and *L. viticola*, exhibited the highest optimal growth temperature and the fastest growth rates. They were also responsible for the most extensive necrosis and cankers on three *Vitis vinifera* cultivars (Cabernet Sauvignon, Merlot, and Ugni-Blanc) exhibiting different susceptibility to Botryosphaeria dieback, and on two genotypes having resistance to downy and powdery mildew (RV4 and RV5). Identification of the extracellular toxins produced by isolates in culture media showed that the isolate of *N. parvum* had a metabolite profile that was different from the others since it produced terremutin, and salicylic acid derivatives, which are known to be virulent compounds. In a second step, life traits were associated with nondestructive monitoring of gene expression involved in the grapevine defenses of the five cultivars and genotypes, after

inoculation of Botryosphaeriaceae in wood cuttings. The transcript analyses were carried out at different times and were associated with principal component analysis (PCA). Each cultivar presented a specific transcript signature and several transcripts were correlated either with the size of necrosis/cankers or with symptom reduction, thus offering useful markers for breeding or estimating the defense status of plants.

Antagonist interaction between grapevine pathogen *Phaeomoniella chlamydospora* and *Epicoccum* sp. G. DEL FRARI, TERESA NASCIMENTO, R. BOAVIDA FERREIRA and H. OLIVEIRA. *LEAF – Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. E-mail: gdelfrari@isa.ulisboa.pt*

Grapevine wood is colonized by a diverse spectrum of fungi which interact with the plant and with each other. These interactions can be either beneficial for the plant, or neutral or negative. In the latter case, plant pathogen fungi can cause diverse symptoms described as Grapevine Trunk Diseases (GTDs). *Phaeomoniella chlamydospora* (Pch) is one of the described GTD pathogens responsible for Petri disease and commonly associated with the Esca disease complex. The plant-Pch interaction has been widely studied, however, the disease expression and possible changes in Pch colonization in the presence of other common grapevine fungal endophytes is still unclear. The ascomycetous *Epicoccum* genus is commonly associated with grapevines. The antagonistic interaction between *Epicoccum* sp., namely *E. nigrum*, and several plant pathogens has been proved, thus revealing that this fungus may have potential to be used as a Biological Control Agent (BCA) in different crops. In this work nine *Epicoccum* sp. strains were isolated from Portuguese vineyards. Their antagonistic activity was tested *in-vitro* against a Pch strain, CBS 161.90 from South Africa. All the studied *Epicoccum* sp. strains considerably reduced the growth of Pch in confrontation tests on Potato Dextrose Agar (PDA) medium. The most successful strain was also tested *in-planta*, using rooted cuttings of Touriga Nacional variety (*Vitis vinifera* L.), under greenhouse conditions. The plants were inoculated on the stem with a 6 mm diameter mycelium plug of both *Epicoccum* sp. and Pch, taken from the actively growing edge of the colony. Re-isolations of the fungi were made three months following inoculation, on PDA medium, after flame disinfection of the samples. The results of the *in-planta* experiment proved the significant reduction of Pch re-isolations comparing to the control plants. This study suggests that *Epicoccum* sp. plays an active role in the wood of grapevines by acting as antagonist against Pch spread. Further studies might result in identifying *Epicoccum* sp. as a possible BCA for Petri and Esca diseases.

The proteome of two strains of *Lasiodiplodia theobromae*. C. FÉLIX[1], J. JORRÍN-NOVO[2], A. ALVES[1] and A. C. ESTEVES[1]. *Department of Biology, CESAM, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro (Portugal); [2]Agricultural and Plant Biochemistry and Proteomics Research Group, Department of Biochemistry and Molecular Biology, University of Cordoba, Cordoba (Spain). E-mail: acesteves@ua.pt*

One of the most widely cultivated and economically important fruit crop in the world is grapevine (*Vitis vinifera* L.). In Portugal, grapevine plays a significant role as a border culture or as an extensive crop. Of the primary factors that can limit vineyard longevity and productivity, canker diseases caused by fungal pathogens are a major issue. Over the past decades, several species of the Botryosphaeriaceae have been recognized as important pathogens of grapevine, with *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. Being one of the species found in vineyards, especially in subtropical regions. *Lasiodiplodia theobromae* is a phytopathogenic fungus with a large capacity to adapt to different environments, infecting hosts over a wide range of temperatures. The nature of symptoms caused by this species on grapevines suggests that phytotoxic metabolites may be involved in the host-pathogen interaction, but also that extracellular enzymes and other proteins may have important roles in the infection mechanism. By using a proteomics approach we attempted to gain knowledge on the mechanisms of infection and virulence of *L. theobromae* on grapevines. The protein profile of the extracellular medium (secretome) and intracellular mycelium of two strains differing in virulence is being characterized using two-complementary approaches, gel-based and gel-free/shotgun.

Pathogen-Host-Interaction: Analysis of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* PKS KO-mutants due to impact on pathogenicity. J. FISCHER[1], F. FONTAINE[2] and E. THINES[1,3]. [1]*Institut für Biotechnologie und Wirkstoff-Forschung gGmbH, Erwin-Schrödinger-Straße 56, 67663 Kaiserslautern, Germany.* [2]*Université de Reims Champagne-Ardenne, Unité de Recherche de Vigne et Vin de Champagne, Laboratoire Stress, Défenses et Reproduction des Plantes, Bât. 18 BP 13039, 51687 Reims Cedex 2, France.* [3]*Mikrobiologie und Weinforschung am Institut für Molekulare Physiologie, Johann-Joachim-Becherweg 15, 55128 Mainz, Germany. E-mail: fischer@ibwf.de*

Several gen-deletion mutants of Esca-associated fungi (*Phaeomoniella chlamydospora* (*Pch*) and *Phaeoacremonium aleophilum* (*Pa*l, *Tmi*)) lacking the gene sequences of the Polyketide synthase 1 and 3 (ΔPKS1 and 3), the Tetrahydroxynaphthalene Reductase (ΔT4HNR) and the L-Ornithine-N5-Monooxigenase (ΔLOMO), respectively, were tested for their ability to infect grapevine cuttings as well as *in-vitro* plants. To compare the pathogenicity of those mutants an infection assay involving wild-type strains and mutant strains was conducted. Therefore grapevine cultivars (*V. vinifera* grapevine cuttings cv. Cabernet sauvignon and *in-vitro* plants were inoculated and monitored. The differing susceptibility of the plants and the pathogenicity of the fungal insertion mutants were then determined. Twenty one days after the inoculation with spores and incubation of the plants, all mutants were reisolated from the *in vitro* plants (roots, stems, leaves). Of these strains *Pal* ΔPKS3 and *Tmi* ΔT4HNR showed a strongly reduced infection ability rate. In general, the infection rates in the cuttings test system were much lower than in the system based on *in vitro* plants, whereby even the wild-type strains were only mainly reisolated from the leaves of the grapevine cuttings. It is estimated that the herbal response to the fungal infection is more efficient in the case of the cuttings compared to the protective mechanism of *in vitro* plants. Furthermore, the results indicate that none of the alterations in the fungal genomes were able to reduce the ability of the fungi to infect the grapevine cuttings of 'Cabernet sauvignon'. The influence of those mutations on the growth rate of the KnockOut-strains was more severe when those strains were previously tested under stress conditions on agar plates. Those stress tests included osmotic stress, heat stress, and oxidative stress. The results gathered during those pre-tests indicated that the ability to adapt to environmental stresses is highly reduced in the mutant strains.

Whole-plant defense during the early stage of infection to the wood-canker pathogen *Neofusicoccum parvum* (Botryosphaeria dieback). E. R GALARNEAU[1], M. MASSONNET[2], D. LAWRENCE[1], R. TRAVADON[1], C. WALLIS[3], D. CANTU[2] and K. BAUMGARTNER[4]. [1]*University of California-Davis Plant Pathology Department, 1 Shields Ave Davis, California, 95616, United States of America.* [2]*University of California-Davis Viticulture and Enology Department, 595 Hilgard Lane Davis, California, 95616, United States of America.* [3]*United States Department of Agriculture-Agricultural Research Service-Crops Disease, Pests, and Genetics Research Unit, 9611 S Riverbend Ave Parlier, California, 93648, United States of America.* [4] *United States Department of Agriculture-Agricultural Research Service-Crops Pathology and Genetic Research Unit, 430 G Street #4160 Davis, California, United States of America. E-mail: egalarneau@ucdavis.edu*

Botryosphaeria dieback (causal fungus *Neofusicoccum parvum*) is a wood-canker trunk disease that limits vineyard productivity. Early detection of the disease is hampered by the internal nature of the canker and absence of leaf symptoms. A detection tool for the early stage of infection, and from leaves instead of wood, would minimize the propagation of infected vines in the nursery. We previously defined the early stage of infection of inoculated vs. non-inoculated (both wounded and non-wounded) plants, based on microscopic investigation of fungal colonization at and beyond the inoculation site, and plant anatomical responses to such colonization. From both the canker region of the wood and the newly-expanding leaves, we identified differentially-expressed genes, which were specific to the early stage of infection, based on RNA-seq. Candidate genes significantly induced during the early stages of infection were associated with the following plant-defense responses: 1) cell wall modification, 2) altered primary metabolism, and 3) secondary metabolism compounds. Secondary metabolites such as stilbenoids, which are abundant in grapevine and act as phytoalexins, were altered in inoculated grapevines. Stilbenoid-related gene modification was observed

in both the wood and leaves of inoculated plants versus non-inoculated and non-wounded plants, and confirmed in wood with High-Performance Liquid Chromatography. This research is an introduction to the whole plant's response to infection by *Neofusicoccum parvum* during the early stage of infection, and will further elucidate the metabolic interactions of Botryosphaeria dieback of grapevine.

Was sodium arsenite more than a "simple" fungus killer? M. GELLON[1], M.-L. GODDARD[1,2], C. TARNUS[2], C. BERTSCH[1] and S. FARINE[1]. [1]*Laboratoire Vigne, Biotechnologies et Environnement, Université de Haute-Alsace, 33 rue de Herrlisheim, 68008 Colmar Cedex, France.* [2]*Laboratoire de Chimie Organique et Bioorganique, Université de Haute-Alsace, 3bis rue Alfred Werner, 68093 Mulhouse Cedex, France. E-mail: sibylle.farine@uha.fr*

Grapevine trunk diseases (GTDs) have since the early 2000s become the subject of major concern for the wine industry. We have recently demonstrated that extracellular proteins produced by *Neofusicoccum parvum* isolate Bourgogne (involved in Botryosphaeria dieback) can induce different defense gene expression patterns and necrosis in cells of *Vitis vinifera* cv. Chardonnay. These results suggested that the symptoms observed in leaf and berry could be caused by the extracellular compounds produced by fungi in the discolored trunk tissues translocating to the leaves via the transpiration stream. Since the use of sodium arsenite, the only compound registered to control GTDs, was prohibited in France more than 15 years ago, crop losses caused by this disease have increased and now represent at least 15% of the vineyard potential. Investigations into the mechanisms involved in the control and reduction of these diseases are currently required. As part of this research, the impact of sodium arsenite on these extracellular metabolites secreted by *N. parvum* isolate Bourgogne was conducted to observe their actual influence on pathogen's virulence factors. In this project, supported by the University of Haute-Alsace, we have observed calli necrosis and expression of grapevine defense genes (Rt-qPCR) when proteins secreted by the fungus grown in the presence of sodium arsenite ($NaAs0_2$) were added to the calli culture medium. The initial results indicate the induction of a specific pathway in grapevine calli suggesting that sodium arsenite has an effect on the virulence factors of the GTDs pathogens and, apart from being a fungicide, could have other effects.

Early response characterization of grapevine wood inoculated with *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*. R.J.G. PIERRON[1,2], J. POUZOULET[3], C. COUDERC[1], E. JUDIC[1], S. COMPANT[4] and A. JACQUES[1]. [1]*Unité P.P.G.V., Département des Sciences Agronomiques et Agroalimentaires, Institut National Polytechnique de Toulouse – Ecole d'Ingénieurs de PURPAN, Université de Toulouse, Toulouse, France.* [2]*Département BIOSYM, LGC UMR 5503 (CNRS/UPS/INPT), INP-ENSAT Université de Toulouse, Castanet-Tolosan, France.* [3]*Department of Botany and Plant Sciences, University of California, Riverside, CA, USA.* [4]*AIT Austrian Institute of Technology GmbH, Bioresources, Center for Health & Bioresources, Tulln, Austria. E-mail: alban.jacques@purpan.fr*

Defense mechanisms in woody tissue infected by fungi associated with grapevine leaf stripe disease (GLSD, esca complex) are still poorly understood. Several studies have showed some of the molecular mechanisms in perennial tissues of *Vitis vinifera* L. that are involved in trunk defense reactions. Here we investigated plant response in woody tissue inoculated with different combinations of GLSD-associated fungi (*Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*). The inoculations were carried out in cuttings of Cabernet Sauvignon (clone 15). Firstly, we analyzed plant responses using optical microscopy. The histological analyses of the plants, six weeks after inoculation, showed that *P. chlamydospora* significantly inhibited the production of newly formed vascular tissues, while *P. minimum* did not. The co-inoculation of both GLSD-associated fungi resulted in an intermediate reaction. Secondly, cellular responses in woody tissues were investigated by determining the relative expression of 11 defenses related genes (i.e. *PAL*, *PR10.3*, *TL*, *TLb*, *Vv17.3*, *STS*, *STS8*, *CWinv*, *PIN*, *CAM*, *LOX*) by RT-qPCR at 10, 24, 48, and 120 h post-inoculation (hpi). The gene set was differentially expressed after wounding without inoculation with the fungi, except for genes *CAM* and *LOX*, revealing that wounding caused significant background responses at the gene expression level. The expression of the grapevine genes (*PAL*, *PR10.3*, *TL*, *TLb*, *Vv17.3*, *STS*, *STS8*, *CWinv*, and *PIN*)

however significantly differed upon pathogens inoculation, suggesting the perception of mycelium by the plant. A specific pattern also emerged from the different expression regulation associated with different treatments. We concluded that *Vitis vinifera* L. woody tissue might perceive the presence of GLSD-associated fungi differently depending on the inoculated species and their combination, suggesting that a defense orchestration takes place in the perennial organs of a woody plant like grapevine.

The role of grapevine plant volatiles on conidial germination and germ tube development of Botryosphaeriaceae species. J. SAMMONDS[1], M. JASPERS[1], M. ROSTAS[2] and E. JONES[1]. [1]*Department of Ecology, Faculty of Agriculture and Life Sciences* [2]*Bio-Protection Research Centre, Lincoln University, PO Box 85084, Lincoln, Canterbury, New Zealand. E-mail: Eirian.Jones@lincoln.ac.nz*

Botryosphaeriaceae spp. pathogens infect grapevines mainly through wounds. This study aimed to identify the main volatiles released from wounded grapevine shoots and to determine the effect on germination and/or directional growth responses of Botryosphaeriaeae spp. conidia. GCMS analysis of volatiles released from wounded and non-wounded grapevine shoots showed 2-hexenal, cis-3-hexenal and hexanal were emitted at the highest concentrations. Emission rates of the green leaf volatiles (GLV's) 2-hexenal and trans-2-hexen-1-ol increased significantly after wounding. GLVs are reported to play important roles in signalling and have both antibacterial and fungicidal activity. In addition, the emission rates of toluene, citral, geraniol (isomer of citral) and limonene increased significantly after wounding. Exposure to volatiles directly released from freshly wounded stem tissue reduced germination for *Neofusicoccum luteum* isolate MM558 conidia compared with non-wounded tissue, but not *N. luteum* isolate CC445 or *N. parvum* isolate G652. Presence of wounded tissues significantly reduced germ tube growth in the presence of wounded compared with non-wounded shoots for both *N. luteum* MM558 and CC445, but not for *N. parvum* G652. Liquid volatile mixtures from GCMS collection from wounded or non-wounded shoots did not have a major effect on the germination or germ tube growth of conidia of *N. luteum* MM558. Exposure to selected pure volatile compounds reduced germination of both *N. luteum* MM558 and *N. parvum* G652, with almost complete inhibition in the presence of 2-hexenal and cis-3-hexenyl acetate. No chemotropic growth response was observed in germ tubes of *N. luteum* MM558 due to the volatiles released from wounded grapevine stems, however microscopic observations on grapevine stems appeared to show directional growth for germlings very close to wound sites. The results showed that volatiles from wounded grapevine tissue can affect the germ tube growth of some Botryo-sphaeriaceae isolates and that growth seems more sensitive to volatiles than germination.

The analysis of wood anatomy reveals the colonization strategies of fungi associated with grapevine trunk diseases. H.-H. KASSEMEYER[1], A. BOEDDINGMEYER[1], R. STREIT[1], S. FINK[2], J. GRÜNER[2], K. BUNK[3], T. SPECK[3], E. BIELER[4] and M. DUERRENBERGER[4]. [1]*Staatliches Weinbauinstitut Freiburg Dept. Biology, Merzhauser Str. 119, 79100 Freiburg Germany.* [2]*Albert-Ludwigs-Universität Freiburg, Chair for Forest Botany, Bertoldstr. 17, 79085 Freiburg Germany.* [3]*Albert-Ludwigs-Universität Freiburg Plant Biomechanic group, Schänzlestr. 1, 79104 Freiburg, Germany.* [4]*University Basel, Swiss Nano Institute, Nano Imaging Lab, Klingelbergstr. 50, 4056 Basel, Switzerland. E-mail: hanns-heinz.kassemeyer@wbi.bwl.de*

Grapevine trunk diseases, in particular Esca, cause various symptoms in the wood cylinder of the stem such as white rot and brown-wood streaking. Different methods of microscopy and digital imaging are used to characterize the micro-structures of asymptomatic and symptomatic xylem and to get insight into the interaction between the wood colonizing fungi and the host tissue. The wood cylinder of grapevine consists of a ring-porous xylem and pith rays. Early wood in the xylem wide vessels (tracheae) are perforated by scalariform pits, the late wood is composed of tracheids. Both are surrounded by thick-walled libriform fibers with three distinct cell-wall layers which are pervaded by tiny pits. The thick central layer (L2) of the cell-wall is enclosed by a thin outer (L1) and interior (L3) layer. The lumina of these xylem elements are empty except some vessels with thyllosis. The xylem is crossed by primary and secondary pith rays with living parenchymatic cells filled with starch granules. In cross sections of

wood showing brown wood streaking the cell-wall seems to be intact. However, in the cell lumina of completely infested xylem elements (tracheae, tracheids and fibers) and the parenchymatic cells of the pith rays fungal hyphae occur. The parts of the trunk showing white rot are separated from the undamaged wood by a sector of cells filled with a dark matrix. At the beginning of the white rot the L2 layer of the cell wall of the xylem elements, particularly of the fibers, shows the beginning of digestion. In the advanced stage of cell wall disintegration the L2 layer is perforated. At the end the central layer is totally eroded and only the L1 and L2 remain. In addition, in diseased and healthy trunks the water conduction and the water regime was analyzed. The preliminary results also indicate sufficient water supply to the canopy in Esca diseased vines. Hence we can postulate that disintegrated or plugged vessels can be excluded as a cause of the Esca syndrome in the canopy.

Searching for resistance to grapevine trunk disease pathogens among Spanish germplasm collections. M. P. MARTÍNEZ-DIZ[1], E. DÍAZ-LOSADA[1], J. MARTÍNEZ-GASCUEÑA[2], J.L. CHACÓN[2], P. M. IZQUIERDO[2], E. BARAJAS[3], J.A. RUBIO[3], C. BERLANAS[4] and D. GRAMAJE[4]. *[1]Estación de Viticultura y Enología de Galicia (INGACAL-EVEGA), Ponte San Clodio s/n 32428-Leiro-Ourense, Spain [2]Instituto de la Vid y el Vino de Castilla-La Mancha (IVICAM), Ctra. Toledo-Albacete s/n 13700-Tomelloso, Spain. [3]Instituto Tecnológico Agrario de Castilla y León (ITACyL), Ctra. Burgos km 19, Finca Zamadueñas, 47071 Valladolid, Spain. [4]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, 26071 Logroño, Spain. E-mail: david.gramaje@icvv.es*

Planting disease-resistant cultivars is a time-tested and sustainable approach for disease management. There have been limited reports of resistance or tolerance of *Vitis vinifera* cultivars to trunk diseases. The aim of this study was to evaluate grapevine germplasm collections from three regions in Spain (Castilla y León, Castilla-La Mancha and Galicia) for resistance to trunk disease pathogens. In total, grapevine cuttings of 70 cultivars were tested for susceptibility to infection caused by *Eutypa lata*, *Neofusisoccum parvum* and *Phaeomoniella chlamydospora* in three independent experiments: Exp 1; 12 cultivars from the germplasm collection of the Instituto de la Vid y el Vino de Castilla-La Mancha (IVICAM), Exp 2; 14 cultivars from the germplasm collection of the Instituto Tecnológico Agrario de Castilla y León (ITACyL) and Exp 3; 44 cultivars from the germplasm collection of the Estación de Viticultura y Enología de Galicia (EVEGA). In all experiments, dormant canes were cut into uniform lengths containing four buds. Potted grapevine cuttings were then wounded between the two upper internodes with a cork borer and inoculated with a 5-mm mycelium PDA plug of each fungus. Negative controls were inoculated with uncolonized agar plugs. Twelve cuttings per treatment were used and the experiment was repeated. Inoculated cuttings were immediately planted in July 2016 in a field site (Exp. 1), or placed in two greenhouses at 25 °C (Exp. 2 and 3). Six months after inoculation, cuttings were collected and inspected for lesion development. All cultivars were susceptible to infection by *E. lata*, *N. parvum* and *P. chlamydospora*, but the severity of internal wood discolouration varied significantly, suggesting possible disease tolerance in some accessions. These cutting assays are being repeated, and pathogen isolation will be performed, to confirm whether some cultivars are less susceptible to colonization by fungal trunk pathogens.

Physiological, molecular and chemical alterations related to esca-leaf symptoms in cv. Tempranillo. L. MARTÍN[1], F. FONTAINE[2], R. FERRER-GALLEGO[1], R. RODA[1] and F.J. CASTAÑO[1]. *[1]Fundació Parc Tecnològic del Vi (VITEC), Ctra. de Porrera km 1, 43730 Falset, Spain. [2]Université de Reims Champagne-Ardenne, Unité de Recherche de Vigne et Vin de Champagne, Laboratoire Stress, Défenses et Reproduction des Plantes, Bât. 18, BP 13039, 51687 Reims cedex 2, France. E-mail: laura.martin@vitec.cat*

Esca is a destructive fungal disease that reduces productivity and longevity of grapevines worldwide. Fungi colonization causes both wood lesions and "tiger stripe" leaves. There are no curative treatments available and infected plants eventually die. Some grapevine cultivars have been observed to be more susceptible to esca disease than others. Among those, cv. Tempranillo, which is the most widespread cultivar in Spain, is known as being susceptible. Understanding foliage alterations produced by

esca disease may help to identify potential markers of susceptibility or tolerance. Our study compared physiological, molecular and chemical changes occurring in vines that did or did not express foliar esca-symptoms. Four Spanish regions with dissimilar climate conditions and irrigation availability were considered. Foliar esca symptoms of Tempranillo vines were evaluated from 2013 to 2015. Two groups of plants were identified per experimental site: (i) vines which showed no esca symptoms, and were considered visually healthy; and (ii) vines which developed "tiger stripe" symptoms and were considered as diseased plants. *Diplodia seriata*, *Phaeoacremonium minimum*, *Phaeomoniella chlamydospora* and *Fomitiporia* sp. were isolated and identified from diseased plants. Net photosynthesis, transpiration rate and stomatal conductance were determined using a portable infrared gas analysis system. Furthermore, water potential was measured with a pressure chamber. Induction of the expression of 10 genes related to photosynthesis, water stress and defence response were tracked by quantitative reverse transcription-PCR methodology. The concentration of phenolic compounds (stilbenes and flavonols), chlorophylls and carotenoids were also determined by HPLC-MS and spectrophotometric methods, respectively. At water potential values lower than -1.4 MPa, a significant decrease in the physiological activity was detected in esca diseased plants. Induction of the expression of defence-related genes appeared to be correlated with foliar esca symptoms. Likewise, esca symptomatic leaves showed a lower concentration of chlorophylls and carotenoids.

Does flowering influence the grapevine defense response towards botryospaeriaceous fungi infection? V. MONDELLO[1], A. SPAGNOLO[1], P. LARIGNON[2], S. VILLAUME[1], F. RABEONELINA[1], C. CLÉMENT[1] and F. FONTAINE[1]. [1]*Université de Reims Champagne-Ardenne, Unité de Recherche de Vigne et Vin de Champagne, Laboratoire Stress, Défenses et Reproduction des Plantes, Bât. 18, BP 13039, 51687 Reims cedex 2, France.* [2]*Institut Francais de la Vigne et du Vin, Pôle Rhône-Méditerranée, 7 Avenue Cazeaux, 30230 Rodilhan, France. E-mail: florence.fontaine@univ-reims.fr*

In a preliminary study aimed at identifying the sensitivity of grapevine to the Botryosphaeria dieback agents *Neofusicoccum parvum* and *Diplodia seriata* infection during the growth season, the flowering phase was designated as being the period of highest weakness. This could be due to a reduced reactiveness of the grapevine as a consequence of the metabolic activity being mainly oriented towards the developing inflorescences during such a phenophase. According to this hypothesis, the goal of this study was to gain further insights into the physiological changes occurring in green stems of adult vines cv. Mourvèdre artificially infected with the Botryosphaeria dieback pathogens *N. parvum* and *D. seriata*. We analyzed the effect of inflorescences removal at the F stage (visible clusters) on the evolution of artificial inoculations with both pathogens, on the onset of G (separated clusters) and I stages (flowering) and at the M stage (veraison). At all three stages, lesion size was measured and real-time reverse-transcription polymerase chain reaction (RT-qPCR)-based analysis were carried out. Sixteen genes, including those encoding components of the phenylpropanoid pathway, PR and other plant defense proteins, as well as those involved in the detoxification processes and in primary metabolism or water stress were studied. The results clearly show the importance of inflorescences in the development of lesions associated with Botryosphaeria dieback pathogens inoculated on green stems of adult vines, especially at the onset of flowering. At flowering, the biggest necroses were observed with the inflorescences present, as well as an activation of the studied defense responses. Thus, an ineffective response to the pathogen could be consistent with a possible metabolic reprogramming linked to the host phenophase.

Intraspecies genomic diversity of Eutypa lata. A. MORALES-CRUZ[1], K. BAUMGARTNER[2] and D. CANTU[1]. [1]*Department of Viticulture and Enology, University of California Davis, One Shields Ave, Davis CA 95616, USA.* [2]*Department of Plant Pathology, University of California Davis, One Shields Ave, Davis CA 95616, USA. E-mail: abmora@ucdavis.edu*

Eutypa lata is the most common species causing Eutypa dieback symptoms in old vineyards around the world, where it is responsible for significant reductions in productivity and vineyard longevity. Toxins and cell wall degrading enzymes have been described specifically in *E. lata*, as they are thought to be essential virulence components for disease de-

velopment. Although this accumulated knowledge is important to hypothesize which enzymatic activities might be involved in pathogenesis or virulence, there still no relation between these functions and specific genes sequences or gene products. With the objective of closing this gap, over the past few years we have taken advantage of short-read sequencing technologies to create a profile of virulence functions for *E. lata*. The annotation of the genome has provided a first comprehensive view on the virulence potential of this pathogen, such as plant cell wall oxidative functions and gene clusters potentially involved in toxin biosynthesis. More recently, we utilized long-read sequencing technology, which allowed the assembly of the complete genome of *E. lata* isolate EN209. We assembled ten chromosome-length contigs with flanking telomeric repeats. In addition, we re-sequenced ten isolates from different hosts and known to exhibit variable levels of virulence. Initial comparisons of the genomes of these isolates to the reference genome showed relatively high similarity, ranging from 89.9 to 91.9%. Two large fragments from chromosome 1 and 5 seem to be the most variable regions in the genome among isolates. When looking at genes, we observed that on average 83.01% were part of highly conserved orthologous blocks, 14.93% were in variable orthologous blocks and 1.7% seemed to be unique to each isolate. Interestingly, the latter group of genes have a high number of virulence-related functions such as polyketides, P450s and detoxification transporters; which in some degree might explain the virulence difference among isolates.

New insights in the vessel diameter mediated resistance toward Esca disease of Grapevine. J. POUZOULET[1], E. SCUDIERO[2], M. SCHIAVON[1] and P.E. ROLSHAUSEN[1]. *[1]Department of Botany and Plant Sciences, University of California, Riverside, CA, 92521, USA; and [2]USDA–ARS, US Salinity Laboratory, 450 West Big Springs Rd., Riverside, CA, 92507-4617. E-mail: philrols@ucr.edu*

Our study presents new findings on the mechanisms of resistance to *Phaeomoniella chlamydospora*, a causal agent of the Esca disease. First, we established a parallel between Esca and other wilt diseases that have been described in perennial hosts, and propose that Esca should also be viewed as a vascular wilt disease. We confirmed that differences in the distribution of xylem vessel diameter of grapevine cultivars could explain the level of susceptibility to Esca disease, as observed under field conditions. Based on our *in planta* bioassays, we further identified that the density of wide diameter xylem vessels correlated with the ability to limit the *P. chlamydospora* movement in the stem of four cultivars (*Vitis vinifera* cv. Merlot, Chardonnay, Cabernet Sauvignon and Thompson Seedless). Our results highlighted how the host xylem vessel diameter impacted both the quality and the speed of vessel occlusions. Our observations indicated that the fungus thrived in pectin rich spaces found in between tyloses, that are found to be more abundant in vessels of wide diameter than in vessel of narrow diameter. In addition, using a dye injection experiment performed over a period of one week, we showed that vessel diameter also affected significantly the speed of vessel occlusion, with times that can vary from one day to up to seven days. We discuss the practicality of these concepts for the development of new management strategies for Esca disease.

Study of melanin produced in the grapevine phytopathogenic fungus *Lasiodiplodia theobromae*. E. A. RANGEL-MONTOYA[1], M. PAOLINELLI-ALFONSO[1] and R. HERNÁNDEZ-MARTÍNEZ[1]. *[1]Centro de Investigación Científica y de Educación Superior de Ensenada. Carretera Ensenada-Tijuana 3918, Zona Playitas, 22860 Ensenada, B.C. E-mail: ruhernan@cicese.edu.mx*

Melanin is a pigment produced by oxidation of phenolic and indole compounds. There are different types depending of their precursor. *Lasiodiplodia theobromae* is a fungus from the Botryosphaeriaceae family and one of the most aggressive pathogens in grapevine, which cause grapevine decline. Associated with infection by this fungus, melanin is deposited on the inner surface of cell walls, but its participation in the process of infection of grapevine is unknown. The search for factors related with the pathogenicity of vascular pathogens is important, to enable an understanding of their interaction with their hosts. The aim of this work was to study the *in vitro* behavior of *L. theobromae* str. UCD256Ma in the presence of inhibitors of melanin and under different stress conditions. Firstly, the fungus was grown in

the presence of phthalide, tropolone and nitisinone, inhibitors of DHN-melanin, DOPA-melanin and pyomelanin, respectively. Then, the combined effect of these inhibitors with enzymatic lysis, H_2O_2, and UV radiation was evaluated and lastly, the ability of the fungus to use tyrosine as a carbon and nitrogen source. The growth of *L. theobromae* in the presence of tropolone at 15 $\mu g \cdot mL^{-1}$ was inhibited and it lost the ability to degrade tyrosine. Exposure to H_2O_2 only affected the growth of the fungus in the presence of 15 $\mu g \cdot mL^{-1}$ tropolone. Total growth inhibition was observed with a combination of an enzymatic extract of *Trichoderma asperellum* and 15 $\mu g \cdot mL^{-1}$ tropolone. Finally, the viability of non-melanized spores was reduced under UV radiation. Based on these results, *L. theobromae* str. UCD256Ma is able to use tyrosine as carbon and nitrogen source for its growth and perhaps as a melanin precursor; melanin protects the fungus against abiotic stress conditions and this fungus produces three types of melanin, with DOPA-melanin being the principal type produced.

Histopathology of grapevine infected by the fungus *Lasiodiplodia theobromae*. E.A. RANGEL-MONTOYA[1], J. POUZOULET[2], P.E. ROLSHAUSEN[2] and R. HERNÁNDEZ-MARTÍNEZ[1]. [1]*Departamento de Microbiología, Centro de Investigación y de Educación Superior de Ensenada, Baja California, México.* [2]*Department of Botany and Plant Sciences, University of California, Riverside, CA, USA. E-mail: ruhernan@cicese.edu.mx*

Lasiodiplodia theobromae is a fungus from the Botryosphaeriaceae family and the causal agent of degenerative diseases, dieback and plant death in a wide range of woody plants. This fungus has been reported as one of the most aggressive pathogens in grapevine. Little is known about *L. theobromae*-plant host interaction. Thus, the aim of this work was to get a better understanding of the colonization strategy of *L. theobromae* used to cause disease in grapevine. One year old plants of cv. Cabernet sauvignon were inoculated with *L. theobromae* str. UCD256Ma through a mechanical injury and then incubated in a growth chamber with temperature day-night cycles from 10 to 30 °C. Samples were taken at 28 days post inoculation and cross and longitudinal sections were made. Histological observations showed that the fungus could induce the production of phenolic compounds in the vascular cambium as a response of the plant to the infection as well as the mechanical injury. *L. theobromae* could use the starch deposited in the ray cells as carbon source. The fungus did not degrade lignin but with epifluorescence microscopy it was observed that there was an induction in the production of suberin, which result in the compartmentalization of the xylem by the suberized cell walls that surround the ray parenchyma. SEM images showed that the fungus colonized the vascular cambium and ray parenchyma, as well as the vascular bundles. This study will provide the foundation for future work aim at identifying resistant varieties to important disease of grapevine in hot and arid climates.

On the phytotoxins produced by some *Lasiodiplodia* strains colonising grapevine wood in Brasil. P. REVEGLIA[1,2], T. CINELLI[3], M. MASI[2], A. CIMMINO[2], M. A. SILVA[4], L. MUGNAI[3], S. J. MICHEREFF[4], G. SURICO[3] and A. EVIDENTE[2]. [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]*University of Naples Federico II, Department of Chemical Sciences Complesso Universitario Montesant'Angelo, Via Cintia 4, 80126, Naples, Italy.* [3]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Sez. Patologia vegetale ed entomologia, Università di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy.* [4]*Departmento de Agronomia, Universidade Federal Rural de Pernambuco, 52171-900 Recife, Brazil. E-mail: preveglia@csu.edu.au*

Botryosphaeriaceae fungi are cosmopolitan plant pathogens causing fruit rot, leaf spot, dieback, wood cankers and even root rot of various Angiosperms and Gymnosperms. Many Botryosphaeriaceae species are important pathogens of grapevine in all the main grape-growing areas worldwide, where they cause wood cankers, stunted growth, dieback, light-brown stripes under the bark, and sectorial necrosis in the wood. Eleven species of *Lasiodiplodia* including *L. brasiliense*, *L. crassispora*, *L. egyptiacae*, *L. euphorbicola*, *L. jatrophicola*, *L. hormozganensis*, *L. missouriana*, *L. parva*, *L. pseudotheobroma*, *L. theobromae* and *L. viticola* have so far been reported as pathogenic on grapevine. Among them, *L. theobromae* appeared to be the most widespread and aggressive in the tropical, subtropical and warmer regions. Several toxic metabolites were produced *in vitro* from *L. theobromae* and another *Lasidiplodia* spp., *Lasiodiplodia mediterranea,* which

was recently isolated from a grapevine in Sardinia and which is closely related to *L. pseudotheobromae*, including lasiojasmonates A-C, and 16-*O*-acetylbotryosphaerilactones A and C, (1*R*,2*R*)-jasmonic acid, which is its methyl ester, and also botryosphaerilactone A, (3*S*,4*R*,5*R*)-4-hydroxymethyl-3,5-dimethyldihydro-2-furanone, and (3*R*,4*S*)-botryodiplodin. In a recent study on grapevines growing in Brazil and showing Botryosphaeria dieback symptoms, *L. brasiliense*, *L. euphorbicola*, *L. hormozganensis* and *L. jatrophicola* were isolated, together with other *Lasiodiplodia* species previously reported on grapevine. These isolates were investigated for the production of high molecular weight and low molecular weight phytotoxins. *L. euphorbicola, L. egyptiacae*, *L. hormozganensis* and *L. pseudotheobroma* produced esopolysaccharides (EPSs) that were fully characterized. This study will report the isolation and chemical characterization of low molecular weight lipophilic phytotoxins produced by six strains of the above-mentioned fungal species and in particular those synthesized from *L. euphorbicola* and *L. hormozganensis* and those coming from the work in progress on *L. egyptiacae* CMM 0349 and *L. jatrophicola* CMM 849.

Characterisation of secondary metabolites produced by Australian species of Botryosphaeriaceae involved in grapevine trunk diseases. P. REVEGLIA[1,2], R. BILLONES BAAIJENS[1], A. CIMMINO[2], A. EVIDENTE[2] and S. SAVOCCHIA[1]. [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]*University of Naples Federico II, Department of Chemical Sciences Complesso Universitario Montesant'Angelo, Via Cintia 4, 80126, Naples, Italy. E-mail: preveglia@csu.edu.au*

Botryosphaeria dieback (BD) is considered a serious problem of grapevines worldwide including Australia. This disease is caused by several Botryosphaeriaceae species causing cankers, dieback and eventually death of vines. In Europe, this disease is also associated with foliar symptoms including yellowish-orange spots on white cultivars or wine-red spots on red cultivars. These symptoms were not reported in Australian vineyards to date. Many foliar symptoms are usually associated with the production of phytotoxic metabolites produced by the pathogen that are translocated into the leaves. Thus, there is a need to investigate the role of phytotoxins in the symptom expression of Botryosphaeria dieback pathogens in Australian vineyards. These preliminary studies investigated the production of phytotoxins from the most widespread and virulent Botryosphaeriaceae species associated with BD in Australia. Three isolates each of *Neofusicoccum parvum, Dothiorella iberica, Spencermartinsia viticola* and four *isolates of Diplodia seriata* were screened for their ability to produce phytotoxins using thin layer chromatography (TLC). All isolates were cultured in Czapek Dox broth and the culture filtrates were harvested after 14 and 21 days. All filtrates and organic extracts were assayed using grapevine leaves and tomato seedlings. Preliminary results showed all were phytotoxic to both plants but the degree of toxicity differed between isolates, species and assay conditions. None of the controls (H_2O and Czapek Dox broth) caused any toxicity to both plants. TLC analyses showed all organic extracts from the 13 culture filtrates produced secondary metabolites. *D. seriata* (H141a), *Do. iberica* (L5) and *S. viticola* (L19) were further selected, and the isolation, chemical and biological characterisation of their secondary metabolites is in progress. This study will elucidate the role of phytotoxins in the pathogencity and symptom development of Botryosphaeriaceae species that may assist with field diagnosis and the development of control strategies for BD in Australian vineyards.

Phenolic responses in different brown spots associated with ESCA fungi in grapevine wood. D. RUSJAN[1], M. PERŠIĆ[1], M. LIKAR[2] and M. MIKULIČ PETKOVŠEK[1]. [1]*Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia.* [2]*Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia.E-mail: denis.rusjan@bf.uni-lj.si*

The study was carried out on the Esca susceptible grapevine variety 'Cabernet Sauvignon' (*Vitis vinifera* L.) in 2015. Changes in phenolic contents were determined in relation to visible symptoms (healthy - HLT, necrotic - Nec and rotten - Rot) separately on the heads, trunks and rootstocks of both symptomatic (Sym) and non-symptomatic (Non-sym) vines. In the HLT wood of the heads of Non-sym vines no fungi were identified, while the Nec wood

was colonised by *Alternaria alternata*. In the HLT wood from the heads of Sym vines Botryosphaeriaceae sp. and *Aureobasidium pullulans* were identified, while in the Nec wood on the same heads, trunks and rootstocks *Fomitiporia mediterranea* and in the Rot wood *Fomitiporia mediterranea* and *Phaeomoniella chlamydospora* were determined. Irrespective of the wood condition and vine part, the significantly highest content of total analysed phenolics was measured in Sym vines, especially in Nec wood of the trunks and rootstocks. Esca caused a significantly higher content of gallic acid (GA), total flavonols (FLO) and stilbenoids (STB), especially in Nec, but lower in Rot wood. Moreover, in Non-Sym vines the FLO content in the Nec wood of the heads was higher in comparison to that of the HLT wood, but not observed in the trunks. On the other hand, in Sym vines higher FLO contents were measured in the Nec wood on the trunks and rootstocks in comparison to the HLT wood, however this was not observed in the heads. Procyanidin dimers were the most abundant phenolic, the content of which significantly increased with Esca infection, especially in the trunk and rootstock Nec wood. Necrosis, in general, also significantly increased the content of resveratrols, especially in the heads and just minimally in the rootstocks of Sym plants. The obtained results suggested that Esca infection caused different responses in phenolics in different parts of the plant, occurring gradually along the longitudinal spread of the pathogens through the vine.

Effect of temperature on metabolites production by *Lasiodiplodia theobromae* a fungus causing canker and dieback of grapevine. M.M. SALVATORE[1], C. FELIX[2], M. DELLAGRECA[1], F. SALVATORE[1], D. NAVIGLIO[1], M. GALLO[3], M. GUIDA[4], A. ALVES[2], A.C. ESTEVES[2] and A. ANDOLFI[1]. [1]*Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte Sant'Angelo, via Cintia, 4, 80126 Napoli, Italy*. [2]*Departamento de Biologia, CESAM, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*. [3]*Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II via Pansini 5, 80131 Napoli, Italy* [4]*Dipartimento di Biologia, Università degli Studi di Napoli "Federico II", Complesso Universitario Monte Sant'Angelo, via Cintia, 21, 80126 Napoli, Italy*. *E-mail: mari.salvatore@yahoo.it*

A large number of species of the family Botryosphaeriaceae have been associated with Botryosphaeria canker and dieback of grapevines. *Lasiodiplodia theobromae* is the prevailing disease causing agent among species in the genus *Lasiodiplodia*, which can infect a wide range of plants growing in a variety of climate zones. *Lasiodiplodia theobromae* has also been associated with foliar chlorosis of host plants and may occasionally act as an opportunistic pathogen for humans. Pathogenicity of fungi is usually associated with the expression of several compounds, such as enzymes and other metabolites, involved in host/pathogen interactions. Accordingly, a number of secondary bioactive metabolites belonging to different classes are produced by *Lasiodiplodia* spp. For instance, in a recent study of strains of *L. mediterranea* (associated with grapevine decline in Sardinia and Sicily, Italy) new metabolites have been isolated and identified: lasiojasmonates A-C; 16-*O*-acetyl-botryosphaeriolactones A and C; and lasiolactols A and B. The aim of this study was to characterize the effect of temperature on the expression of secondary metabolites by different strains of *L. theobromae* isolated from grapevine. Preliminary investigations (via GC/MS, NMR and other analytical techniques) show that the production of secondary metabolites strongly depends, both in quality and quantity, on cultural conditions. The final objective is to isolate and characterize chemically the full spectrum of compounds produced by *L. theobromae* under a variety of experimental conditions and to investigate their biological activity and toxicity to plants and the environment.

Evaluating grapevine germplasm for tolerance to grapevine trunk diseases. M.R. SOSNOWSKI[1,2], M.R. AYRES[1], T.R. WICKS[1], M. McCARTHY[1] and E.S. SCOTT[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 5064. E-mail: mark.sosnowski@sa.gov.au*

Eutypa and botryosphaeria dieback are the most widespread and devastating trunk diseases of grapevine around the world. Vines are infected through pruning wounds when spores of the causal fungi land on, germinate and colonise exposed wood, causing dieback and eventual vine death. Previous reports of varying susceptibility of *Vitis vinifera*

cultivars to trunk disease prompted this research to identify potential sources of resistance or tolerance. A germplasm collection located in the Barossa Valley, South Australia was utilised for this study. It consists of 178 mature *V. vinifera* winegrape cultivars sourced from around the world, planted between 1977 and 1982. Vines were assessed for severity of trunk disease symptoms, including stunted shoots, distorted leaves, dead spurs, cordon die-back and trunk cankers. Eighteen cultivars assessed as having less than 20% disease severity were selected and compared with the susceptible cultivars Shiraz and Sauvignon Blanc (>80% severity). Experiments were conducted using a detached cane assay, and on mature vines in the field, to evaluate response to inoculation with *Eutypa lata* (eutypa dieback) and *Diplodia seriata* (Botryosphaeria dieback). All cultivars were susceptible to wound infection by the pathogens, but the extent of fungal growth within woody tissue varied significantly, suggesting possible tolerance or resistance in some cultivars. The fungus grew ahead of the stained wood, in apparently healthy tissue, up to 193 mm (*E. lata*) and 94 mm (*D. seriata*) suggesting that, during remedial surgery, vines should be cut at least 200 mm beyond any stained wood. Furthermore, there was no correlation between staining and pathogen recovery, casting doubt over the usefulness of extent of staining as an indicator of disease severity. Further investigation is required to determine the mechanisms by which some cultivars appear to restrict the growth of the fungus. Future research will focus on the susceptibility of clones and rootstock/scion combinations.

The effect of water stress on infection of grapevine canes *in situ* by *Eutypa lata* and *Diplodia seriata*. M.R. SOSNOWSKI[1,2], M.R. AYRES[1] and E.S. SCOTT[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 5064. E-mail: mark.sosnowski@sa.gov.au*

Water stress is thought to contribute to increased severity of Eutypa and Botryosphaeria dieback in Australia, particularly through drought and deficit irrigation practices. Although previous research indicated that foliar symptom expression and infection of wounds by *Eutypa lata* increased under water stress, information about colonisation of grapevine tissue in such conditions is lacking. Trials were established in South Australia to investigate the effect of deficit irrigation on colonisation of grapevine tissue by trunk disease pathogens. In the Riverland (mean annual rainfall 261 mm), Cabernet Sauvignon vines were subjected to four irrigation treatments between 100 and 12.5% of the standard irrigation program from 2011. In the Barossa Valley (rainfall 472 mm), Shiraz vines, on a range of rootstocks, were subjected to 100 or 0% irrigation from 2008. In winter 2013 and 2014, vines were pruned and inoculated with spores of *E. lata* or *Diplodia seriata*. During summer, pressure chamber readings confirmed various degrees of stress; leaf water potential ranged from mean values of 6 to 12 Bar for irrigation treatments 100 to 0%. One year later, treated canes were removed and assessed by re-isolation of pathogens from consecutive sections cut along canes. *E. lata* colonised canes further than *D. seriata*, and there was no correlation between distance of recovery and staining. Both pathogens colonised canes further in fully irrigated vines than in those receiving little or no irrigation, with the exception of *D. seriata* in the Riverland, having no difference among irrigation treatments. There was an interaction between irrigation treatment and rootstock on colonisation by both species in the Barossa Valley. Colonisation was not exacerbated by water stress and, in some cases was decreased, suggesting that drought and deficit irrigation practices are not likely to contribute to increased prevalence of grapevine trunk disease in vineyards.

Defense responses induced by extracellular proteins produced by Botryosphaeriaceae on *Vitis* cells. E. STEMPIEN[1], M.-L. GODDARD[1,2], Y. LEVA[1], M. BENARD-GELLON[1], H. LALOUE[1], S. FARINE[1], F. KIEFFER-MAZET[1], C. TARNUS[2], C. BERTSCH[1] and J. CHONG[1]. [1]*Université de Haute-Alsace, Laboratoire Vigne Biotechnologie et Environnement LVBE EA 3991, 33 rue de Herrlisheim, 68008 Colmar Cedex France.* [2]*Université de Haute-Alsace, École Nationale Supérieure de Chimie, Laboratoire de Chimie Organique et Bioorganique EA 4566, 3 rue Alfred Werner, 68093 Mulhouse Cedex France. E-mail: elodie.stempien@gmail.com*

Grapevine trunk diseases are devastating for the viticulture industry. Eutypa dieback, Esca and Botryosphaeria dieback are caused by a complex

of xylem-inhabiting fungi generating severe yield reduction in vineyards (Bertsch *et al.*, 2013). Botryosphaeria dieback is known to be associated with a wide range of Botryosphaeriaceae and in particularly *N. parvum* and *D. seriata*. To explain the foliar symptoms in absence of pathogens in leaves, it has been hypothesized that these fungi secrete toxins in the infected wood, which are then translocated via the xylem sap. Recent toxicity studies on extracellular proteins produced by Botryosphaeriaceae showed that these induce necrosis on grapevine cells as well as defense gene expression. Plant resistance depends on the ability to quickly and efficiently mount early signaling events of defense responses. In this context, induction of early defenses is an important step. Therefore, we were interested to compare defense reactions induced in grapevine cells of two different genotypes, *V. vinifera* cv. Gewurztraminer and *V. rupestris*, by purified Botryosphaeriaceae extracellular proteins showing differential aggressiveness. We used cell suspensions to study ions fluxes that lead to oxidative burst, cell death, phytoalexins production as well as defense gene expression. By comparing two different Botryosphaeriaceae *(N. parvum* and *D. seriata*), we found that for early events, *N. parvum* induces a more intense defense response than *D. seriata*. For later responses such as stilbene production and expression of defense genes, the phenomenon is reversed. At every stage of the defense reaction, we observed a higher response of *V. rupestris* cells, except for stilbene production. We hypothesize that inhibition of a defense signaling step by protein effectors could explain the virulence of *N. parvum*. Likewise, the differences between the two grapevine genotypes could be related to a lower susceptibility of *V. rupestris* to Botryosphaeriaceae.

Secondary metabolites produced by *Neofusicoccum parvum* differentially contribute to the trunk disease expression in grapevine. P. TROTEL-AZIZ[1], E. ABOU-MANSOUR[2], B. COURTEAUX[1], F. RABENOELINA[1], J.-F. GUISE[1], C. CLEMENT[1], A. AZIZ[1] and F. FONTAINE[1]. [1]*Université de Reims Champagne-Ardenne, Unité de Recherche de Vigne et Vin de Champagne, Bât. 18, BP 1039, 51687 Reims cedex 2, France.* [2]*Plant Biology Department, University of Fribourg, 1700 Fribourg, Switzerland. E-mail: patricia.trotel-aziz@univ-reims.fr*

Botryosphaeriaceae, including *Neofusicoccum parvum*, produce phenolic phytotoxins such as terremutin (Trm) and mellein (Mln) in the grapevine pathosystem (Abou-Mansour *et al.*, 2015). Untill now, the relationship between the production level of both toxins and their contributions to GTD symptoms is unclear. Here, we first investigated the capacity of grapevine plants to detoxify purified Trm and Mln. We further examined whether these toxins are involved in the Botryosphaeria dead arm (BDA) symptom expression by using cuttings inoculated with *N. parvum* (Np) strains producing different levels of Mln or Trm. The impact of Trm and Mln on grapevine immune responses was also explored. We showed that grapevine plantlets can remove both toxins from their culture medium with greater and faster efficacy for Mln than Trm, without exhibiting any physiological disorder. Interestingly, a NpB mutant impaired in Trm, but producing more Mln, showed increased canker size on cuttings compared to NpB Wt (wild type), indicating a strong implication of Mln in the canker expression. Moreover Np Bt67, which produces high levels of Trm, strongly induced BDA-symptoms including typical dead branches within a few days. Application of purified Trm to NpB Wt-inoculated plants clearly increased canker and stem lesion sizes, while it did not modify the phenotype of the NpB mutant-inoculated plants. This highlights the implication of Trm as a virulence factor in BDA expression. Our data also provides evidence that only a few defense responses are slightly upregulated by phytotoxins and depend on their structural moiety. Nevertheless, results indicated that Trm, as the Wt fungus Bt67, enhanced the expression of Salicylic Acid-dependent defense responses. Overall, these findings uncover the implication of Trm (more than Mln) in the mechanism by which *N. parvum* overcomes the weak grapevine immunity to promote disease expression. Current experiments are focused on boosting grapevine immunity by using beneficial microbes to improve detoxification of phytotoxins and then to control GTD.

Beneficial bacteria improve detoxification of secondary metabolites produced by Botryosphaeriaceae and enhance grapevine resistance against trunk diseases. P. TROTEL-AZIZ[1], E. ABOU-MANSOUR[2], B. COURTEAUX[1], F. RABENOELINA[1], J.-F. GUISE[1], C. CLEMENT[1], F. FONTAINE[1] and A. AZIZ[1]. [1]*Université de Reims Champagne-Ardenne, Unité de Re-*

cherche de Vigne et Vin de Champagne, Bât. 18, BP 1039, 51687 Reims cedex 2, France. [2]*Plant Biology Department, University of Fribourg, 1700 Fribourg, Switzerland. E-mail: patricia.trotel-aziz@univ-reims.fr*

Fungal phytotoxins are considered as primary determinants of disease spread in infected plants. In grapevine, the contribution of secondary metabolites produced by the Botryosphaeriaceae to GTD symptoms is highly suspected. Therefore, detoxification of these metabolites might be an efficient strategy to control GTD diseases, and that can be easily improved with the application of beneficial microorganisms. In this study, we investigated whether grapevine plants inoculated or not with the beneficial bacterium *Bacillus subtilis* PTA-271 can eliminate two major phytotoxins produced by GTD fungi, terremutin (Trm) and mellein (Mln). We also examined the effect of both toxins on plant immune response upon interaction with PTA-271. We found that grapevine plantlets can eliminate both toxins from the external medium to different extents, with a strong accumulation of Mln in the roots. Pretreatment of grapevine plant with the beneficial bacterium PTA-271 also led to a rapid removal of phytotoxins from the medium. Results provide information about the difference in the removal rate of Mln and Trm by PTA-271-treated plants, and also underscored the ability of bacterized plants to detoxify Mln more efficiently than Trm. These results are consistent with the PTA-271-induced expression of various plant defense responses including GST1 which is involved in the redox status and in detoxification pathways. Over-expressed genes may be strongly repressed by an additional supply of Mln and/or Trm. Interestingly, PTA-271 itself can also directly eliminate the two phytotoxins from its incubating medium without any growth disturbance. Additional experiments performed with cuttings inoculated with PTA-271 confirmed the potential of this bacterium to protect cuttings against *Neofusicoccum parvum*. This protection appears to involve fungistatic effect and induction of various defense responses including detoxification pathways.

***Pestalotiopsis*: A pictorial of symptomology of a quiescent and ubiquitous grape disease in the eastern United States.** D.S. VOLENBERG[1] and L. MORTON[2]. [1]*University of Missouri Grape and Wine Institute, 214 Waters Hall, Columbia, MO, 65211.* [2]*Morton Viticulture, PO Box 5607, Charlottesville, VA, 22905. E-mail: volenbergd@missouri.edu*

Pestalotiopsis and related genera fungi are important phytopathogens that can cause trunk disease, fruit rot, and foliage blight in *Vitis* spp. In Missouri and Arkansas *Pestalotiopsis uvicola* and *P*. sp. were first identified being associated with vascular symptoms of trunk diseases. More recently (2015), in Missouri, *Pestalotiopsis* spp. caused early season berry rot in the cultivar Norton. *Pestalotiopsis* spp. also resulted in rapid defoliation of a Norton vine from another Missouri vineyard in 2015. In 2016, in Missouri, *Pestalotiopsis* spp. appeared as a leaf blight on the cultivars Chambourcin and Norton. The symptomology of the leaf blight varied among cultivars. The leaf blight resulted in leaf fall of infected leaves. *Pestalotiopsis* was identified as the causal agent by recovering the unique appendage-bearing conidia from symptomatic tissue after 24-hrs of moist incubation. The species were identified from sequence data of internal transcribed spacer-rDNA region. The sequence data was compared to GenBank sequences and *P. sydowiana* and *P. microspora* were identified. Near Burkittsville, Maryland (2014 to 2016), vines of primarily, but not exclusively Cabernet sauvignon had the following symptomology; reddening of the pedicel and peduncle, leaf stripe in lower lateral leaves (not primary), followed by berry shrivel and cluster stem wilt. Visual examination in the field revealed acervuli on the canes and occasionally the peduncle, but no fungal sporulation was evident. *Pestalotiopsis* was consistently isolated from symptomatic canes and clusters after moist incubation. An independent laboratory confirmed *Pestalotiopsis*. In most instances, in the cases of *Pestolotiopsis* described, strobilurin fungicides were not applied in these commercial vineyards. The literature in other crops suggest that strobilurin fungicides applied at bloom can be effective in controlling *Pestolotiopsis*. Portrayed here is a pictorial of symptomology associated with *Pestalotiopsis* in both American and European *Vitis* species.

Microbial ecology

Comparison of the wood-microbiome from Grapevine Trunk Disease-plants, treated or not with sodium arsenite. E. BRUEZ[1], P. LARIGNON[2], C. BERTSCH[3], P. REY[1,3] and F. FONTAINE[4]. [1]*SAVE,*

INRA, Institut National de Recherche Agronomique, BSA, ISVV, 33882, Villenave d'Ornon, France. [2]IFV Pôle Rhône-Méditerranée, 7 avenue Cazeaux, 30230 Rodilhan, France. [3]Université de Bordeaux, Bordeaux Sciences Agro, UMR1065 SAVE, 33140 Villenave d'Ornon, France. [4]SFR Condorcet, URCA, URVVC EA 4707, Laboratoire SDRP, BP 1039, 51687 Reims Cedex 2, France. E-mail: emilie.bruez@inra.fr

Before its ban in the early 2000s, sodium arsenite had been used in many European vineyards for decades to control Esca and Botryosphaeria dieback. Although this fungicide was frequently described as very effective in controling these diseases, its mode of action is relatively unknown. The present experiment has been carried out to address this puzzling question by studying its effect on the wood-microbiome. Grapevines that had previously shown GTD-foliar symptoms from three cultivars planted in Alsace (Gewurztraminer), Champagne (Chardonnay) and Languedoc-Roussillon (Merlot) were treated with sodium arsenite in 2014 and 2015. All the plants were free of GTD-foliar symptoms after sodium arsenite application. In the treated- and control-plants, the microflora of the plants sampled in September 2014 and 2015, which colonised these wood-tissues (non-necrotic, white-rot, sectorial necrotic and border necrotic tissues) were compared. For the three cultivars, the fingerprinting method (Single Strand Conformation Polymorphism, SSCP) confirmed that the fungal and bacterial communities were specific to each type of tissue. Using classical-microbiology, GTD-pathogenic fungi were isolated from all the tissues. However, Botryosphaeriaceae spp. were more isolated in the border of the necroses, *Phaeomoniella chlamydospora* in the non-necrotic and necrotic tissues, and *Fomitiporia mediterranea* in white-rot. Microbiological results also showed that sodium arsenite applications significantly modified the wood-inhabiting fungal communities. For instance, fewer Esca-pathogenic fungi (*P. chlamydospora*, *F. mediterrannea*) were isolated in the treated plants but potential plant-beneficial fungal species, such as *Trichoderma* spp., were more frequently isolated. Mi-Seq results also showed differences in the microbiome after sodium arsenite application. For instance, in the border of necrotic tissue and white-rot, more genera were identified in the treated grapevines. Finally, it was hypothesized that, while sodium arsenite modified the pathogenic microflora, potential plant-beneficial fungi took advantage of that situation to colonize the woody tissues.

Effect of the hot water treatment used in nurseries on pathogenic fungi inhabiting grapevine wood and involved in GTDs. E. BRUEZ[1], P. LARIGNON[2], S. COMPANT[3] and P. REY[1,4]. *[1]Université de Bordeaux, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), Bordeaux Sciences Agro, F-33140, Villenave d'Ornon, France. [2]Institut Français de la vigne et du vin (ENTAV-ITV France), pôle Rhône-Méditerranée, 7 avenue Cazeaux, 30230 Rodilhan, France. [3]INRA, ISVV, UMR1065 SAVE, F-33140, Villenave d'Ornon, France. [4]AIT Austrian Institute of Technology GmbH, Bioresources Unit, Health & Environment Department, Konrad Lorenz Strasse 24, 3430 Tulln, Austria. E-mail: emilie.bruez@inra.fr*

Hot water treatment (HWT) is used in nurseries to control the pathogenic fungi involved in Grapevine Trunk Diseases (GTDs), as well as other pathogens, such as phytoplasmas. The long-term impact of this treatment on the microflora, especially on the fungal microbiota inhabiting the wood-tissues, still remains unknown. In this study, the fungal microflora of grapevines, treated or not 14 and 15 years earlier by HWT, were compared. Comparisons were made at different plant part levels. The fungal microflora was relatively abundant in the different types of wood tissues. Certain fungal genera were isolated and identified on the basis of their ITS-DNA sequencing. Independently of the HWT, significant changes in the fungal microflora were observed in 2010 and 2011, the two years of sampling. Although the HWT may have affected the cuttings microflora at the nursery stage, this had not persisted after several years of HWT treatment for the fungi, especially the pathogenic ones, thereby demonstrating that HWT does not durably control GTDs. HWT is useful for controlling microbial pathogens in nurseries, but does not have a significant long-term control effect on GTD pathogens in mature plants in the vineyards. Similarly, it has no subsequent impact on fungal communities colonizing the wood tissues of grapevine.

Cartography of the wood-microbiome of various grapevine cultivars planted in 7 French regions

and expressing or not Esca-foliar symptoms. E. BRUEZ[1], D. RENAULT[1], A. SAYEH[1], S. BASTIEN[1,2], P. LECOMTE[1], B. DIARRA[1], J. VALLANCE[1,2] and P. REY[1,2]. [1]*INRA, ISVV, UMR1065 SAVE, F-33140, Villenave d'Ornon, France.* [2]*Université de Bordeaux, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), Bordeaux Sciences Agro, F-33140, Villenave d'Ornon, France. E-mail: emilie.bruez@inra.fr*

The microbiome colonizing the wood tissues of grapevine, expressing or not Esca-foliar symptoms, has been studied in recent years. However, this is been done for a relatively small number of cultivars such as Cabernet Sauvignon or Baco blanc. Here, the microbiome of 8 cultivars planted in 6 wine regions of France was analyzed by using (i) a fingerprinting method, Single Strand Conformation Polymorphism (SSCP) and (ii) a high throughput sequencing technique, Mi-seq. In Burgundy, the cultivar sampled was Pinot noir; in Champagne, Chardonnay; in Jura, Chardonnay, Savagnin and Trousseau; in Charentes, Ugni blanc; in Val de Loire, Cot Malbec and Chenin and in Languedoc-Roussillon, Mourvèdre. Grapevines were uprooted and the microbiome of the non-necrotic and necrotic woody tissues of the trunk and cordon were compared. Generally, the microbiome from one cultivar differed from another cultivar. For instance, in the Jura, for the three different cultivars sampled, the fungal microflora colonizing the non-necrotic woody tissues in the trunk were different in the Trousseau, Savagnin and Chardonnay cultivars. However, for the bacterial communities, the communities from the Trousseau differed from the two others. Results obtained by Mi-seq are discussed with regards to the composition of the microbiome, particularly the Esca-pathogens and the potential plant-beneficial microorganisms, which were specifically detected or not in each cultivar.

Comparison of fungal and bacterial microflora of grapevines expressing or not esca-foliar symptoms in North-eastern Spain. E. BRUEZ[1], G. ELENA JIMENEZ[2], J. LUQUE[3] and P. REY[1,4]. [1]*INRA, ISVV, UMR1065 SAVE, F-33140, Villenave d'Ornon, France.* [2]*Instituto Agroforestal Mediterráneo (IAM), Universitat Politècnica de València (UPV), Camino de Vera S/N, 46022-Valencia, Spain.* [3]*IRTA, Ctra. de Cabrils km 2, 08348 Cabrils, Barcelona, Spain.* [4]*Université de Bordeaux, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), Bordeaux Sciences Agro, F-33140, Villenave d'Ornon, France. E-mail: emilie.bruez@inra.fr*

This study describes the microbial communities found in a 30 year-old 'Cabernet-Sauvignon' vineyard naturally affected by grapevine trunk diseases (GTDs) in Spain. In summer 2014, six symptomatic and six asymptomatic vines were uprooted in El Pla del Penedès (Catalonia, NE Spain). The symptomatic vines had expressed esca-foliar symptoms at least twice over the last four years, whereas asymptomatic plants did not show any symptom within the same period. Necrotic, white-rot and non-necrotic tissues were sampled in rootstocks and trunks in both types of plant. Combined fungal isolations and molecular techniques were used to investigate the endophytic microflora and describe the fungal diversity of the vines. Correspondence and Principal Components analyses were carried out to explore the associations between the health status of vines, the part of the plant and the type of tissue being sampled, and their associated fungal and bacterial communities. Molecular techniques, namely the fingerprinting Single Strand Conformation Polymorphism method, showed that differences were observed between the different types of tissue in the two types of plant. Pathogenic fungi involved in GTD were identified in all types of tissue from asymptomatic and symptomatic vines. However, differences in the pathogenic mycoflora were observed in the different types of tissue; *Phaeomoniella chlamydospora* and *Diplodia seriata* were more abundant in non-necrotic tissues, whereas *Fomitiporia mediterranea*, *Neofusicoccum parvum* and *Eutypa lata* were mainly found in white-rot and necrotic tissues, respectively. Several *Alternaria* and *Trichoderma* species were isolated, mainly from non-necrotic tissues. Differences were also observed in fungal communities of non-necrotic tissues from the rootstock and trunk. This study showed that a complex and diverse microflora can colonize the various woody tissues of 30 year-old grapevines.

***Agrobacterium*-transformation of Ascomycota associated with grapevine trunk diseases for visualization of colonization niches.** S. COMPANT[1], G. ELENA JIMENEZ[2], K. HÖNIG[1], R.J.G. PIERRON[3,4], A. JACQUES[3], J. ARMENGOL[2], M. RIEDLE-BAUER[5] and M. GORFER[1]. [1]*AIT Austrian Institute of Technology GmbH, Bioresources, Center for Health & Bioresourc-*

es, Tulln, Austria. [2]Instituto Agroforestal Mediterráneo (IAM), Universitat Politècnica de València (UPV), Camino de Vera S/N, 46022-Valencia, Spain. [3]Unité P.P.G.V., Département des Sciences Agronomiques et Agroalimentaires, Institut National Polytechnique de Toulouse – Ecole d'Ingénieurs de Purpan, Université de Toulouse, Toulouse, France. [4]Département BIOSYM, LGC UMR 5503 (CNRS/UPS/INPT), INP-ENSAT Université de Toulouse, Castanet-Tolosan, France. [5]Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau Klosterneuburg, Wienerstraße 74, Klosterneuburg, Austria. E-mail: stephane.compant@ait.ac.at; markus.gorfer@ait.ac.at

Grapevine plants can host several ascomycetes, leading by their infection to trunk diseases. The behavior, niches, and sources of colonization of these fungi within the plants are however not well known. Using *Agrobacterium*–mediated transformation, several fungi were recently transformed with green or red fluorescent protein markers using binary vector pCBCT or pBHt2-TdTom, respectively, containing fluorescent genes under the control of a *toxA* promotor and Hygromycin resistance marker *hph*. *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora* were transformed easily with *gfp* gene and their colonization niches were followed in planta. Several strains of *Phomopsis oblonga* (*Diaporthe eres*), *Phomopsis viticola* (*Diaporthe ampelina*), and *Dactylonectria* spp. were also transformed successfully with green or red fluorescent gene to further monitor differential plant colonization by these fungi and to determine how they interact with the plant tissues within the wood. The herein utilized vectors and transformation protocols proved to be valuable tools to study plant colonization with a broad range of phylogenetically diverse ascomycetes.

Grapevine fungal residents: Boon and bane in the same vine. M. HAUSTEIN and A. KORTEKAMP. *State Education and Research Center DLR Rheinpfalz, Institute for Plant Protection, Breitenweg 71, 67435 Neustadt an der Weinstrasse, Germany. E-mail: andreas.kortekamp@dlr.rlp.de*

External and internal symptoms of two distinct sets of grapevines (standard and high grafted vines) were examined first visually (in cooperation with A. Becker DRL RNH). Then, the cultivatable mycoflora was analysed in four cross sections. Results indicate that the fungal microflora is characterised by a high diversity. As expected, well-known GTD pathogens were frequently found. However, the presence of *Diplodia* spp., *E. lata*, and *P. chlamydospora* was significantly higher in standard grafted vines. Analysis of species composition of both young and old vines also revealed an unequal distribution of distinct species and the simultaneous presence of pathogens and putative antagonists, such as *Trichoderma* species. More than 80 *Trichoderma* strains belonging to 11 species were successfully isolated and identified based on morphological, microscopical, and molecular analysis (ITS, TEF-1a), followed by database alignments. Most frequent species were *T. harzianum* (51%), *T. atroviride* (14%), *T. citrinoviride* (10%), *T. koningiopsis* (5%), and *T. virens* (5%). The putative antagonistic behaviour of several *Trichoderma* strains was characterised by using different test sets. Four species (*T. harzianum*, *T. citrinoviride*, *T. cf. priscilae*, *T. sinuosum*/*T. cremea*) had no significant effects on pathogen growth. One species (*T. harzianum*) showed some degree of plasticity with regard to the strains tested, whereas another species (*T. atroviride*) inhibited the growth of *Botryosphaeria* sp. but not of *Ilyonectria* ssp. and *N. rubicola*. All isolates of *T. gamsii*, *T. hamatum*, *T. koningiopsis* and *T. viride* inhibited the growth of all pathogens tested and were therefore used for further tests. Effects of volatile compounds produced by *Trichoderma* were demonstrated for *T. gamsii* and *T. viride* which induce significant growth inhibition in case for *P. chlamydospora*, *P. minimum* and *I. liriodendri*. In addition, exudates obtained from liquid cultures of *T. koningiopsis* and *T. gamsii* altered or inhibited spore germination of all pathogens tested.

Influence of the training system on the incidence of Esca and the fungal community of grapevine in German vineyards. C. KRAUS[1], R. VÖGELE[2] and M. FISCHER[1]. *[1]Julius Kühn-Institute, Institute for Plant Protection in Fruit Crops and Viticulture, Siebeldingen. [2]University of Hohenheim Department of Phytopathology Hohenheim. E-mail: christian.kraus@julius-kuehn.de*

Semi minimal pruned hedge (SMPH) is a novel and innovative grapevine training system, which is more cost-effective than the traditional vertical shoot positioning (VSP) system. A grapevine trained in SMPH shows more woody canes, a wider leaf canopy and

more bunches carrying fewer berries, compared to VSP. Whether and how those changes influence the emergence of grapevine trunk diseases (GTDs), such as Esca, is mostly unknown. For that reason one aim of the present three-year-study is to investigate the development of Esca-related symptoms in vineyards trained in SMPH and VSP, respectively. In addition we study the composition and development over time of the endophytic fungal community in grapevine canes. Thereby the temporal emergence of GTD-related fungi is monitored to evaluate the related risk for SMPH plants. For this purpose fungi from canes of different age (2 months to 8 years) are isolated and identified. Based on external symptoms on leaves and berries our results indicate a slightly higher susceptibility against Esca in SMPH trained vines compared to VSP plants. In 2016 for instance, 17,3% of the SMPH vines in a 32-year old 'Müller-Thurgau' plot showed Esca-related symptoms, while the incidence rate of the VSP trained plants was 9,6%. The analysis of the fungal community in the canes revealed a distinctive change corresponding with the age of the canes. With ongoing time the number of isolated fungal species increased from 16 (in 2-month canes) to 31 (in 8-year canes). Also the abundance of GTD-associated fungi within the endophytic community raised from 8,5% to 29,5%. The most frequently isolated GTD-associated pathogen was *Diplodia seriata*, followed by *Eutypa lata*, *Eutypa leptoplaca* and *Cadophora luteo-olivacea*. Additional studies will be necessary to get a more concise idea about the possible influence of the training system on the incidence of GTDs.

Exploring endophytic bacteria as potential biocontrol agents against grapevine trunk disease pathogens. J. NIEM, R. BILLONES-BAAIJENS and S. SAVOCCHIA. *National Wine and Grape Industry Centre, School of Agricultural and Wine Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga NSW 2678, Australia. E-mail:jniem@csu.edu.au*

Grapevine trunk diseases are responsible for loss in productivity of grapevines worldwide. The main trunk diseases responsible for this loss in Australia are Eutypa dieback and Botryosphaeria dieback. Currently these diseases are mainly managed by remedial surgery and the application of fungicides to wounds. However, fungicides only offer short-term protection. Furthermore, registered fungicides are limited and others that were effective have been removed from the market. Biocontrol may be used as an alternative or supplement to fungicides and can be part of an integrated trunk disease management strategy. Preliminary studies in our laboratory revealed 10 bacterial grapevine endophytes to exhibit inhibitory activity against Botryosphaeriaceae pathogens. The presence of these bacteria in the grapevine microbiota leads us to explore their biocontrol potential as they may have adaptive and competitive potential due to their natural residence in grapevine tissue. Preliminary *in vitro* and dual culture assays of the bacterial isolates showed varying degrees of mycelial growth inhibition against three grapevine trunk disease pathogens: *Diplodia seriata, Neofusicoccum parvum,* and *Eutypa lata.* From these results, three bacterial isolates were further selected for *in planta* assays using detached grapevine canes. Canes were wounded and inoculated with the three selected bacterial isolates. After 24 hrs the canes were inoculated with *Neofusicoccum luteum* (200 conidia). Re-isolations from canes after 4 weeks showed that bacterial isolate BCA13 significantly reduced disease incidence by 72%, compared to 11% and 0% by isolates BCA11 and BCA18, respectively. All control cuttings (*N. luteum* inoculated, no biocontrol treatment) had 100% disease incidence. These preliminary results indicate that some endophytic bacteria may be potential biocontrol agents that can be used as an alternative or in conjunction with chemical fungicides for managing grapevine trunk diseases. Identification and biochemical characterisation of these bacteria is ongoing.

Deep analysis of the grapevine associated microorganisms: their biocontrol capacity against Botryosphaeria dieback agents, grapevine colonisation and biotechnological potential. C. PINTO[1,2], V. CUSTODIO[2], A. SONGY[1], M. GANTET[1], C. CLÉMENT[1], A.C. GOMES[2] and F. FONTAINE[1]. [1]*Université de Reims Champagne-Ardenne, Unité de Recherche de Vigne et Vin de Champagne EA 4707, Laboratoire Stress, Défenses et Reproduction des Plantes, Bât.18, BP 13039, 51687 Reims cedex 2, France.* [2]*Genomics Unit – Biocant, Technology Transfer Association, Biocant Park, Nuc.4 Lt8, 3060-197 Cantanhede, Portugal. E-mail: catia.pinto@biocant.pt*

Grapevine trunk diseases (GTDs) are the most destructive grapevine diseases and treatments are not available. Thus, the development of control solutions is urgently needed and friendly-environmental solutions are of utmost interest. Grapevine is a natural source of associated microorganisms and some of them may play specific roles on plant protection against phytopathogens. The present challenge is to understand how such microorganisms interact with plants and their biotechnological potential for development of innovation strategies based on the sustainability. Here we report the grapevine population and its natural biocontrol potential. For this, grapevine isolates were obtained from soil, roots, leaves, stems and grapes and tested for their antagonistic potential against Botryosphaeria dieback agents namely, *Diplodia seriata* and *Neofusicoccum parvum*. One of the most promising biocontrol candidates was selected and a deep characterization by means of biochemical analysis, grapevine colonisation and genome sequencing was performed. A total of 202 isolates were obtained from Bairrada appellation (Portugal) over 2010 and 2011 and the most commonly isolates belonged to the genera *Bacillus* (55%), *Streptomyces* (14%) and *Aureobasidium* (12%). Each plant structure showed to be confined to a set of specific microorganisms which confirms their preference and adaptability to specific plant organs. A pre-screening antagonistic analysis showed that strains from *Bacillus* genus had the major inhibitory activity against Botryosphaeria dieback and, thus *B. amyloliquefaciens* strain Fito_F321 was further characterized. This strain reduced significantly the mycelium growth of all pathogenic fungi ($p<0.05$), with an average of 24% of inhibition, showed to produce a high range of extracellular enzymes as amylase, cellulase, lipase, pectinase and proteases, siderophores and to solubilise phosphate. It colonised also the grapevine at both epiphyte and endophyte level. The genome analysis showed that this strain is a source of bioactive compounds of biocontrol value. Overall, this work highlights an advance on the characterization of the natural beneficial microorganisms associated with grapevine with a special focus on the strain *B. amyloliquefaciens* Fito_F321, as it represents an opportunity to develop new strategies for GTDs management.

High concentration of copper in vineyard soil favours the development of wood necrosis caused by the Botryosphaeria dieback pathogen *Neofusicoccum parvum*. E. BRUEZ[1], L. ANATOLE-MOUNIER[1], J. VALLANCE[1], S. BUSSIÈRE[2], L. DENAIX[2], P. REY[1] and D. THIÉRY[1]. [1]*INRA, ISVV, UMR1065 SAVE, Bordeaux Sciences Agro F-33882, Villenave d'Ornon, France.* [2]*INRA, UMR ISPA, Bordeaux Sciences Agro, F-33882, Villenave d'Ornon, France. E-mail: emilie.bruez@inra.fr*

After decades of copper applications on grapevines to control downy mildew, high copper accumulation has been measured in many French vineyard soils. Copper can induce phytotoxicity, and its accumulation in plants modifies the morphology, biochemistry or physiology of plant organs. It may also disturb the plant-pest and pathogen interactions. The effect of copper contamination in vineyard soils on the plant sensitivity to pathogenic attacks has so far received very limited attention. The present study investigated the sensitivity of potted grapevines to inoculation of *Neofusicoccum parvum,* a pathogenic fungus involved in Botryosphaeria dieback, at 3 different concentrations of copper in the soil (3, 108 and 325 mg $Cu.kg^{-1}$). We also studied the microflora associated with the wood trunk tissues using (i) a fingerprinting method, Single Strand Conformation Polymorphism (SSCP) and (ii) a next-generation sequencing method, pyrosequencing 454. Results show that high doses of copper in soil promoted the development of wood necrosis caused by *N. parvum*, and modified the composition of the associated microflora. In particular, the bacterial communities of woody tissues were different in the plants inoculated or not with *N. parvum*, unlike the global fungal communities, which seemed to be relatively similar in both types of plant. Cartography of the microbiote colonizing wood tissues of grapevines, inoculated or not by *N. parvum,* are presented. In conclusion, high concentration of copper in soil has an influence on the fungal microbiota and permits more *N. parvum* attacks. Further studies have been developed to verify whether high soil concentrations of copper and GTD development are correlated in vineyards.

Differentiation of bacterial and fungal diversity in the different types of trunk wood tissues of mature grapevines revealed by metagenomics. J. VALLANCE[1,2], E. BRUEZ[2], A. GAUTIER[3], V. LAVAL[3], S. BASTIEN, M.H. LEBRUN[3] and P. REY[1,2]. [1]*Université de Bordeaux, Bordeaux Sciences Agro, UMR 1065 Santé*

et Agroécologie du Vignoble, F-33175 Gradignan, France. [2]INRA, UMR 1065 Santé et Agroécologie du Vignoble, ISVV, F-33140 Villenave d'Ornon, France. [3]INRA, UMR 1290 Bioger, F-78850 Thiverval-Grignon, France. E-mail: jessica.vallance@inra.fr

Fungal and bacterial microfloras inhabiting the trunk wood tissues of mature (25-year-old) grapevines cv. Cabernet Sauvignon, having expressed or not esca-foliar symptoms, were investigated by molecular fingerprinting (Single-Strand Conformation Polymorphism - SSCP) and tag-encoded 454 pyrosequencing. For both symptomatic and asymptomatic vines, five kinds of tissue were sampled and analysed, based on their status, healthy or necrotic, and their location in the trunk, outer (under the bark once removed) or inner part (heart of the trunk): brown stripe (BS), sapwood (SW), central necrosis (Nec), white rot (WR) and heartwood (HW). Complex microbial communities, as shown by SSCP analyses, colonized the wood; their repartition was dependent on the location and type of tissue they inhabited within the trunk, i.e. healthy or necrotic wood in the outer or inner part of the trunk. Bacterial and fungal diversity per type of vine and tissue were then assessed by using tag-encoded 454 pyrosequencing of the 16S rRNA V5-V6 region, and the nuclear ribosomal ITS1 region, respectively. No fewer than 10,056 and 56,837 quality sequences were respectively obtained for bacteria and fungi, corresponding to 127 and 228 Operational Taxonomic Units (OTUs). OTUs mostly belonged to the *Proteobacteria* phylum (79.1%) for bacteria, and to the *Basidiomycetes* (68.1%) and *Ascomycetes* (24.9%) phyla for fungi. Alpha diversity analyses revealed that greater diversity was detected in the brown stripe (BS) for bacteria, and in the sapwood (SW) for fungi. Beta diversity analyses demonstrated, like the SSCP fingerprinting, that differentiation of the structure of the wood-colonizing microbial communities was tissue- and trunk zone-dependent (healthy vs. necrotic), rather than between esca-symptomatic and asymptomatic plants.

Disease management in nursery and vineyard

Six years of trials on the activity of Remedier® against Esca disease complex in young and mature Italian vineyards C. ALOI[1], G. BIGOT[2], P. BORTOLOTTI[3], M. COTROMINO[1], S. DI MARCO[5], F. FACCINI[1], A. MONTERMINI[3], L. MUGNAI [4], F. OSTI[5], R. NANNINI[3] and F. REGGIORI[1]. *[1]Isagro S.p.A., Via Fauser 28, 28100 Novara, Italy – Novara, [2]Perleuve, via Isonzo 25 – 34071 Cormons (GO), [3]Consorzio Fitosanitario di Modena e Reggio Emilia,* Via Gualerzi, 32 - 42124 - Reggio Emilia, Italy, *[4]Dipartimento di Scienze delle Produzioni Alimentari e dell'Ambiente (DiSPAA) – Sezione Patologia vegetale ed Entomologia, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy, [5]IBIMET, Via Gobetti, 101, 40129 Bologna, Italy. E-mail: caloi@isagro.com*

Esca disease complex is probably the greatest threat for wine grapes nowadays, due to its severity on most cultivars, the complex interactions of the fungi associated with the disease and the lack of effective control protocols. Control of grapevine leaf stripe disease (GLSD), the most common disease in Europe within the esca disease complex on young vineyards (2 years old), was achieved by spraying pruning wounds with Remedier®, a bio-fungicide containing two selected strains of *Trichoderma asperellum* and *Trichoderma gamsii* developed by Isagro Company. A single aerial application carried out with machine sprayers at the end of winter or early spring on pruning wounds every year soon after pruning (1–2 days) on ten extremely susceptible cultivars and the evolution of the disease was monitored over 6 years. The results were significantly positive, showing a reduction in the number of symptomatic vines by 45–65% in the treated plots, compared with untreated control plots. In the same way, trials were performed in older vineyards 5–26 years old, to verify the efficacy of the product in controlling the spread of symptomatic plants and plant death. After 6 years of treatment and monitoring, not only the presence of vines showing leaf symptoms (GLSD) was reduced, but also the incidence of dead plants in the treated plots. These trials, both on young and older vineyards, confirm the importance of application with Remedier® to pruning wounds in order to reduce infection by pathogens. Remedier® was effective in reducing the occurrence of vines showing symptoms for the first time, presumably because they create a biological barrier against the pathogen, as shown in previous research with artificial inoculation. Applications must be repeated every year when environmental conditions (mainly T° higher than 10–12 °C) are suitable for a fast and effective fresh wound colonization by the strains of *Trichoderma*.

Critical timing of application of fungicides for the prevention of wound infection by *Eutypa lata* and *Neofusicoccum luteum*. M. R. AYRES[1], R. BILLONES-BAAIJENS[2], S. SAVOCCHIA[2], E.S. SCOTT[3] and M.R. SOSNOWSKI[1,3]. [1]*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001.* [2]*National Wine and Grape Industry Centre, Charles Sturt University, School of Agricultural and Wine Sciences, Locked Bag 588, Wagga Wagga NSW 2678.* [3]*School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond SA 5064. E-mail: matthew.ayres@sa.gov.au*

The grapevine trunk diseases Eutypa and Botryosphaeria dieback are caused by fungal pathogens that infect pruning wounds, leading to vine decline and eventual vine death. Research has shown that fungicides applied to grapevine pruning wounds can prevent infection by trunk disease pathogens, but in order to maximise the efficiency of pruning wound protection, there is a need to determine the critical timing of fungicide application following pruning. Two field trials were conducted each in South Australia ('Shiraz') and New South Wales ('Cabernet Sauvignon') between 2013 and 2015. Three fungicides, namely tebuconazole (Folicur), pyraclostrobin (Cabrio) and fluazinam (Emblem), were applied to fresh grapevine pruning wounds made in winter 1, 6 or 14 days prior to inoculation with *Eutypa lata* (South Australia) or *Neofusicoccum luteum* (New South Wales), to assess their ability to prevent infection. The same fungicides were also applied 1, 3 and 6 days after inoculation with the pathogens to assess their curative effect. In the following winter of each trial, the treated canes were harvested, and assessment of colonisation of the canes by the pathogens was conducted in the laboratory by isolation on agar media. All three fungicides provided preventative control of *E. lata* for at least 1 week and *N. luteum* for up to 2 weeks after pruning and application. The fungicides also exhibited curative properties by reducing infection by both pathogens when applied up to 6 days after pruning and inoculation. Therefore, if applied 1 week after pruning, a single fungicide application could provide up to 3 weeks of wound protection, thereby providing improved trunk-disease management options for growers.

Susceptibility of grapevine pruning wounds to the trunk disease pathogens *Eutypa lata, Diplodia seriata* and *Neofusicoccum luteum*. M.R. AYRES[1], R. BILLONES-BAAIJENS[2], S. SAVOCCHIA[2], E. S. SCOTT[3] and M.R. SOSNOWSKI[1,3]. [1]*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001.* [2]*National Wine and Grape Industry Centre, Charles Sturt University, School of Agricultural and Wine Sciences, Locked Bag 588, Wagga Wagga NSW 2678.* [3]*School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond SA 5064. E-mail: matthew.ayres@sa.gov.au*

Eutypa and Botryosphaeria dieback, caused by fungal pathogens that infect grapevine pruning wounds, pose a serious threat to wine-grape production throughout the world. Limited information exists on the duration of susceptibility of wounds to infection at different times during the pruning season. Field trials were conducted in South Australia (cv Shiraz) and New South Wales (cv Cabernet Sauvignon) between 2013 and 2015 to evaluate grapevine wound susceptibility following early (June), mid (July) and late (Aug) winter pruning, by inoculating with spores of *Eutypa lata*, *Diplodia seriata or Neofusicoccum luteum* up to 16 weeks after each pruning time. The following winter of each trial, canes were assessed for infection by isolation on agar media. Wounds were generally more susceptible to infection by *E. lata* when pruned early in June compared with the later pruning times, and were most susceptible to infection for 2 weeks regardless of pruning time, after which susceptibility decreased sharply. For *D. seriata*, there was no difference between pruning times but susceptibility was extremely high in the first 2 weeks compared with the other pathogens, followed by a steady decline thereafter. With *N. luteum*, susceptibility decreased at the late pruning time, and the most susceptible period was the first week, with a sharp decline in the following weeks. Detached cane assays using *E. lata* and *N. luteum* were also established in the greenhouse to compare wound susceptibility between six cultivars: Shiraz, Chardonnay, Cabernet Sauvignon, Merlot, Sauvignon Blanc and Semillon, and revealed no difference following infection by both pathogens 1, 7 and 14 days after pruning. This study highlights the importance of protecting pruning wounds, regardless of the pruning time or grapevine cultivar.

A new agronomic approach to control GTD: the sylvestris way. C. BERTSCH, J. CHONG and C.

TARNUS. *Université de Haute-Alsace; Laboratoire Vigne Biotechnologies et Environnement et Laboratoire Chimie Organique et Bio-organique, Colmar France. E-mail: christophe.bertsch@uha.fr*

In Europe, from ages past to the mid-19th century, *Vitis vinifera* was apparently growing in balance with its environment but unsafe exchanges of vine species between Europe and America led to the introduction of pathogens that clearly shaped the vineyards. The introduction of powdery mildew, downy mildew and phylloxera, caused major phytosanitary crises. However, from these issues emerged strategies that led to the founding of modern plant pathology and plant protection. The vine has been saved from phylloxera by one of the most elegant and effective systems ever found in plant protection: the use of American rootstocks resistant to phylloxera. Grapevine trunk diseases (GTDs) are amongst the major challenges for viticulture and are economically significant diseases caused by a complex of xylem-inhabiting fungi, no efficient treatment is available to control these diseases. The different fungi associated with GTD are located in the wood but not in the leaves. As the roots for phylloxera, the trunk represents the weakness of the vine concerning GTD. Recent works of our group has shown a large variation of susceptibility to fungi associated with Botryosphaeria dieback, with good performance in several accessions from *V. vinifera* subsp. *sylvestris*, the ancestor of cultivated grapevine. In order to fight against GTD we propose to test a new architecture of our vine plants by the introduction of a *sylvestris* trunk between the rootstock and the *vinifera* varieties. The substitution of *V.v.* varieties wood by *V. vinifera* subsp. *sylvestris* wood could lead to the decrease of optimal conditions for GTD pathogen multiplication.

Trichoderma atroviride I-1237 as a biocontrol solution to manage Grapevine Trunk Diseases; ESQUIVE® WP field approaches. F. BOULISSET, A. HENNEGUEZ, P. LETOUSEY, R. KEMPF and C. PROFIZI. *Agrauxine Lesaffre Plant Care, 2&4 rue Henri Becquerel, 49070 Beaucouzé, France.* E-mail: *fboulisset@agrauxine.fr*

Grapevine Trunk Diseases (GTD) are widely spread around European vineyards, and represent a major threat for vine-growing with economic impact, due to limited lifespan, yield and wine quality. Biocontrol products represent one of the ways to limit these diseases in vineyards. ESQUIVE® WP, whose active substance is *Trichoderma atroviride* I-1237, has been registered for vineyards in France against Esca and Botryosphaeria dieback (often reported as black dead arm, BDA). Agrauxine Lesaffre Plant Care has built a national experimental network, with more than 20 multi-year field trials, an average of 700 vines per trial, and individual plant disease monitoring. Results obtained so far have showed that ESQUIVE® WP applied at 4 kg ha^{-1}, and compared to an untreated control, reduced significatively Esca and BDA symptom expression and associated vine plant death under field conditions. Thus, ESQUIVE® WP is now considered as a real option for GTD management, that could be combined with other prophylactic and agronomic measures. Moreover, the field trial network and individual plant disease monitoring also provides better understand of disease development and the multi-year symptom expression from a global to a plant scale.

***Trichoderma* strains isolated from vineyards in Castile-Leon (Spain) against *Phaeoacremonium aleophilum in* "in vitro" assays.** G. CARRO HUERGA[1], S. MAYO[1], A. RODRIGUEZ-GONZÁLEZ [1], S. ÁLVAREZ-GARCÍA[1], S. GUTIÉRREZ[2] and P.A. CASQUERO[1]. [1]*Research Group of Engineering and Sustainable Agriculture, Natural Resources Institute, University of León, León, Spain,* [2]*Research Group of Engineering and Sustainable Agriculture, Area of Microbiology, University School of Agricultural Engineers, University of León, Ponferrada, Spain. E-mail: gcarh@unileon.es; pacasl@unileon.es*

Trichoderma is widely used in agriculture as a BCA (Biological Control Agent). This fungus is an opportunistic secondary invasive, which is fast growing and produces a large number of spores, enzymes and compounds with antimicrobial activity. *Trichoderma* has been reported as an effective BCA against pathogens which cause GTD´S (Grapevine trunk diseases). Co-evolution between pathogens and *Trichoderma* have occurred over many years in vineyards, so we have selected *Trichoderma* strains from old vineyards in Castile-Leon country (Spain). *Trichoderma* strains have been isolated from soils and vines (trunk, cordon and spur). In this assay, the pathogen *Phaeoacre-*

monium minimum was used, which is one of the first fungi that colonize grapevine pruning wounds and has been isolated from diseased vines. Dual culture evaluation was done as the first *in vitro* assay. An antagonistic interaction was observed between cultures of *P. minimum* and *Trichoderma* strains. We evaluated *Trichoderma* sporulation, inhibitory capacity, medium staining and overgrowing capacity; to determine the best *Trichoderma* strain. Then the ability of strains to colonize the plant (translocation along the plant and persistence in vines) was evaluated. Disinfected grape wood was used in an *in vitro* assay evaluation. *Trichoderma* strains were isolated from vine wood 25 days after inoculation and 2 cm below the inoculation point. Future research will examine how long *Trichoderma* can persist and the distance it can colonise the vine.

Effect of plant defense stimulators or biostimulants on Botryosphaeriaceae *in planta* and on grapevine trunk disease in the vineyard. A. BELLÉE[1], G. COMONT[1], A. NIVAULT[1], L. GUÉRIN-DUBRANA[2], O. ANDRE[2], M.-C. DUFOUR[1] and M.-F. CORIO-COSTET[1]. [1]*INRA, UMR Santé et Agroécologie du Vignoble (1065), ISVV, Labex Cote, CS 20032, 33882 Villenave d'Ornon, France.* [2]*Université de Bordeaux, ISVV, UMR SAVE, Bordeaux Sciences Agro, F-33175 Gradignan, France,* [3]*DE SANGOSSE - « Bonnel » B.P. 5 - 47480 Pont-du-Casse, France. E-mail: marie-france.corio-costet@inra.fr.*

Grapevine trunk decline is complex and involves many factors such as the presence of grapevine trunk disease pathogens, but also the genetic background of vines and in particular their response to various stresses. Rooted cuttings of Cabernet sauvignon inoculated with *Neofusicoccum parvum* and *Diplodia seriata* were treated with plant defense stimulator (PDS) or biostimulant-PDS. A decrease of both the length of cankers and necrosis were observed, depending on the PDS applied. Depending on the application mode, one or two led to a significant decrease of necrosis. The expressions of defense genes in leaves of inoculated or un-inoculated plants were followed with Neovigne96 chip. As an example given, a foliar treatment led to repression and over-expression of different genes involved in the defenses. More interesting, treatments applied in vineyard in 2014 and 2015 on symptomatic and asymptomatic plants showed that it was possible to reduce the expression of foliar symptoms in symptomatic plants on different plots and on different cultivars (Sauvignon blanc, Cabernet-Sauvignon). In fact, PDS applied only once in vineyard reduced symptom expression of dieback diseases and increased the yield. The use of PDS in the nursery or on young plants may also limit the appearance of grapevine trunk disease in vineyard, but requires further evaluation.

Hot-water treatment and fungicide sensitivity of *Diaporthe ampelina* isolates causing wood canker of grapevine in Turkey vineyards. Q. NAWAZ AWAN and D. SONER AKGÜL. *University of Çukurova, Faculty of Agriculture, Department of Plant Protection, 01330, Balcalı, Adana / Turkey. E-mail: sakgul@cu.edu.tr*

Diaporthe ampelina, causing wood canker of grapevine, is a common fungal trunk disease pathogen in Turkish vineyards. The aims of this study were to evaluate the sensitivity of *D. ampelina* isolates to hot-water treatment (HWT) and some fungicides using *in vitro* assays. Sensitivity of six fungal isolates was tested against HWTs and eight fungicide treatments (azoxystrobin, azoxystrobin + tebuconazole, boscalid, cyprodinil, fludioxonil, cyprodinil + fludioxonil, propiconazole + azoxystrobin + cyproconazole and tebuconazole). Ten-day-old mycelial plugs were subjected to HWTs (in centrifuge tubes containing sterile distilled water at 45-50°C for 30 and 45 min) by dry block heater, and then transferred to potato dextrose agar (PDA) media. In another assay, PDA plates containing five different concentrations (1, 5, 10, 25, 50 $\mu g \cdot mL^{-1}$) of fungicides were prepared and fresh mycelial plugs were placed onto fungicide-amended PDA. To promote mycelial growth, all plates were incubated at 24°C for 10 days and colony diameters were recorded daily. Thermal death points and half maximal effective concentration (EC_{50}) values were determined for each isolate. Sensitivity of the isolates differed from each other for thermal death point and fungicide sensitivity. Thermal death point was fixed at 48°C for 30 min (isolates 62, 101 and 144) or 45 min (isolates 70, 78 and 97). On the other hand, tebuconazole and cyprodinil + fludioxonil were the most effective fungicides (EC_{50} <0.001 $\mu g \cdot mL^{-1}$) inhibiting mycelial growth completely, while azoxystrobin and boscalid were ineffective (EC_{50} >100 $\mu g \cdot mL^{-1}$). It may

be inferred from the results that HWT at 48°C for 45 min and fungicide combinations (tebuconazole and cyprodinil + fludioxonil) may be useful to effectively reduce *D. ampelina* infections in hydration tanks before grafting in grapevine nurseries.

Using tractor mounted optical sensors to assess grapevine canopy decline due to eutypa dieback. K. DeGARIS[1,2], P. BALNAVES[1,3], C. KIDMAN[1,2] and M.R. SOSNOWSKI[4]. [1]*Limestone Coast Grape and Wine Technical Council, PO Box 28, Coonawarra SA 5263, Australia.* [2]*Wynns Coonawarra Estate, Memorial Drive, Coonawarra SA 5263, Australia,* [3]*Balnaves of Coonawarra, PO Box 16, Main Road, Coonawarra SA 5263, Australia.* [4]*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia.*
E-mail: mark.sosnowski@sa.gov.au

Eutypa dieback is an important disease affecting vineyards worldwide, causing significant yield and quality reduction through stunted shoots, distorted leaves, shrivelled fruit and loss of productive canopy. Assessment of eutypa dieback to determine its impact on production and assist in decision making for implementing management strategies has relied on visual assessments which are labour intensive and expensive. The application of Greenseeker® to assess grapevine canopy decline in vineyards was evaluated against visual assessments by trained personnel. Greenseeker provides a normalized difference vegetation index (NDVI) using optical sensors in the visible and near-infrared bands of the electromagnetic spectrum to detect the presence of green vegetation. In the Limestone Coast region of South Australia, two Cabernet Sauvignon single-cordon vineyards, aged 16 and 18 years, were surveyed by walking along rows and visually assessing each vine from 0–100% reflecting the percentage of healthy canopy. On the same day, a tractor mounted with a Greenseeker, passed through rows of each vineyard, taking horizontal readings across the canopy every 50 cm. NDVI data was interpolated by kriging using Farmworks® software and data was analysed and compared with visual assessments using Microsoft® Excel®. Comparing assessments of two different personnel for one of the vineyards, there was no significant difference between assessors, with mean overall canopy ratings of 81.1 and 79.8%. There was a strong correlation between visual assessments and the Greenseeker data, with R^2 value of 0.73 when data from both vineyards were combined. This research has provided support for the use of Greenseeker to assess loss of productivity due to eutypa dieback. Further research is required to expand evaluations to different environments, varieties and training systems. The use of unmanned aerial vehicles fitted with multi-spectral sensors may also provide alternative assessment tools for trunk disease management, and will be the focus of future research.

What can the OIV do to help in Grapevine Trunk Diseases control? A real challenge for the vitivinicultural sector. M. DE LA FUENTE, *Head of Unit Viticulture of the International Organisation of Vine and Wine (O.I.V.), 18 Rue d'Aguesseau, 75008 Paris, France.*
E-mail: viti@oiv.int

Grapevine trunk diseases (GTD) are not a new challenge, but they still remain as an unsolved problem to the vitivinicultural sector. The International Organisation of Vine and Wine (OIV) has been working in different actions since the appearance of the VITI 02/2006 resolution, which focused on measures used to prevent or limit the proliferation of wood diseases. This text recognised some problems as the fact that the players in the viticulture world are not very familiar with the symptomatology induced by these diseases; that this symptomatology is often little known and confusing; and finally that, at the present time, there are no corrective measures sufficiently effective. On the other hand, this resolution aims to adopt some preventive measures: planting, pruning and formation of vines and rootstocks, remove or restore diseased vine plants or dead plant material, training on viticultural practices, etc. More recently, the OIV (in collaboration with the ICGTD) has published a new expertise document concerning the development and impact of the main trunk diseases, and alternatives to control or mitigate of their spread and damage at an international level. Lastly, during 2015–2016, the OIV has supported two fellowship grants in this topic: one about the degree of resistance/susceptibility of grapevine rootstocks towards grapevine trunk disease pathogens, and another about the beneficial microflora colonizing the wood of *Vitis vinifera* (protection against esca). Although these actions will help to understanding GTD in vines, they will not be enough for our pur-

poses. Handbooks, impact data and vine selection models will be revised by the OIV for managing future actions in order to progress work on this very important topic.

Activity of electrolyzed acid water for the control of trunk disease pathogens of grapevine in the nursery. S. DI MARCO[1], F. OSTI[1], M. NOCENTINI[2] and L. MUGNAI[2]. [1] *IBIMET, CNR, Via Gobetti 101, 40129 Bologna, Italy.* [2]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DiSPAA)- Sez. Patologia vegetale ed Entomologia, University of Florence, Piazzale delle Cascine 28, 50144 Florence, Italy. E-mail: s.dimarco@ibimet.cnr.it*

Phaeomoniella chlamydospora (Pch) and *Phaeoacremonium minimum* (Pmi) are among the main grapevine trunk disease pathogens in the nursery. These pathogens can cause latent infections of the propagation material often appearing as dark wood streaking, and are associated with decline occurrence in young vineyards. The infection may occur during the grafting process, particularly during hydration. Promising preliminary results were obtained in the nursery using electrolyzed acid water (EAW) for the hydration of cuttings; EAW is characterized by low values of pH (2.5), high oxidation reduction potential (ORP>1000) and a certain amount of free chlorine. No differences in absorption kinetics were recorded between EAW and tap water, nor among 1103P, K5BB and SO4 rootstocks. In all combinations no additional absorption was observed after 7 hours of immersion. In order to assess the characteristics of EAW after the contact with the inner plant tissues ORP and pH were measured from the liquid extracted from rootstocks after immersion in EAW. The values of the liquid, named "weakened" EAW (wEAW) (625–650 mV ORP and 3.1-3.3 pH), were significantly different from the values of both EAW and the liquid extracted after immersion in tap water. When the propagation material had been hydrated for 7 hr in EAW no significant effect on vegetating growth performance and percentage of No. 1 quality plants was ever observed for SO4 or 1103P, whereas a reduction in both vegetative growth and No. 1 quality plants was observed in K5BB rehydrated vines at 12hr hydration. EAW and wEAW gave no significant reduction of *in vitro* mycelial growth, whereas a consistent decrease in conidial germination was observed in Pch and Pmi. The three-year experiments carried out in the nursery on cuttings inoculated with Pch and re-hydrated in EAW showed a remarkable reduction of the infection level in the treated plants.

Application of *Trichoderma* in nurseries as a kind of vaccination to avoid early infections with GTD pathogens. J. EDER, M. ZINK, M. HAUSTEIN, M. KUBE and A. KORTEKAMP. *State Education and Research Center DLR Rheinpfalz, Institute for Plant Protection, Breitenweg 71, 67435 Neustadt an der Weinstraße. E-mail: joachim.eder@dlr.rlp.de*

Application of *Trichoderma* at grapevine grafting was tested in a multi-year trial at the experimental station for plant propagation at the DLR Rheinpfalz, to obtain high colonization rates of the antagonist and to avoid subsequent GTD pathogen infections. Grafted vines obtained from scions and rootstocks previously disinfected with 8-Hydroxychinolin (BELTANOL-L) were treated using commercial *Trichoderma*-products. The *Trichoderma* colonization on treated vines was assessed by analysis of wood slices taken from different portions of the plant and expressed as incidence and severity. Colonization rates reached up to the 100%, when vines were treated with *Trichoderma* immediately after grafting and before waxing. All *Trichoderma*-treated vines developed well in nursery fields and no visible abnormalities were observed when compared to the untreated plants. Percentages of plants suitable for sale were at similar levels for both *Trichoderma*-treated and untreated control vines. Fresh weight and root length analyses revealed that the treatment did not interfere negatively with vine quality. To prove the beneficial effect of *Trichoderma* on the health status of vines, both treated and non-treated vines were planted in the field, allowing a long term evaluation of both plant growth and external or internal symptom expression of GTD´s. First observations revealed no differences between treated and non-treated plants with regard to plant vigour. In order to develop practical methods, when a *Trichoderma* application is implemented as additional step in the plant propagation process, rootstock, scions, and vines were treated at different times during the plant production procedure. Application of *Trichoderma* on scion and rootstock cuttings before grafting and on grafted vines after callusing resulted in high colonization rates. Thus, first tests

of *Trichoderma* application in German nurseries were successfully established.

Sodium arsenite application on plants expressing Grapevine Trunk Diseases-foliar symptoms: impact on grapevine physiology. J. VALLET[1], G. ROBERT-SIEGWALD[2], C. GUILLIER[3], M.-L. GODARD[4], E. BRUEZ[5], M. ADRIAN[3], S. TROUVELOT[3], L. JACQUENS[3], A. SONGY[1], C. CLEMENT[1], P. REY[5], C. TARNUS[4], C. BERTSCH[6], P. LARIGNON[7], M.-H. LEBRUN[2] and F. FONTAINE[1]. *[1]SFR Condorcet, URCA, URVVC EA 4707, Laboratoire SDRP, BP 1039, 51687 Reims Cedex 2, France. [2]UMR 1290 BIOGER, INRA AgroParisTech, Ave. L. Bretignières, 78850 Thiverval Grignon, France. [3]Agroécologie, AgroSup Dijon, CNRS, INRA, Univ. Bourgogne Franche-Comté, INRA, 17 rue Sully, BP 86510, 21000 Dijon, France. [4]Laboratoire COB, 3 rue Alfred Werner, 68200 Mulhouse, France. [5]UMR 1065 SAVE, INRA/Bordeaux Sciences Agro, 33883 Villenave d'Ornon, France. [6]LVBE EA 3391, UHA, 33 rue de Herrlisheim, BP 50568, 68008 Colmar cedex, France. [7]IFV Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France. E-mail: florence.fontaine@univ-reims.fr*

After decades of sodium arsenite applications in many European vineyards, the sole product registered to control Grapevine Trunk Diseases (GTDs), it has been banned in Europe since 2003. No other efficient chemical molecules are currently available. A French project was launched in 2013 to decipher the mode of action of sodium arsenite on grapevine physiology and its microbiome, and the distribution of arsenite in the various plant organs. During the 2014 and 2015 winters, a sodium arsenite treatment (1250g hl^{-1}) was applied to grapevines a few weeks before bud burst in three vineyards with: Chardonnay (in Champagne), Gewurztraminer (in Alsace) and Merlot (in Languedoc-Roussillon). Each year, three arsenite treated-vines and three untreated ones were uprooted before flowering and before harvest, then leaves, stem, trunk and root samples were collected. For the three cultivars, the expression of grapevine genes involved in detoxification and plant defense response to pathogens (*GTS1, PR1, STS, CHV5, SOD*) were studied. A differential response for these selected targeted genes was observed depending on the cultivar, the organ within the plant, the phenological stages (flowering, harvest), and the year. To better characterize the physiological changes, global analyses of transcriptomic and metabolomic data were made with the Chardonnay samples. In grapevine-treated plants, a modulation of plant physiology with distinct metabolomic and transcriptomic profiles was observed. Metabolomic approach pointed out organ-dependent fingerprints in response to sodium arsenite treatment. Discrimination between healthy-untreated and sodium arsenite-treated plants could also be pointed out. The most important differences were located in the trunk and concerned CHO compounds from plant and microbial origin. In addition, half of the genes expressed in infected, but arsenite-treated plants, and healthy-untreated plants have a similar expression pattern, suggesting sodium arsenite partially cured grapevine from GTDs. This hypothesis was supported by microbiological and metagenomic analyses of trunk-microbiome: the number of pathogenic fungi is reduced and the whole microflora displays significant changes in GTDs-sodium arsenite-treated vines compared with controls.

A new mode of treatment to cure GTDs: vegetal endotherapy? M. GELLON[1], J. FUCHS[1], S. FARINE[1], F. MAZET[1], H. LALOUE[1], M.-L. GODDARD[1,2], M. FISCHER[1], J. CHONG[1], C. TARNUS[2] and C. BERTSCH[1]. *[1]Laboratoire Vigne, Biotechnologies et Environnement, Université de Haute-Alsace, 33 rue de Herrlisheim, 68008 Colmar Cedex, France. [2]Laboratoire de Chimie Organique et Bioorganique, Université de Haute-Alsace, 3bis rue Alfred Werner, 68093 Mulhouse Cedex, France. E-mail: melanie.gellon@uha.fr*

Grapevine trunk diseases are devastating vineyards. The main diseases (Eutypa dieback, Esca and Botryosphaeria dieback) are caused by a complex of fungi which attack perennial organs leading to vine death. Since the use of sodium arsenite was banned in 2001, no curative treatment has been offered to winegrowers. The situation is such that today, these diseases are increasing and the regulations require more environmentally sensitive practices and safe products. A solution may come from vegetal endotherapy, an alternative treatment technique by directly injecting plant protection products into the vascular system of trees. This technique actually offers good results in pest management in fruit trees like apple tree and avocado or in urban areas and has numerous technical, economic and environmental interests. Negative

impacts on environment are reduced by using lower quantities of products. Chemical drift in the soil, air and water are limited, thus reducing the impact on non-target organisms. Trunk injection also has lower risk for the applicator. It was recently shown that Esca and Botryosphaeria dieback (referred by some authors as black dead arm) symptom expression can decrease the following years after curing the white rot. Moreover, after sodium arsenite application on the trunk during the vegetative cycle, the population of pathogen fungi decreased and no GTD foliar symptoms were observed. The study of sodium arsenite distribution has shown that the molecule is concentrated in the white rot after spraying application. We adapted trunk injection to the vine by drilling a vertical hole in the trunk to reach the white rot and inject fungicides. The interest of this method is to target fungi involved in grapevine trunk diseases, like *Fomitiporia mediterranea*, and attack them where they multiply and produce different kind of toxic metabolites.

qPCR and RT-qPCR assays for the quantification *Trichoderma asperellum* icc012 and *Trichoderma gamsii* icc080 used as biocontrol agents against *Phaeomoniella chlamydospora* in grapevine nursery. D. GERIN[1], C. RAGUSEO[1], N. TANESE[1], C. ROTOLO[1], R. M. DE MICCOLIS ANGELINI[1], C. DONGIOVANNI[2], M. DI CAROLO[2], G. FUMAROLA[2], S. POLLASTRO[1] and F. FARETRA[1]. [1]*Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, via Amendola, 165/A, 70126 Bari, Italy.* [2]*Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile Caramia", via Cisternino, 284, 70010 Locorotondo (BA), Italy. E-mail: stefania.pollastro@uniba.it*

Trichoderma asperellum strain icc012 (TA) and *Trichoderma gamsii* strain icc080 (TG) (Remedier, Isagro S.p.A., Italy) are biological control agents (BCAs) proposed against esca disease on grapevine. This research was aimed at evaluating their effectiveness against esca in the nursery with particular regard to *Phaeomoniella chlamydospora* population and at developing a fast and reliable method to quantify the two BCAs. The effectiveness of TA and TG was evaluated on 1103 P. rootstocks, artificially inoculated at the hydration stage with conidia of the benomyl-resistant mutant C1.A43.1 of *P. chlamydospora* and successively treated or not with Remedier at different time combinations (end of hydration, at planting, and three times during plant growth). Eight different combination programs were compared. At uprooting of rootstocks, wood fragments were sampled at 1 cm and 2–15 cm over the crown from at least 90 rootstocks per program. The incidence of fungi, including the C1.A43.1 mutant and the BCAs, was evaluated on malt extract agar (MEA) and MEA amended with 10 mg L^{-1} of benomyl. Natural infections of *P. chlamydospora* were rarely recovered while the population of the artificially inoculated mutant C1.A43.1 was always reduced by BCAs ($P \leq 0.05$, ranging from 18% with only one application at hydration to 36% with four applications). *Fomitopsis* sp., *Acremonium* sp., and *Fusarium* sp. were also reduced by BCAs. qPCR and RT-qPCR detection methods based on specific primers and TaqMan probes were set up and applied to quantify singularly and in duplex the populations of TA and TG. The primers/probe sets were designed on the polymorphic region of the *rpb2* gene and the method allows to specifically detect up to 10 pg of DNA and 150 pg of RNA. Further studies are in progress to evaluate the effectiveness of the BCAs and define the most appropriate usage in grapevine nursery for reducing infection of esca-associated fungi.

Arsenite experiments in the program CASDAR V1301 - Arsenic speciation in grapevines and impact on fungal population. M.-L. GODDARD[1,2], A. BOOS[3], P. LARIGNON[4], F. FONTAINE[5], C. BERTSCH[2] and C. TARNUS[1]. [1]*Laboratoire de Chimie Organique et Bioorganique, Université de Haute-Alsace, 3bis rue Alfred Werner, 68093 Mulhouse Cedex, France ;* [2]*Laboratoire Vigne, Biotechnologies et Environnement, Université de Haute-Alsace, 33 rue de Herrlisheim, 68008 Colmar Cedex, France;* [3]*Institut Pluridisciplinaire Hubert Curien (IPHC), UMR 7178 CNRS, Université de Strasbourg, 25 rue Becquerel, 67087 Strasbourg Cedex 2, France.* [4]*IFV Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France.* [5]*SFR Condorcet, URCA, URVVC EA 4707, Laboratoire SDRP, BP 1039, 51687 Reims Cedex 2, France. E-mail: mary-lorene.goddard@uha.fr*

Sodium arsenite has been used for decades to treat grapevines against various pathogens and has particularly proved its efficacy against GTD fungi. This fungicide was prohibited in the 2000s, without any

substitute for winegrowers. In the program CASDAR V1301 (2013-2016), the mode of action of sodium arsenite on vineyards was investigated in order to understand its efficacy and find a substitute. A series of experiments was carried out on vineyards over two years using three cultivars (Chardonnay, Gewurztraminer and Merlot) in three regions of France. After a sodium arsenite treatment in February 2014 & 2015 before bud break, grapevines were extracted in June and September and various organs from the roots to the leaves, were analysed for sectorial and central necrosis or brown streaking. A large number of analyses have been performed, i.e. arsenic localisation, transcriptomic, metabolomic, morphologic and microbiologic analyses. In the present work, we will report on the arsenic distribution as well as the arsenic forms (inorganic and methylated As(III) and As(V)) in the different tissues of grapevines. The correlation between the arsenic concentration and the GTD fungal populations will be discussed.

Screening and modes of action of antagonistic bacteria to control two fungal pathogens, *Phaeomoniella chlamydospora* and *Neofusicoccum parvum*, involved in grapevine trunk diseases. R. HAIDAR[1,3], E. BRUEZ[1], J. ROUDET[1], A. DESCHAMPS[1], P. REY[1,2] and M. FERMAUD[1]. [1]*SAVE, INRA, Institut National de Recherche Agronomique, BSA, ISVV, 33882, Villenave d'Ornon, France.*[2]*Université de Bordeaux, Bordeaux Sciences Agro, UMR1065 SAVE, 33140 Villenave d'Ornon, France.*[3]*Tichreen University, Faculty of Science, Biology Department, PO Box 2231, Latakia, Syrian Arab Republic. E-mail: patrice.rey@inra.fr*

Grapevine trunk diseases (GTDs), such as Esca and Botryosphaeria dieback, markedly impact the worldwide winegrape and tablegrape industry. Detection and development of antagonistic microorganisms, particularly bacteria, to achieve biological control of GTDs, would be of prime importance as a future innovative alternative practice in viticulture. The antagonistic activity of 46 bacterial strains, isolated from Bordeaux vineyards, were evaluated against *Phaeomoniella chlamydospora* and *Neofusicoccum parvum*, two major pathogens involved in GTDs. Different bioassays, under greenhouse conditions, with foliar grapevine stem cuttings have shown that the protection efficacy depends on the bacterial strain, the targeted pathogen species and, for *N. parvum*, on the application mode of the bacterial strain. A significant reduction in length of the necrosis due to *P. chlamydospora* and/or *N. parvum,* ranging between 40 and 64% in non-grafted grapevine cuttings, resulted from three bacterial strains: *Pantoea agglomerans* (S1), *Paenibacillus sp.* (S19) and *Bacillus pumilus* (S32). Against *P. chlamydospora*, the bacterial efficacy did not depend on the application method: co-inoculation, preventive inoculation in the soil and preventive inoculation in the wood. Preventive application of the bacteria in the wood was, however, the most efficient method against *N. parvum*. All three strains were subsequently further investigated to determine their major mode(s) of action by i) antibiosis, ii) production of antifungal volatile organic compounds, and/or iii) induction of grapevine systemic resistance. The volatile compounds secreted by these strains were identified by gas chromatography/mass spectroscopy (GC/MS). Finally, the induction of grapevine systemic resistance was studied by quantification associated with the expression of 10 major grapevine defense genes by real-time PCR.

Mycoparasitic behaviour of a *Microdochium* sp. isolate against *Dothiorella* sp., a fungus associated with Botriosphaeria dieback of grapevine. Z. KARÁCSONY, S. LENGYEL, Á. JUHÁSZ and K. VÁCZY. *Eszterházy Károly University of Applied Sciences, Food and Wine Knowledge Centre, 3300 Eger, Hungary. E-mail: karacsony.zoltan@uni-eszterhazy.hu*

The control of trunk diseases (including Botriosphaeria dieback) is a challenging task in the disease management of grapevine, since the ban of sodium arsenite. Thus many studies focus on the examination of alternative chemicals and techniques. However no experiments with fungicides showed satisfying results, only labour intensive canopy management techniques were able to reduce the occurance of these infections. A promising way to control grapevine trunk diseases is the application of mycoparasitic fungi as biological control agents. In the present study, we examine a possible control agent against Botryosphaeria dieback. The mycoparasitic fungus was isolated from the surface of a colony of a *Dothiorella* sp. isolate which derived from a grapevine (Eger wine region, Hungary) showing the symptoms of trunk diseases. According to the sequence of the ITS (internal transcribed spacer) region

of ribosomal RNA, the isolated fungus belongs to the *Microdochium* genus. Mostly plant pathogenic fungi are present in this group, mycoparasitic behaviour has not been reported yet in the case of this genus. Microscopic examinations on coverslip cultures of mixed mycelia showed mycoparasitic interaction between the *Microdochium* sp. and *Dothiorella* sp. isolates. The *Microdochium* sp. developed papilla-like structures on the surface of *Dothiorella* sp. mycelia and penetrated the cell wall. The intracellular expansion of the mycelia of the mycoparasite resulted in the perishment of host cells. However dual culture experiments on complete medium did not show antagonism between the two fungi. The mycelia of *Microdochium* sp. colonized the *Dothiorella* sp. colonies only a week after the contact established. This indicates that the mycoparasitic behaviour of *Microdochium* sp. is strongly dependent on nutrient availability. Our results extend the knowledge about *Microdochium* species and might provide the basis for the development of biological control techniques against grapevine trunk diseases.

Esca of grapevine and training practices in France: results of a 10-year survey. P. LECOMTE[1], B. DIARRA[1], A. CARBONNEAU[2], P. REY[3] and C. CHEVRIER[4]. *[1]INRA, UMR SAVE, Université de Bordeaux, ISVV, 71 av. Edouard Bourleaux, CS 20032, 33882 Villenave d'Ornon cedex, France. [2]Chambre Régional d'Agriculture du Languedoc-Roussillon - CS 30012 - 34875 LATTES, France. [3] BORDEAUX SCIENCES AGRO, UMR SAVE, Université de Bordeaux, ISVV, 71 av. Edouard Bourleaux, CS 20032, 33882 Villenave d'Ornon cedex, France. [4] UMR AGAP, 2 Place Viala, F-34060 Montpellier, France. E-mail: lecomte@bordeaux.inra.fr*

Esca largely contributes to the grapevine decline observed in France. Different biotic and abiotic factors may explain this situation. A survey was undertaken from 2007 by the 'Unité Mixte de Recherches en Santé et Agroécologie du Vignoble' at Bordeaux to identify relevant cultural predisposing factors that may influence the development of esca. This aetiological study was first based on local observations in Aquitaine. Then, it was expanded in a national project financed via public and private fundings, from 'Compte d'affectation spéciale pour le développement agricole et rural' and from 'Comité National des Interprofessions des Vins à appellation d'originerespectively. Thirty eight comparable plots of varieties with similar levels of susceptibility, the same age and similar soil and climatic environments, but with varying training or pruning systems, were examined up to 2016. Esca was the most prevalent trunk disease in these plots and incidence was assessed from all visible symptoms (leaves and wood). Results indicated a similar trend, that training decisions leading to long arms were generally less affected than the forms with short or no arms. For example, an experiment on cv Merlot in Gironde, showed that vines trained as lyra had 14 % esca incidence compared with those trained as Guyot, having 36 %. The pruning decisions also played a major role. For example, in Jura, on Guyot trained cv Chardonnay, vines pruned normally had 16% esca incidence compared with 38% for those subjected to a severe pruning regime. The study also showed that foliar symptoms reveal the presence of the disease but are not a reliable indicator of impact. On the whole, this study confirms that vine training and pruning decisions may greatly influence the amount of esca disease and that simplification of trunk structure observed in recent decades may have favoured the disease.

A questionnaire on the interest of cultural practices in the vineyard for the management of GTDs in Europe. F. OSTI[1], P. LECOMTE[2], B. DIARRA[2], D. GRAMAJE[3] and S. DI MARCO[1]. *[1]INRA, UMR SAVE, Université de Bordeaux, ISVV, 71 av. Edouard Bourleaux, CS 20032, 33882 Villenave d'Ornon cedex, France. [2]CNR IBIMET, Via Gobetti 101, 40129 Bologna Italy. [3]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, 26071 Logroño, Spain. E-mail: lecomte@bordeaux.inra.fr; s.dimarco@ibimet.cnr.it*

Grapevine trunk diseases (GTDs) of mature vines (Botryosphaeria and Eutypa diebacks and Esca) largely contribute to the grapevine decline in all viticultural regions of Europe. Different biotic and abiotic factors may explain this situation. Among them, cultural practices may increase or decrease the incidence of GTDs. Under the framework of the COST action FA1303 'Sustainable control of grapevine trunk diseases', an anonymous questionnaire was designed and sent to growers representing the most important viticultural regions in Europe (plus

Israel). The objective of this survey was to know more about the current interest for prophylactic measures, including cultural practices, recently or traditionally recommended within a control strategy towards GTDs. Questions were divided in four categories: general questions to know whether growers applied cultural practices to control GTDs or not, questions to know more about the practices used to remove or reduce inoculum sources, to prevent GTDs, or finally to control infection. Results indicated that growers generally have a better knowledge on the application of remedial strategies for disease control, than on preventative practices that can be used for the control of GTDs. A small number of growers are aware of pruning systems that can have the potential to reduce GTDs, such as the Guyot Poussard pruning system. Therefore, a major effort seems necessary to increase the knowledge and training on the principles and methods to prevent and to reduce infection by GTDs in mature vineyards.

ADVANTAGE: a French collaborative project for the development of new protection products and services for the setting up of a durable protection strategy against Grapevine Trunk Diseases, from nursery to vineyard. P. LETOUSEY[1], C. BENETREAU[2], M. BONNISSEAU[3], F. BOULISSET[1], M. CADIOU[1], M. CARRIER[4], C. COPPIN[5], P. DUBOURNET[6], F. FONTAINE[5], C. GERMAIN[7], E. GOULET[3], M.-C. GROSJEAN-COURNOYER[6], M.-J. LAMBERT[4], P. LARIGNON[3], P. LECOMTE[2], E. PAGOT[8], C. PROFIZI[1], F. RANCON[7], P. REY[2], M. SINEUX[9], L. VALETTE[8], A. YACOUB[2] and O. ZEKRI[9]. [1]*AGRAUXINE LESAFFRE PLANT CARE, 2&4 rue Henri Becquerel, 49070 Beaucouzé, France.* [2]*INRA, UMR SAVE, Université de Bordeaux, ISVV, 71 avenue Edouard Bourleaux, CS 20032, 33882 Villenave d'Ornon Cedex, France.* [3]*Institut Français de la Vigne et du Vin, 42 rue Georges Morel, 49071 Beaucouzé, France.* [4]*CYBELETECH, Campus Ter@tec, 2 rue de la Piquetterie, 91680 Bruyères le Chatel, France.* [5]*SFR Condorcet, URCA, URVVC EA 4707, Laboratoire SDRP, BP 1039, 51687 Reims Cedex 2, France.* [6]*BAYER S.A.S., 16 rue Jean-Marie Leclair, CP310, 69337 Lyon Cedex 09, France.* [7]*Université de Bordeaux, IMS UMR 5218, F-33400 Talence; Bordeaux Sciences Agro, F33170 Gradignan, France.* [8]*TELESPAZIO, Geo-Information Business Unit, Aerocampus, 1 route de Cénac, 33360 Latresne, France.* [9]*Pépinières Viticoles MERCIER, 16 rue de de la Chaignée, 85770 Vix, France. E-mail: pletousey@agrauxine.fr*

Face with an increasing epidemic context and to important economic losses, the wine network is strongly waiting for tools and solutions to control Grapevine Trunk Diseases (GTD). Since 2015, a French consortium of nine industrial, technical and academic partners is engaged into a 4-year program for nursery and vineyard protection against GTD. The main objective of the ADVANTAGE project is to develop new technical management tools adapted to both nurseries and vineyards in order to limit significantly GTD impact and for a global management of these diseases. The innovation of the protection strategy proposed by ADVANTAGE results in the implementation of a combinatorial management by the association of complementary protection solutions for a better efficacy and the development of decision support tools (DST) for the end-users, from nursery to vineyard. These DST either could be molecular tools or based on imaging and modelling. In nurseries, products and tools developed by ADVANTAGE will be maintained for the evaluation and the control of young plant health quality. In vineyards, the ADVANTAGE protection products could be coupled to a monitoring of survey health. The proposed tools, based on innovative imaging and modelling techniques, will allow to perform a diagnostic assessment at the level of plot GTD infestation, to estimate GTD development risk (early detection of GTD) and to quantify associated yield losses, in order to support phytosanitary treatment recommendations and to improve treatment positioning.

Biological and chemical protection of grapevine pruning wounds against *Phaeomoniella chlamydospora* in Chile. M. LOLAS[1], A. CASTRO[2], Y. MORENO[3] and G. A. DÍAZ[1]. [1]*Fruit Pathology Lab, Facultad de Ciencias Agrarias, Universidad de Talca, Chile.* [2]*UC Davis Chile, Life Science Innovation Center, Chile.* [3]*Grapevine and Wine Technological Center, Facultad de Ciencias Agrarias, Universidad de Talca, Chile. E-mail: mlolas@utalca.cl*

Trunk diseases cause decline and death of grapevine in Chile and worldwide. In Chile, the trunk diseases are associated with a fungal complex where *Phaeomoniella chlamydospora* is one of the most important caus-

al agents. Pruning wounds are considered the main point of infection, therefore, their protection is crucial in order to maintain a healthy vineyard. A study was conducted in the Maule region of Chile during winter (July) 2016. Several chemical and biological protection sprays and pastes were evaluated on pruning wounds of cvs. Cabernet Sauvignon and Sauvignon Blanc, for their efficacy to control *P. chlamydospora*. Treatments included chemicals: pyraclostrobin (Comet, BASF), fluazinam (Shirlan, Syngenta), tebuconazole (Apolo, Anasac) and fluopyram + tebuconazole (Luna Experience, Bayer) applied as sprays; pyraclostrobin (Podexal, BASF) and tebuconazole + kresoxim methyl (Pasta Poda Full, Anasac) applied as pastes; and biologicals: *Trichoderma virens* + *Bacillus licheniformis* + *Bionectria ochroleuca* (Coraza, Bionsumos Nativa) applied as paste; *T. virens* + *T. gamsii* + *B. ochroleuca* (Mamull, Bionsumos Nativa) and *T. virens* native strain (Universidad de Talca property) applied as sprays. A control treatment without any protection was included. Twenty four hours post-protection, wounds were inoculated using mycelial plugs colonised with *P. chlamydospora*. The necrotic lesion was measured 4 months post-inoculation (November). Results showed that all treatments in both cultivars significantly reduced the length of the necrotic lesion compared with the control treatment, with 48 (Apolo) - 58% (Coraza) control in Cabernet Sauvignon, and 48 (Poda Pasta Full) and 55% (Shirlan) control in Sauvignon Blanc. This study showed that fungicides currently registered to prevent powdery mildew and grey mould in grapevines in Chile, are also able to reduce the necrosis caused by *P. chlamydospora*. The biological agents showed similar control to chemical fungicides.

Pruning wound protection for control of esca causal agents. L. MARTÍN[1], F.. J. CASTAÑO[1] and P. ALUJA[1]. [1]*Fundació Parc Tecnològic del Vi (VITEC), Ctra. de Porrera km 1, 43730 Falset, Spain. E-mail: laura.martin@vitec.cat*

Esca is a major fungal disease causing decline and death of grapevines all over the world. In infected vineyards, fungal colonization occurs through injuries caused by pruning. Because no curative fungicides exist, pruning wound protectants provide options for growers to manage and reduce losses due to esca disease. Nowadays, limited fungicide products are registered for this use. Novel biological or alternative control strategies need to be evaluated for efficient control of the esca disease. During 2015 and 2016, five vineyards located in the Spanish regions: Rioja, Catalunya, Castilla-La Mancha, Aragón and Galicia were monitored. A total of 103 symptomatic esca vines (cv. Tempranillo, Cabernet Sauvignon and Albariño) were collected and their wood analysed to isolate pathogenic fungi. The causal agents of esca were identified by morphological and molecular tools. Detached cane assays were conducted to evaluate a range of 10 products for efficiency as pruning wound protectants. Natural formulations, fungicides and biological control agents were included in the assay. Each product was tested against three pathogen species (*Diplodia seriata*, *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*) as a preventative to avoid mycelial colonization. After two months of incubation, re-isolation of both pathogen and biological agents were performed. Moreover, percentage and length of vascular discoloration were estimated. The most commonly isolated pathogens from esca symptomatic grapevines were *Diplodia seriata*, *Phaeoacremonium* spp., *Phaeomoniella chlamydospora* and *Fomitiporia* sp. Protection with *Trichoderma spp.* resulted in failure to recover *Phaemoniella chlamydospora* and reduced re-isolation of *Phaeoacremonium minimum* and *D. seriata*. Quitosan and copper oxychloride decrease the infection of *D. seriata* and *Phaeoacremonium minimum*, respectively.

Rootstock impact and graft type on foliar symptom expression of Esca on *Vitis vinifera* cv. Cabernet sauvignon. M. SEVERINE[1], L. PASCAL[2], L. CORALIE[1] and J.-P. ROBY[3]. [1]*Université de Bordeaux, Vitinnov, ISVV, 1 cours du Général De Gaulle, 33170 Gradignan, France.* [2]*INRA, UMR1065 SAVE, Université de Bordeaux, ISVV, BP 81, 33883 Villenave d'Ornon Cedex, France.* [3]*Université Bordeaux, ISVV, Ecophysiology and functional genomics of grapevines, UMR 1287, F-33140 Villenave d'Ornon, France. E-mail: severine.mary@agro-bordeaux.fr*

Trunk diseases, Esca in particular, represent a major threat to vineyard sustainability. Most surveys or plot monitoring generally show that the levels of Esca foliar symptoms may depend on the cultivar and on environmental factors such as soil and climate. There is also evidence that soil has a major effect on

grapevine water status, and the interface between the ground and the vine is provided by the rootstock. The first study presents the results of a 4-year experiment carried out in the Bordeaux region (France) on a plot of Cabernet Sauvignon grafted onto four different rootstocks (Gravesac, 101-14, 3309 C, RGM). Results suggest that one of the four tested rootstocks significantly reduced Esca foliar symptoms under the conditions relative to this region. Among the three other rootstocks, there were some differences that were related weather conditions of the year. This could be considered as an extra-element to add to all the criteria required in choosing a rootstock. In a second study we investigated the role of three grafting methods, namely Omega, Whip and Tongue and Full cleft graft in Esca foliar symptom expression on the two cvs Cabernet Sauvignon and Mourvèdre in two French vineyards with different climatic conditions. For both varieties, we found a significant effect of the grafting type on the development of Esca foliar symptoms. The study assessed, for the first time, the differences among the three considered grafting methods revealing a higher incidence of Esca on Whip and Tongue grafted vines. The spread pathogens during mechanical grafting may be one of the factors explaining the increase of the incidence of Esca in vineyard.

Behavior of different grapevine cultivars to infection by Botryosphaeriaceae. J. MONTEALEGRE[1], L. M. PÉREZ[2], J. MOLINA[1], M. RAMÍREZ[1], V. ARRIAGADA[1]. [1]*Universidad de Chile, Facultad de Ciencias Agronómicas, Departamento de Sanidad Vegetal, Santiago, Chile.* [2]*Asesorías e inversiones Biostrategy Limitada Santiago, Chile. E-mail: jmonteal@uchile.cl*

Diplodia seriata and *D. mutila* are among the fungal species causing Botryosphaeria dieback "dead arm" disease in vineyards in Chile. Many grapevine crops are cultivated on their own roots, although some are grafted on rootstocks. It seemed important to analyze the susceptibility of different vine cultivars currently in use, to infection by these fungi. One-year-old canes of wine grape cvs. Cabernet Franc, Malbec, Merlot, Sauvignon Blanc and Syrah (all on their own roots), and of table grapes cvs. Thompson Seedless, Flame Seedless, Crimson Seedless, and Red Globe (all rootstock Harmony), were evaluated. Ten cane sections per cv. were inoculated with *D. seriata* and *D. mutila* (wine grape) or with *D. mutila* (table grape) and incubated in the dark at 25ºC and 95% humidity. The length of necrotic lesions observed at the internode region, was measured from both sides of the inoculation point with a vernier, after careful removal of the bark. Results showed that the mean length of lesions caused by *D. mutila* (2.9±0.1 cm) were three times greater than those produced by *D. seriata* in grapevine canes. Cvs. Cabernet Franc and Syrah were the most susceptible to *D. mutila* and Merlot was the least susceptible. No significant differences were observed between grapevine cvs. inoculated with *D. seriata*, or between table grape cvs. inoculated with *D. mutila*. Field experiments (four plants with five repeats for each cultivar) on Cabernet Sauvignon and on Chardonnay on their own roots, inoculated with *D. seriata*, showed that lesions in the Cabernet Sauvignon cv. were larger than those produced in the Chardonnay cv. These last results agree with those obtained in grapevine seedlings, where the cv. Chardonnay had greater activity of several pathogenesis related proteins than cv. Cabernet Sauvignon.

Development of an application device for efficient and effective application of a BASF wound protectant. M. NOLTE[1], S. HENKES[1], D. MORAN[1], C. KEPES[1], L. LUDWIG[1], S. DÖRR[1], A. KÜHN[1], R. ZITO[1], R. REHKUGLER[2], J. MOGILEWSKI[2], B. STOCKBURGER[2] and C. WINTER[3]. [1]*BASF SE, Agricultural Center, Speyerer Strasse 2, 67117 Limburgerhof, Germany.* [2]*MESTO Spritzenfabrik Ernst Stockburger GmbH, Ludwigsburger Str. 71, 71691 Freiberg am Neckar, Germany.* [3]*FELCO SA, Mélèzes 4, 2206 Les Geneveys-sur-Coffrane, Switzerland. E-mail: marc.nolte@basf.com*

To help combat grapevine trunk diseases (GTDs), BASF has developed in Europe a wound protectant (Tessior®) containing the fungicides pyraclostrobin and boscalid in a ready-to-use film-building formulation. To efficiently apply the protectant during winter pruning, a special application device was jointly developed by BASF, MESTO and FELCO. The device achieves an optimum between targeted manual application and classical spray application. Application to pruning wounds can be made at temperatures as low as -3°C, while not exceeding the proposed maximum application rate. To achieve good coverage on single pruning wounds ranging from 10,000–60,000

wounds/ha in a typical European vineyard, the application must target the wound precisely. Therefore, the most efficient application is manual. Several prototypes were developed and tested under practical field conditions. After several prototype generations, the device was optimised for comfort and safe use, and also for efficiency regarding time needed and amount of product applied per ha. The device presented here is lightweight, ergonomic and runs on electric power. It consists of a backpack unit that contains the electronics and the power supply. The product container is neatly integrated and connected to the backpack unit. The connected container serves as the spray tank and can be quickly exchanged when empty. A handheld quick-acting valve with a trigger is used to target the wounds and apply the product. Due to the range of wound sizes and different growing systems used in practice, different spray volumes per wound are needed. Dosages of 0.15–0.3 mL/shot can be adjusted in 4 steps by a control switch during application. The spray nozzle was selected to generate an accurate spray of the viscous formulation under typical working conditions. This pointed spray application results in a retention on simulated wound targets up to 75%.

Highlights on ecophysiological changes in Esca-diseased grapevines in comparison to healthy plants. L. OUADI[1], E. BRUEZ[1], S. BASTIEN[1,2], J.-C. DOMEC[2] and P. REY[1,2]. [1]*INRA, ISVV, UMR1065 SAVE, F-33140, Villenave d'Ornon, France.* [2]*Université de Bordeaux, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), Bordeaux Sciences Agro, F-33140, Villenave d'Ornon, France. E-mail*: *patrice.rey@inra.fr*

Grapevine Trunk Diseases (GTDs) such as Esca, are caused by a broad range of taxonomically unrelated fungal pathogens that attack the inner woody tissues of the grapevine. Fungal colonization of the wood can reach a critical point when the functional tissues are severely damaged, thus interfering with the vine physiology, leading to esca-foliar symptom expression or ultimately to death. The purpose of this study is to better understand the ecophysiological changes inside the Esca-infected vines that show foliar symptoms, especially regarding the sap flow, the stomatal conductance and the foliar transpiration rate. Using these parameters, the influence of Esca *in planta* was evaluated, regarding both the circulatory activity of the whole plant and the efficiency of gas exchanges in leaves. The experiments were conducted in 16-year-old vines of the cultivar Cabernet Sauvignon (*Vitis vinifera*), that had been monitored for Esca symptoms since 2015, at the Luchey-Halde vineyard in Pessac-Léognan, (Bordeaux, France). During the summer of 2016, heat sensors were installed on 5 asymptomatic and 5 Esca-symptomatic vines, to continuously measure the sap flow. Over the same period, a porometer was used to record stomatal conductance and foliar transpiration rate. This physiological monitoring showed that the sap flow density in Esca-symptomatic vines dropped significantly a week before the first leaf symptoms appeared. When water demand was the highest, the sap flow density was about two times lower in Esca-symptomatic vines than in asymptomatic vines. Equally, a similar trend was recorded with the stomatal conductance and the leaf transpiration rate of symptomatic vines. These parameters could be useful physiological indicators to assess the health status of a vine before the development of Esca-foliar symptoms.

Empirical attempt to control GTDs in vineyard: result of Winetwork Project. F. PREZMAN[1], C. ABIDON[2] and E. SERRANO[1]. [1]*Institut Français de la Vigne et du Vin, V'Innopôle Sud-Ouest, 81310 Lisle sur Tarn, France.* [2]*Institut Français de la Vigne et du Vin, Biopôle, 28, rue de Herrlisheim, 68000 Colmar. E-mail: fanny.prezman@vignevin.com*

The European project Winetwork started in 2015 and aims to stimulate knowledge exchange and collaborative innovation in the wine sector on Grapevine Trunk Diseases and Flavescence Dorée. The approach is mainly based on the interactions between a network of facilitator agents, several regional technical working groups from ten regions of seven countries representing more than 90% of the EU wine production and one European scientific working group. As many winegrowers are testing innovative and sustainable approaches to fight these diseases, it is very beneficial to capture innovative practices and to share them between EU countries. Practices were synthesized, tailored and translated to become fully accessible to innovation support services and to winegrowers. The project delivers a vast reservoir of existing scientific and practical knowledge related

to sustainable vineyard management. In order to collect practical information on GTDs management, 220 interviews were made in the ten wine regions. The interviews focused on three main grapevine trunk diseases: Esca, Botryosphaeria dieback and Eutypa dieback. Practices implemented in field are diverse and some are not validated scientifically but can present an interest in GTD control. Some practices, such as trunk renewal or *Trichoderma* application, are very popular among European winegrowers and some atypical practices as hydrogen peroxide injection or cooper nanoparticles were also reported. It appears from this consultation that winegrowers are well aware of GTDs, trying, with their available means, to limit GTDs incidence and request for concrete effective solutions.

***Trichoderma atroviride* strain I-1237 protects pruning wounds against grapevine wood pathogens**. P. REIS[1], P. LETOUSEY[2] and C. REGO[1]. [1]*Centro de Investigação em Agronomia, Alimentos, Ambiente e Paisagem (LEAF), Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.* [2]*Agrauxine Lesaffre Plant Care, 2&4 Rue Henri Becquerel - 49070 BEAUCOUZ, France. E-mail: pedroreis@isa.ulisboa.pt*

Neofusicoccum parvum and *Phaeomoniella chlamydospora* are among the grapevine pathogens associated to Botryosphaeria dieback and Grapevine leaf stripe disease (one of the diseases within the Esca complex), respectively. These trunk diseases are of major importance worldwide. They are associated with wood necrosis and affect both young and mature vines. Infection by these fungal pathogens occurs primarily through pruning wounds, with highest risk of infection from late fall to early spring, due to the high number of wounds made during pruning and the frequency of rain events that occur during that period. Therefore, in order to test the formulation of *Trichoderma atroviride* strain I-1237 (Esquive® WP, Agrauxine Lesaffre Plant Care) as a wound protectant, trials in two different Portuguese vineyards (cvs. Pinot Noir and Aragonez) were carried out. The effectiveness of the treatments was evaluated through the ability of the product in reducing the infections on pruned canes artificially inoculated with the two pathogens. Seven months after inoculation, reisolations were performed from the treated canes. The biofungicide was able to reduce the disease incidence by as much as 37% for *P. chlamydospora* and 57% for *N. parvum* and severity by 15.3% and 57.5%, respectively. The efficiency of the treatment was confirmed when compared to the reisolation percentages recorded in the positive controls, 68% and 89% for *P. chlamydospora* and *N. parvum*, respectively. Incidence of *T. atroviride* was also evaluated and recorded at different levels in the canes in one vineyard, where it was found to be higher on the canes artificially infected with *N. parvum* ranging from 66% to 81% than with *P. chlamydospora*, ranging from 41% to 56%. In conclusion, *T. atroviride* strain I-1237 can be regarded as an effective biological control agent for wound protection of grapevines against both fungi in this study.

The antagonistic effect of *Bacillus subtilis* and *Pantoea agglomerans* on two grapevine cultivars, Cabernet Sauvignon and Muscat d'Italie, against two Grapevine Trunk Disease (GTD) pathogens, *Neofusicoccum parvum* and *Phaeomoniella chlamydospora*. A. REZGUI[1,2], J. VALLANCE[2,3], A. B. GHNAYA CHAKROUN[1], E. BRUEZ[2,3], M. ARFAOUI[1,2], N. SADFI ZOUAOUI[1] and P. REY[2,3]. [1]*Laboratoire Microorganismes et Biomolécules Actives, Faculté des Sciences de Tunis, Université de Tunis El Manar, 2092, Tunisia.* [2]*INRA, UMR 1065 Santé et Agro-écologie du Vignoble (SAVE), ISVV, 33140 Villenave d'Ornon, France.* [3]*Université de Bordeaux, Bordeaux Sciences Agro, UMR 1065 SAVE, 33140 Villenave d'Ornon, France. E-mail: foufarezgui07@gmail.com*

Biological control assays against *Phaeomoniella chlamydospora* and *Neofusicoccum parvum*, two pathogens commonly associated with esca and the brown wood-streaking of grapevine, were carried out on two grapevine cultivars, Muscat d'Italie (widespread in Tunisia) and Cabernet Sauvignon (widespread in France). The two fungi were inoculated, either individually or in combination with potential antagonistic bacterial strains, *Bacillus subtilis* B6, *Pantoea agglomerans* S5 and B6+S5, through wounds in the trunks of young vines of the two cultivars. Internal symptom severity, assessed as the length of wood necrosis varied, depending on the pathogen and cultivar used. Cabernet Sauvignon grapevines were more susceptible to necrosis by *P. chlamydospora* infection than Muscat d'Italie grapevines, whereas

no differences in necrosis were recorded between the two cultivars inoculated with *N. parvum*. In terms of plant protection, the combination of both bacterial species was the most efficient in reducing the *in planta* necrosis caused by both pathogens. However, bacterial treatments were significantly more efficient in reducing the necrosis caused by *N. parvum* and/or *P. chlamydospora* in Muscat d'Italie than in Cabernet Sauvignon vines.

Biological control of the Botryosphaeriaceae in grapevines using endophytes from *Leptospermum scoparium*. W. WICAKSON, E. JONES, J. MONK and H. RIDGWAY. *Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln, Canterbury, New Zealand. E-mail: Hayley.Ridgway@lincoln.ac.nz*

Botryosphaeriaeae species are significant problems in vineyards where they cause canker and dieback of vines. Spores are consistently present and this, coupled with the frequent wounding of vines, provides near year round opportunities for infection. To be effective a biocontrol agent must colonise the infection site and persist in the vine. Thus, endophytic bacteria are ideal candidates as biocontrol agents as they share the same niche with the phytopathogen. *Leptospermum scoparium* is a New Zealand native plant recognised for antimicrobial properties. Although Botryosphaeriaceae species are commonly isolated from *L. scoparium* there are no reports of disease. A subset of 56 (17%) of 230 cultured endophytic bacteria from *L. scoparium* inhibited the radial growth of *Neofusicoccum luteum in vitro*. Of these, ten strains showed broad inhibition of multiple species within the Botryosphaeriaceae, reducing the radial growth of *N. luteum, N. parvum, N. ribis, N. australe, Diplodia mutila,* and *D. seriata* by 20–65% after 3 days on Waksman agar. One of these strains, *Pseudomonas* sp. I2R21, was applied as a 10 μL aliquot of bacterial culture (10^8 CFU/mL) to a wound made on the main trunk of rooted 1 year old Sauvignon blanc cuttings 10 cm above the potting mix surface. Negative controls were inoculated with phosphate buffer saline. *Pseudomonas* sp. I2R21, was successfully transmitted to Sauvignon blanc 1 year old canes, where it colonised the wound site for ≥ 6 months. Inoculation of the same wound site in endophyte colonised grapevines with *N. luteum* showed that the endophyte treatment significantly decreased lesion length (P= 0.038) and pathogen colonization of tissue beyond the lesion (P=0.047) by approximately 40%. This worked showed that endophytes with biocontrol activity reside as a natural resource in *L. scoparium* and these can be transferred to a heterologous host and exert their biocontrol activity.

Grapevine Trunk Disease associated pathogens in one year old dormant grapevine material and effects of hot water treatment and fungicides on their elimination. M. RIEDLE-BAUER, M. MADERČIĆ, L. WIELAND and K. HANAK. *Federal College and Research Institute for Viticulture and Pomology Klosterneuburg, Wienerstraße 74, 3400 Klosterneuburg, Austria. E-mail: Monika.Riedle-Bauer@weinobst.at*

The presence and the frequency of Grapevine Trunk Diseases (GTD) associated pathogens in dormant cane material of *Vitis vinifera* in Austria were studied. One year old dormant canes sampled in four approximately 15 year old vineyards were analyzed by nested PCR and incubation of vine wood on malt extract agar (MEA) agar followed by microscopic examination. Most commonly *Phaeomoniella chlamydospora* (Pch) and *Diplodia seriata* (Ds), were detected, *Botryospaeria dothidea* (Bd) and *Diaporthe* spp. were also present. *Phaeoacremonium minimum* was rarely found. Hot water treatment (HWT, 45 min, 50°C) and vacuum-infiltration of various fungicidal compounds were tested for their capability to eliminate Pch, Pal, Ds, Bd and *Diaporthe* spp. from dormant plant material. The experiments included treatment of i.) healthy 2 bud cuttings vacuum infiltrated with conidial suspensions of Pch and *P. minimum* (conidia from cultures on MEA in sterile distilled water, concentrations adjusted to 5. 10^2 conidia/mL and 10^5 conidia/mL; suction of conidia suspension through the cuttings by aid of a water jet pump until suspension visible on upper cut surface) and ii) dormant canes naturally infected with Ds, Bd, and *Diaporthe* spp. from 3 different vineyards. Right after and up to one year after the treatments the plant material was analyzed for the mentioned pathogens by incubation on MEA and microscopic analysis. After HWT Pch, Bd and *Diaporthe* spp. weren't re-isolated from any of the samples. In case of Ds, however, six months after treatment, the re-isolation rate was higher in the HWT group than in the untreated con-

trol group. HWT did not result in successful removal of *P. minimum*. HWT could be a successful strategy to reduce or eliminate GTD pathogens in propagation material. Additional research, however, is required to investigate unwanted effects such as reduction of natural biocontrol. Elimination of the pathogens was not obtained by vacuum infiltration of the tested fungicides.

Action plan against declining vineyard: an innovative approach. C. RIOU[1,2], D. AGOSTINI[3], P. AIGRAIN[3], M. BARTHE[1], M.-L. DES ROBERT[4], J.-P. GERVAIS[1], E. JOBARD[4], L. LURTON[1], D. MONCOMBLE[1] and C. PRÊTET-LATASTE[1]. *[1] CNIV, 12 rue Sainte-Anne, 75001 Paris, France. [2] IFV Domaine de l'Espiguette, 30200 Le Grau du Roi, France. [3] FranceAgriMer, 12 rue Henri Rol-Tanguy, TSA 20002, 93555 Montreuil Cedex, France. [4] BIPE Le Vivaldi, 11/13 rue René Jacques, 92138 Issy-les-Moulineaux Cedex, France. E-mail: cr@cniv.asso.fr*

Declining vineyards are characterised by decrease in vine productivity over many years and/or its sudden premature or gradual death, based on multiple factors. Since 2015, the French wine sector has been working on a study to identify new research avenues while launching an innovative and coordinated action plan. As a first step, a statistical evaluation was undertaken to assess research efforts in the different countries. Four hundred and fifty publications in 5 languages have been thoroughly analysed. Meanwhile, a comparative study has been carried out on other woody species such as citrus and oak. Seventy factors that contribute to vineyard decline have been identified. There are three main factors: biological, physical or growing practices. While the role of pathogens is fairly well-known, the impact of the land plot or the soil on decline, the impact of climate and the physiology of grafted plants or the effects induced by standards defining viticulture practices still need to be studied. In the second phase, a future-oriented methodology was used to identify better the viticulture system factors and levers affecting vines. The matrix analysis used provided an overview after decomposing the viticulture system into 168 dimensions. It was thus demonstrated that yield and longevity are strongly linked to agronomy and economic variables and are mainly related to plant matter, plant physiology, disease, cultivation practices and development strategies in compliance with standards and regulations. The matrix analysis was then completed with interviews and by statistical data to imagine leverage actions. The result is a strategic action plan focused on four additional objectives: (i) Develop a network of personnel to promote training and transfer of good practices, (ii) Organise plant production in collaboration with vine nurseries, (iii) Coordinate vineyard observations, and (iv) Develop research responding to sector expectations with a renewed partnership with the scientific world.

Remedial surgery for the management of Botryosphaeria dieback. S. SAVOCCHIA[1], R. BILLONES-BAAIJENS[1], M. R. AYRES[2] and M.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: ssavocchia@csu.edu.au*

Remedial surgery techniques have been successfully used to control Eutypa dieback by removing the infected wood from the vine including a section of healthy tissue which may harbour the pathogen. The fresh wound is then sealed with a protectant to minimise reinfection. It was unknown whether this technique could also be used to manage other trunk diseases such as Botryosphaeria dieback. Therefore, trials were established in two vineyards in the Hunter Valley (HV1, HV2) and one in Harden (H1), New South Wales, Australia, in October 2013 and November 2014, respectively. Prior to remedial surgery, the severity of dieback was assessed for each grapevine. The severity of dieback in grapevines from HV1 (grafted vines planted in 1989) varied from 74–77%, for HV2 (own-rooted vines planted in 1993) from 53-65% and for H1 (own-rooted vines planted in 1990) from 16 to 33%. Following the assessment of disease severity, the trunks were cut either high (just below crown), mid (mid-point between ground and crown) or low (20 cm above graft union or ground), cordons and trunks removed, and the cross-section of each trunk assessed for necrotic staining. The severity of internal staining (wedge and/or central necrosis) at all sites varied from 0.6% (low cut) to 26% (high cut).

Botryosphaeriaceae were isolated from all symptomatic wood samples in HV1 and HV2 and while for H1, 27% of samples were positive for Botryosphaeriaceae. The production of water shoots was also assessed at each site. For HV1, 10% (low cut), 32% (mid cut) and 19% (high cut) of vines had produced water shoots and in HV2 and H1, 96-100% of vines had produced shoots at each cut height. These trials have demonstrated the short-term success of remedial surgery for control of Botryosphaeria dieback in own-rooted vines and trials continue to be monitored yearly for disease recurrence.

Preliminary histological observations on grapevine pruning wound. S. SCHIFF[1], C. TANI[1], M. BENANCHI[2] and L. MUGNAI[2]. [1]*Department of Biology, University of Florence, Via Micheli 3, 50121 Florence, Italy;* [2]*Dipartimento di Scienze delle Produzioni Alimentari e dell'Ambiente (DiSPAA) – Sezione Patologia vegetale ed Entomologia, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: silvia.schiff@unifi.it*

It is well known that grapevine trunk diseases spread by infecting pruning wounds. There are many observations on the variables inciting a larger infection incidence and on ways to protect the wounds. Another approach is to determine pruning techniques or protocols that are less inducive of infections. In order to better understand how the grapevine tissue reacts to different wounds applications we are studing the grapevine tissue response to wounds at different times after pruning, and in pruning wounds applied in winter or early spring. Histological observations were carried out on *Vitis vinifera* plants growing in a vineyard located at the Montepaldi farm in the northern part of the production area of Chianti Classico in Tuscany (Italy). The spatiotemporal accumulation of phenols as well as the reinforcement of cell walls with phenolic polymers were histochemically detected at cellular and tissue levels by specific stains and fluorescent microscopy with the aim of following and describing the development and characterization of the necrotic tissue development following the wound. The wide and long vessels in the grapvine were partially and then completely occluded by tylose extending to several millimeters deep after wounding. Even though callose is involved in multiple stress responses and is also present as an essential component of the vine sieve plates, it has not been detected in the wounded tissue. The other reactions of tissues will be described and discussed.

Innovative micro-pathosystem for studying etiology and control of grapevine trunk diseases. M. SELIM[1], S. SCHEDLER[1], C.-D. PATZ[2] and B. BERKELMANN-LOEHNERTZ[1]. [1]*Department of Phytomedicine, Hochschule Geisenheim University, Von-Lade-Straße 1. 65366 Geisenheim, Germany.* [2]*Department of Wine Analysis and Beverage Research, Hochschule Geisenheim University Von-Lade-Straße 1. 65366 Geisenheim, Germany. E-mail: moustafa.selim@hs-gm.de*

Esca, one of the major grapevine trunk diseases (GTDs), is now recognized as an economically important problem in many viticultural regions around the world. It represents a major threat to viticulture due to its increasing incidence within vineyards, especially with no resistant varieties and no effective chemical treatment available for use in Germany so far. However, with latency periods that may extend to several years, research into GTDs such as Esca is difficult with established grapevines. Therefore, we have developed a micro-pathosystem on stem cuttings that allows us to study the etiology of Esca and to screen for innovative control agents *in planta* in short periods (as short as three months). Plant extract (*Glycyrrhiza glabra*) as well as bacterial and fungal antagonists such as *Bacillus subtilis* and *Trichoderma harzianum*, respectively, among others were used to infiltrate stem cuttings in the presence or absence of *Phaeomoniella chlamydospora*. On the one hand, we were able to induce esca-related wood symptoms in stem cuttings within few weeks only and to test the efficiency of the aforementioned control agents. On the other hand, we could provide genuine control cuttings without any GTD pathogens by infiltration with saline or Ringer solution. In addition, vessel sap (after infiltration) was analyzed using nuclear magnetic resonance spectroscopy (non-targeted NMR) analysis to screen for host metabolites that are produced as stress/defense signals and for pathogen-related metabolites that could function as toxins, which could be used as markers for rapid diagnosis of the disease in routine procedures. Indeed, some metabolites from vessel sap were found to be different between stem cuttings that were infiltrated with different agents. Hence, the micro-pathosystem

will shorten and facilitate research in the etiology of GTDs and provide new control strategies.

Practical strategies for controlling infection of pruning wounds by *Eutypa lata* and *Neofusicoccum luteum* in New Zealand vineyards. M.R. SOSNOWSKI[1] and D.C. MUNDY[2]. [1]*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia.* [2]*The New Zealand Institute for Plant & 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 have emerged as major trunk diseases of grapevines in New Zealand, causing significant economic losses as they do worldwide. This has led to concerted efforts, with support from New Zealand Winegrowers, to develop practical strategies for the management of trunk diseases. The fungicides: tebuconazole (Folicur®), carbendazim (Chief®), fluazinam (Gem®), mancozeb (Dithane™) and flusilazole (Megastar™) were evaluated for control of eutypa and botryosphaeria dieback in Sauvignon Blanc, using detached cane assays (DCA) in the laboratory and in vineyard trials in Marlborough over 2 years. All fungicides, when applied at varying rates with a paintbrush, were effective at reducing wound infection by *Eutypa lata* and *Neofusicoccum luteum* compared with untreated controls. Furthermore, DCA experiments revealed that tebuconazole and carbendazim controlled infection when applied up to a week after pruning and inoculation, and once applied, provided protection from infection for a further 2 weeks. Based on data from these experiments, application for label registrations are underway in New Zealand. In addition, recycle, air-shear and tangential sprayers, along with a modified weed sprayer, were evaluated for applying fungicide to wounds on cordon-pruned Cabernet Sauvignon (in Hawke's Bay) and cane-pruned Sauvignon Blanc (in Marlborough) over 2 years. Sprayers were able to deliver sufficient fungicide (carbendazim) to control infection by *E. lata* and *N. luteum*, using water volume rates between 100 and 900 L ha^{-1}, and some nozzle adjustments to focus on the pruning wound zone and achieve maximum coverage. Future research in New Zealand aims to determine the duration of wound susceptibility following pruning at different times, the critical timing of fungicide application (preventative and curative) and timing of spore dispersal, which will assist decision-making for the optimal timing of wound protection.

Economic impact of grapevine trunk disease management in Sauvignon Blanc vineyards of New Zealand. M.R. SOSNOWSKI[1] and G.J. McCARTHY[2]. [1]*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia.* [2]*Sutton McCarthy Limited, 37 Lamb Street, RD3, Cambridge 3495, New Zealand. E-mail: mark.sosnowski@sa.gov.au*

Eutypa and Botryosphaeria dieback are major grapevine trunk diseases worldwide, causing significant yield and quality reduction. They threaten the sustainability of New Zealand vineyards and are becoming an increasing problem as vineyards age. With support from New Zealand Winegrowers, economic analysis was performed based on results from trunk disease surveys and preventative wound treatment trials in New Zealand, in order to provide decision support for managing grapevine trunk diseases. Results showed that early adoption of preventative wound treatments in vineyards will minimise the cost of trunk disease and provide a net present value (NPV) future benefit over the life of a vineyard. Application of pruning wound treatments with a tractor-driven sprayer will further minimise costs compared with hand-application, and ensuring good coverage will maximise efficacy and hence the NPV future benefit. If preventative treatment does not commence until after trunk disease is evident, there will be costs for future crop loss and remediating or replacing vines. The cost of remediating vines by reworking or regrafting will be less than the cost of replacing vines. The sooner the remediation treatment is commenced, the greater the NPV future benefit. The potential "national" value of an effective annual spray treatment to the New Zealand industry is estimated to be at least NZ$20m per annum, increasing by a further NZ$20m per annum when combined with remediation treatments.

Metabolomic and cytological approaches to better understand grapevine trunk diseases disorders. S. TROUVELOT[1], C. GUILLIER[1], L. JACQUENS[1], A. DOUILLET[1,2], S. MARCHAT[1,2], C. GROSJEAN[3], P. SCHMITT-KOPPLIN[4], P. LARIGNON[2], F. FON-

TAINE[5], and M. ADRIAN[1]. *[1]Agroécologie, AgroSup Dijon, CNRS, INRA, Univ. Bourgogne Franche-Comté, INRA, 17 rue Sully, BP 86510, 21000 Dijon, France. [2]IFV Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France. [3]Chambre Régionale d'agriculture de Bourgogne Franche-Comté, 1 rue des Coulots, 21110 Bretenière. [4]Analytical BioGeoChemistry, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany. [5]SFR Condorcet, URCA, URVVC EA 4707, Laboratoire SDRP, BP 1039, 51687 Reims Cedex 2, France. E-mail: sophie.trouvelot@u-bourgogne.fr*

Grapevine trunk diseases (GTD) are devastating diseases involving xylem-inhabiting fungi. Among them, Esca and Botryosphaeria dieback affect vineyards in major worldwide grape-producing areas. These diseases alter both woody (trunk, area of fungal colonization) and green organs (such as leaves). As they are complex and remain partly unknown, different strategies were deployed to further understand symptom expression and plant reactions. Among "omics" strategies, metabolomic is particularly adapted to reveal metabolite signatures in distinct pathological and/or physiological contexts. For example, using this approach we revealed specific metabolic signatures of (i) esca-affected grapevines, (ii) grapevines treated with sodium arsenite and (iii) BDA-affected grapevines. Moreover, we point out organ-dependent signatures and the possibility to discriminate the response of two clones within a same grapevine variety. Despite being highly informative, such global approaches do not allow the described events to be kinked to a specific tissue or cellular localization. In this context, imaging approaches were developed. Thus, histological observations by epifluorescence, scanning- and transmission-electron microscopy were jointly performed. In addition, *in situ* hybridization was developed for markers of interest (plant defense or fungal matrices). Based on these approaches, we have revealed (i) the specific phloem location of a Glutathione-S-Transferase (*GST1*) in leaves of esca-affected grapevines, (ii) vacuolar and chloroplastic alterations in leaves of sodium arsenite-treated grapevines and (iii) alterations in the second meristem performances of Botryosphaeria dieback-affected vines. Altogether, we provide evidence of the interest to combine both global and targeted approaches to further understand the impact of GTD on grapevines.

Greenhouse evaluation of chemical and biological pruning wound protectants against grapevine trunk diseases in British Columbia. J.R. ÚRBEZ-TORRES, J.BOULÉ and D.T. O'GORMAN. *Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Box 5000. Summerland, BC V0H 1Z0, Canada. E-mail: joseramon.urbeztorres@agr.gc.ca*

Grapevine trunk diseases (GTDs) are the main biotic factors limiting both vineyard longevity and productivity in British Columbia (BC). GTDs fungi infect grapevines through wounds and natural openings, primarily pruning wounds. However, there are no products currently available that control the fungi inside the wood once they have infected and colonized the vascular system. Accordingly, the most effective strategy available today to minimize the impact of GTDs on grapevine health relies in protecting the pruning wounds. While other countries possess a few control products, there are currently none registered in Canada against GTDs. Therefore, the main objective of this study was to evaluate both chemical and biological products against the most prevalent GTDs species found in BC as this data is needed to obtain product label extension or new registration. Eight chemicals and four biological products were screened using a detached dormant cane assay in the greenhouse against 10 GTD fungi, including *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum*, *Phomopsis viticola*, *Neofusicoccum parvum*, *Diplodia seriara*, *Diplodia mutila*, *Eutypa lata*, *Cryptovalsa ampelina*, and *Diatrype whitemanensis*. Canes were pruned, immediately treated, and inoculated with either a spore or a mycelium suspension 24 h after treatment. Preliminary results showed that several products within the triazole and thiophanate chemical groups were able to achieve a high level of control under greenhouse conditions. Studies to determine the lifespan of three chemicals and one biological on pruning wounds were also conducted. Results showed mean percent disease control (MPDC) of chemical products to decrease when pruning wounds were challenged with the different pathogens either 7 or 21 days after treatment. Contrary, MPDC significantly increased after 21 days when pruning wounds were treated with a *Trichoderma*-based biological product. Further studies are required to determine the efficacy of these products in the field under BC environmental conditions. We envision this study as the

first step in the process of getting control products registered in Canada to provide grape-growers with control strategies against GTDs.

A protocol proposal for the management of grape vine rootstock mother vines to reduce latent GTDs infection in cuttings. H. WAITE[1], J. ARMENGOL[2], R. BILLONES-BAAIJENS[3], D. GRAMAJE[4], F. HALLEEN[5], S. DI MARCO[6], O. ZEKRI[7] and R. SMART[8]. *[1]Plumpton College, Ditchling Road, near Lewes, East Sussex, BN7 3AE, UK. [2]Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera S/N, 46022-Valencia, Spain.[3]National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW, 2678, Australia.[4]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, 26071 Logroño, Spain.[5]ARC Infruitec-Nietvoorbij (The Fruit, Vine and Wine Institute of the Agricultural Research Council), Private Bag X5026, Stellenbosch, 7599, South Africa. [5]Department of Plant Pathology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa. [6]Istituto di Biometeorologia, Consiglio Nazionale delle Ricerche, Via Gobetti 101, 40129 – Bologna, Italy. [7]Mercier Novatech Le Champ des Noëls, Le Gué de Velluire, 85770, France [8] Smart Viticulture, 31 North Corner, Newlyn, TR185JG, UK. E-mail: helen.waite@plumpton.ac.uk*

This protocol is offered as a guideline for managers of rootstock mother vine blocks, and as a potential research framework for those wishing to reduce Grapevine Trunk Diseases (GTDs) infection in rootstock mother vines and cuttings. Latent GTDs infection in rootstock cuttings is a major source of GTDs contamination in grafted nursery vines and subsequently in new vineyards. The many pruning cuts made at the crown predispose mother vines to infection transmitted to the new shoots via the xylem connection. Direct penetration by epiphytic inoculum on the bark of the shoots / canes can also occur. Older mother vine blocks are typically heavily infected, particularly if not trellised and pruning wounds are not protected. However, availability of effective pruning wound treatments is limited in many countries. The spread of GTDs inocula can be reduced by avoiding sprinkler and flood irrigation, by trellising mother vines, and by spraying fungicides after shoot trimming. Regular trunk renewal also aids in reducing inoculum spread. During harvest, cuttings should not contact the soil. Cuts should be made just below a bud to retain a long internode on the mother vine, and pruning debris should be removed promptly and destroyed. Secateurs should be disinfested regularly and cuttings dipped in a registered fungicide for a maximum of 30 min. Soaking for longer than 30 min is detrimental to the cuttings, increases fungal populations in the basal wounds and softens the bark, favouring penetration by GTDs inoculum. The common practice of dormant bench grafting in nurseries is known to produce more GTDs-symptomatic vines than field chip budding, and likely reasons will be discussed. Improved management for rootstock mother vines is thus more important where dormant cuttings are bench grafted. Here GTDs epidemiology in source blocks is summarised and best practice protocols for mother vine management and pre-grafting stages of propagation suggested.

Grapevine and trunk pathogen transcriptomic changes induced by the root biocontrol agent *Pythium oligandrum*. A. YACOUB[1,2], N. MAGNIN[1], J. GERBORE[3], E. BRUEZ[1,2], S. COMPANT[4], D. EZRA[5], D. GRAMAJE[6], E. KARAFFA[7], L. MUGNAI[8] and P. REY[1,2]. *[1]INRA, UMR1065 Santé et Agroécologie du Vignoble (SAVE), ISVV, 33883 Villenave d'Ornon, France. [2]Bordeaux Sciences Agro, Université de Bordeaux, ISVV, UMR1065 SAVE, 33883 Villenave d'Ornon, France. [3]BIOVITIS, 15400 Saint Etienne de Chomeil, France. [4]AIT Austrian Institute of Technology GmbH, Bioresources Unit, Health & Environment Department, 3430 Tulln, Austria.[5]Department of Plant Pathology and Weed Research, ARO, the Volcani Center, P Dagan 50250, Israel. [6]Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Cientıficas, Universidad de la Rioja, Gobierno de La Rioja26007 Logrono, Spain. [7]University of Debrecen, Institute of Food Processing, H-4032 Debrecen, Hungary. [8]Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Sez. Patologia vegetale ed entomologia, Università di Firenze, 50144 Firenze, Italy E-mail: amira.yacoub@inra.fr*

Pythium oligandrum is an Oomycete whose strains naturally colonize grapevine roots in the Bordeaux region as well as in other countries such as Austria, Hungary, Italy, Israel and Spain. Following root colonization with *P. oligandrum* alone, grape-genome microarray analyses showed significant changes in the

grapevine root transcriptome. Whereas the expression of several transcripts would suggest that the plant sets up defense systems against the Oomycete, certain similarities with symbiotic microorganism/root interactions were also observed, the main one being the stimulation of subtilases. *P. oligandrum* is used as a potential biocontrol agent against various pathogenic fungi of plants, including Esca. Greenhouse assays showed that the necrosis caused by the Esca pathogen *Phaeomoniella chlamydospora* were significantly reduced (40–50%) when *P. oligandrum* colonized the root system of vine cuttings. In order to characterize the mechanisms occurring during this tri-partite interaction, the global transcriptomic grapevine responses at stem level were analyzed, using *Vitis vinifera*-microarrays and RNAseq. Microarray analyses showed that *P. oligandrum* induced a few changes on the plant transcriptome, and also primed the plant responses in presence of the pathogen. Some similarities with Induced Systemic Resistance (ISR) were detected, with specificities at the induction of genes related to jasmonate pathway enzymes and to ethylene-responsive transcription factors. To the best of our knowledge, this result represents the first dataset presenting high-throughput analyses in order to investigate transcriptional changes in the wood tissues of a perennial species induced by a biocontrol agent inoculated at root level. Analysis of the *P. chlamydospora* RNA messenger (mRNA), showed that several genes related to secondary metabolite synthesis, transcription factors implicated in pathway regulations, and certain Carbohydrate Active enZymes (CAZymes), were modulated, when *P. oligandrum* colonized the roots. These results showed that root inoculation with *P. oligandrum* probably induced indirect stress on *P. chlamydospora* transcriptomic responses.

Development of a new BASF wound protectant against grapevine trunk diseases. A. ZAPPATA[1], A. KÜEHN[2], C. AUMONT[3], P. HOFFMANN[4], L. LUDWIG[2], M. MERK[2], K. H. SCHNEIDER[2], B. BLANZ[2], R. ZITO[2] and R. EVAN GOLD[2]. [1]*BASF Italia S.p.A., C306-14, Via Marconato 8, 20811 Cesano Maderno, Italy,* [2]*BASF SE, Agricultural Center, Speyerer Strasse 2, 67117 Limburgerhof, Germany,* [3]*BASF France S.A.S., Division Agro, 21, chemin de la Sauvegarde, 69134 Ecully Cedex, France,* [4]*BASF Hungária Kft., Váci út 96-98, 1133 Budapest, Hungary. E-mail: alessandro.zappata@basf.com*

Grapevine trunk diseases (GTDs) are considered one of the most important economic issues for grape growers around the world. Major fungal pathogens associated with GTDs are *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum,* belonging to the so called 'Esca' complex, but also Botryosphaeriaceae spp. and *Eutypa lata* are pathogens contributing to the GTD syndrome. In the past, GTDs were controlled using arsenite-based products that, among other chemical compounds, were applied to wounds during pruning. After the banning of these products in Europe, a lack of effective chemical control occurred in this region. In recent years, a dramatic upsurge of GTDs has been reported in grape growing countries, where the average annual increase of disease is estimated to be 4–5%. To improve the control of GTDs, BASF developed an innovative wound protectant product*. This product is highly effective in reducing new infections of GTDs via pruning wounds by combining the physical and chemical activity of components in its formulation. The physical activity is ensured by a polymer that hardens after being sprayed on the wound surface. It protects the wound with a durable film that prevents disease spores from entering wood vessels. In addition to this physical barrier, a chemical activity is ensured by two BASF broad spectrum fungicides: pyraclostrobin and boscalid. In collaboration with several European scientific institutes, pioneers in this branch of research, BASF fine-tuned a specific methodology to test its effectiveness under semi-field conditions. Numerous trials performed in European grape growing countries confirmed a high level of protection against GTDs. The product will be introduced in the market as a ready-to-use formulation together with a specifically developed application device that allows accurate point spray applications directly to wounds at temperatures as low as -3°C.

*BASF wound protectant will have Tessior® as registered trademark in Europe.

Uses and efficiency of electrolyzed water in grapevine nurseries for GTDs. M. SINEUX[1] and O. ZEKRI[2]. [1]*Mercier Novatech Le Champ des Noëls, Le Gué de Velluire, France.* [2]*Mercier Frères S.A.R.L, 16 Rue de la Chaignée, Vix, France. E-mail: olivier.zekri@mercier-groupe.com*

Grapevine trunk diseases (GTDs) are currently considered the most destructive disease of vineyards worldwide and are of rapidly growing concern in all wine producing countries. The pathogens responsible for these diseases attack the long-lasting organs, causing the death of vines on shorter or longer term, but also reduce the quality and quantity of grapes and wine production. The life cycle and epidemiology of those fungi are well-known, but the diseases are complex and their symptoms usually take several years to develop. A healthy vine is fundamental to the successful beginning and sustainability of all vineyards. That starts in the grapevine nurseries, which are the first point in the production chain. To produce "healthy/clean" plants, nurseries need a production process that takes care of the control of fungal contamination. Use of electrolyzed water was studied *in vitr*o during several years in Mercier nursery but also *in vivo* directly applied at different steps of production process (hydration, callusing…). Electrolyzed water treatment, used during the nursery process can help to reduce the quantity of fungi inside the young plant at the end of the process. qPCR TaqMan protocol was used to evaluate the effect of electrolyzed water on *Phaeoacremonium aleophilum*, *Phaeomoniella chlamydospora*, *Diplodia seriata*, *Eutypa lata* and *Neofusicoccum parvum* in grapevine material. Reduction of fungi was observed (Ct value). Results of this study showed differences between treated and untreated material. This is the first step for nurseries to select grapevine plant sanitary state using contamination thresholds.

Published online: December 7, 2017