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ABSTRACTS

Abstracts of oral and poster presentations given at the 9th International Workshop on Grapevine Trunk Diseases, Adelaide, Australia, 18–20 November 2014

The 9th International Workshop on Grapevine Trunk Diseases was held in Adelaide, Australia, 18–20 November, 2014. The meeting was attended by 111 participants and 71 papers were presented either as oral or poster presentations in four sessions: Pathogen Identification and Detection, Epidemiology, Host-Pathogen Interactions and Disease Management. A field trip to the Barossa Valley wine region was undertaken to showcase proactive management of grapevine trunk diseases in Australia.
The workshop is the 9th organised by members of the International Council on Grapevine Trunk Diseases (www.icgtd.org), a subject matter committee of the International Society for Plant Pathology (www.isppweb.org).

Pathogen Identification and Detection

The identity, distribution and diversity of botryosphaeriaceous species in New Zealand vineyards – a national perspective. H.J. RIDGWAY[1,*], J. BASKARATHEVAN[1,2], N. AMPONSAH[1,3], M.V JASPERS[1] and E.E. JONES[1]. *[1]Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln University, Lincoln, New Zealand, 7647. [2]Ministry for Primary Industries-Manatū Ahu Matua, PO Box 2095, Auckland 1140, New Zealand. [3]Plant & Food Research, Old Mill Road, RD 3 Motueka, New Zealand. *E-mail: Hayley.Ridgway@lincoln.ac.nz*

In recent years molecular tools have been applied to provide understanding of the population structures of botryosphaeriaceous species in New Zealand vineyards. A national survey of symptomatic material from 43 vineyards showed that 88% had infection by botryosphaeriaceous species. Vine age had the strongest correlation with incidence, with the least infection in grapevines 1–5 years old (30%). Sequencing of taxonomic genes identified nine species. In contrast to other countries, *N. luteum* and *N. parvum* were predominant species with *Lasiodiplodia theobromae* notably absent. As with other countries, research showed that distribution is likely to be related to climate. Analysis of populations demonstrated that, despite predominantly asexual reproduction, the genetic diversity of isolates within species was high. Frequent hyphal anastomoses and fusions were observed in dual culture with weak vegetative compatibility barriers. This indicated the likelihood of frequent parasexual recombination. The isolation of genetically similar isolates from single lesions reinforced this hypothesis. A suite of molecular tools were developed to aid epidemiology studies. Endogenous markers produced for isolates with typical pathogenesis showed they could be dispersed at least 2 m from the site of conidiation in a single rain/wind event. The use of a multi-genus PCR-SSCP system showed that *N. parvum* and *N. luteum* are released year round and this probably contributes to their successful invasion of vineyards. Application of these molecular tools has provided a comprehensive snapshot of New Zealand vineyards revealing a thriving and diverse population of botryosphaeriaceous species that present a serious concern to the industry.

Grapevine trunk diseases studies in British Columbia: the status of long known diseases in a young and emerging grape-growing region. J.R. ÚRBEZ-TORRES*, P. HAAG, P. BOWEN and D.T. O'GORMAN. *Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, 4200 Highway 97, Box 5000, Summerland, BC V0H 1Z0, Canada. *E-mail: joseramon.urbeztorres@agr.gc.ca*

Grapevine trunk diseases (GTD) are caused by a wide range of taxonomically unrelated fungi and are currently recognized as one of the major threats to the industry's future economic sustainability. Previously, there was no information available regarding the status of

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GTD in British Columbia (BC), the second largest grape-growing region in Canada. Therefore, following some preliminary investigations studies to determine the incidence, spread, and significance of GTD in BC started in 2010. Field surveys were conducted in 191 vineyards that included assesment of foliar symptomatology from over 60,000 vines and isolations from 463 symptomatic vines. These studies revealed the presence of all GTD in BC, including Botryosphaeria dieback, esca, Eutypa dieback, young vine decline (black foot and Petri disease), and Phomopsis dieback. Phenotypic characteristics, including colony growth and micromorphology of reproductive structures along with DNA analyses of four genes (ACTIN, β-tubulin, TEF-1α, ITS1-5.8S-ITS2) allowed us to identify i) five different black foot fungi in the genera *Cylindrocarpon* and *Ilyonectria*, ii) seven species associated with esca and Petri disease, including the novel species *Phaeoacremonium canadensis* and *Phaeoacremonium roseum*, iii) several botryosphaeriaceous and diatrypaceous taxa, and iv) species in the *Diaporthe/Phomopsis* genera. Pathogenicity tests for all identified fungi were completed and the characteristic 'tiger-stripe' symptoms commonly observed on esca-infected vines were reproduced when 'Baco noir' on 3309C potted vines were artificially inoculated with *Phaeomoniella chlamydospora* and different *Togninia/Phaeoacremonium* species under greenhouse conditions. This study represents the first attempt to demystify the status of GTD in BC, a young and emerging grape-growing region with unique climatic conditions.

Endophytic and pathogenic fungi associated with Grapevine Trunk Diseases in the Tokaj wine region, Hungary. CS. KOVÁCS[1,*], E. SÁNDOR[1], Z. BIHARI[2] and F. PELES[1]. [1]*University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Food Science Institute, Böszörményi Street 138., H-4032 Debrecen, Hungary;* [2]*Research Institute for Viticulture and Enology, Könyves Kálmán Street 54., H-3915 Tarcal, Hungary. *E-mail: kovacs.csilla@agr.unideb.hu*

Grapevine trunk diseases (GTD) are amongst the most important diseases in vineyards worldwide. GTD are caused by several pathogenic fungi including, *Phaeoacremonium aleophilum, Phaeomoniella chlamydospora, Eutypa lata, Fomitiporia mediterranea, Diplodia seriata* among many others. However, there is currently very limited information about the status of GTD pathogens in the Tokaj wine region in Hungary. In this study, symptomatic vine samples showing GTD foliar symptoms were collected from vineyards in four areas in the Tokaj region in 2013: Szemere (Degenfeld), Dorgó (Disznókő), Szarvas (Kereskedőház) and Várhegy (Patrícius). The woody tissue samples were cut into small pieces, and were disinfected before plating on malt extract agar and incubated at room temperature for 3–8 days. Pathogenic fungi were isolated from 207 symptomatic grape trunks. DNA isolation from fungal colonies was conducted with the NucleoSpin Plant II kit. The ITS4 and ITS5 primers were used for PCR amplification of the ITS1-5.8S-ITS2 region. Purified amplicons were sequenced by Mycrosinth, Austria. Consensus sequences were aligned with reference sequences using Clustal X and manually corrected with GeneDoc. Phylogenetic analyses were performed with MEGA 5.05. To date, 366 pure fungal cultures were isolated and identified. The majority of the isolates (56%) were determined as *Diplodia seriata* (Botryosphaeriaceae). Other fungi, like *Fusarium* sp. (13.9%), *Alternaria* sp. (9.3%), *Aspergillus* sp. (7.9%), *Mucor* sp. (7.1%), *Diaporthe* sp. (3.3%), *Trichoderma* sp. (1.4%), *Epicoccum* sp. (0.5%), *Penicillium* (0.3%) and *Xanthomendoza* sp. (0.3%) were also identified from grapevine trunk samples.

Fungal pathogens involved in Grapevine Trunk Diseases in China. J.Y. YAN[1], W. ZHANG[1], M. LIU[1], X.H. LI[1,*] and K.D. HYDE[1, 2]. [1] *Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry and Sciences, Beijing 100097, China.* [2] *Institute of Excellence in Fungal Research, Mae FahLuang University, Chiang Rai. 57100, Thailand. *E-mail: lixinghong1962@163.com*

The grapevine cultivated area in China (both table- and wine-grapes) has significantly increased from 125,000 hectares to 625,000 hectares between 2002 and 2013. As the cultivation area has increased, diseases became a major problem for the grape industry in China. Apart from grape downy and powdery mildews, grapevine trunk diseases are the main factors limiting both vineyard longevity and productivity. Woody parts of the vine are infected by fungi, which lead to serious decline of the vineyard. In the past 10 years, grapevine trunk diseases have become serious in some regions. Botryosphaeria dieback, being one of the most devastating diseases in China has been studied previously. In this study, another two trunk diseases were studied. Diseased sample survey conducted throughout the country has shown *Diaporthe* spp. and *Pestalotiopsis* spp. are involved in the grape trunk diseases in China. Morphological and molecular phylogeny analysis confirmed four *Pestalotiopsis* species and five *Diaporthe*species associated with grape trunk diseases.

Detection of trunk pathogen inoculum in young vineyards to encourage adoption of preventative practices. D.P. LAWRENCE[1,*], P.T. FUJIYOSHI[1], R. TRAVADON[1], D.A. CANTU[2], A. MORALES-CRUZ[2], P.E. ROLSHAUSEN[3] and K. BAUMGARTNER[1]. [1]*United States Department of Agriculture, Agricultural Research Service, Crops Pathology and Genetics Research Unit, Davis, CA, USA.*

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Because there are no efficient eradicative practices for the wood infections caused by trunk diseases, routine use of preventative practices is critical to maintain vineyard productivity. To help convince California growers that early adoption of such practices is necessary in young vineyards, even in the absence of canopy symptoms, we evaluated spore traps (microscope slides and Rotorod® air sampler, coated in petroleum jelly) as a detection tool. Spores were trapped in three young (3–5 year-old, asymptomatic) and three mature (13–15 year-old, symptomatic) vineyards, 12 sites total, in each of two regions (Napa and San Joaquin Counties) from December 2013 to March 2014. Traps were collected one day after each of eight rain events. For culture-based detection, serial dilutions of spore suspensions were plated on PDA. For DNA-based detection, PCR primers were designed for the most aggressive and prevalent trunk pathogens (*Eutypa lata*, *Neofusicoccum parvum*, *Phaeomoniella chlamydospora*). Culture-based detection revealed *E. lata* in 11 sites, Botryosphaeriaceous fungi (e.g. *N. parvum*) in nine sites, and *P. chlamydospora* in no sites. DNA-based detection revealed *N. parvum* in eight sites, *P. chlamydospora* in two sites, and *E. lata* in no sites. Fewer pathogens were detected in culture and none by PCR from Rotorod® traps compared to microscope slides. Additional years of trapping and more specific primers based on genome sequences of the pathogens may resolve these conflicting results. Regardless, our finding of similar pathogens cultured from microscope slides in both young and mature vineyards suggests that preventative practices should be adopted in young vineyards.

Design, development and implementation of an oligonucleotide array for identification and detection of fungal pathogens responsible for causing young vine decline. J.R. ÚRBEZ-TORRES*, P. HAAG, J. DICK, P. BOWEN and D.T. O'GORMAN. *Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, 4200 Highway 97, Box 5000, Summerland, BC V0H 1Z0, Canada.* *E-mail: joseramon.urbeztorres@agr.gc.ca

Black foot and Petri disease are principle causes of young vine decline (YVD) and mortality in young vineyards. To date, over 45 fungi in the genera *Cadophora*, *Campylocarpon*, *Cylindrocadiella*, *Ilyonectria*/*Cylindrocarpon*, *Phaeomoniella*, and *Togninia*/*Phaeoacremonium* have been associated with YVD. Though significant advances have been made on molecular identification and diagnostics of YVD fungal pathogens during the last decade, many have been PCR-based techniques and are designed for the identification of one or a small number of species at a time. Hence, considering that a wide range of taxonomically unrelated fungal species are involved in the YVD complex and that many of these can be found in the nursery propagated material prior to planting in commercial sites, there is a critical need for a diagnostic approach capable to simultaneously identify and rapidly differentiate a much larger number of YVD fungi at once. Accordingly, we have designed a DNA-based macroarray that contained over 100 species-specific oligonucleotide probes targeting unique polymorphisms of the β-tubulin gene region. Probe specificity was determined by hybridizing over 140 fungal isolates representing 73 different species from 8 genera including ex-type specimens from different geographical grape-growing regions. The DNA macroarray precisely identified and discriminated over 60 different fungal species including those associated with YVD. The array was validated by testing artificially inoculated grapevine cuttings and soil as well as commercial field samples. The high level of specificity demonstrated by this DNA array showed it to be a promising detection system for accurate identification of YVD pathogens in a single test.

Two clonal lineages of *Cadophora luteo-olivacea* in Spain and South Africa revealed by multilocus ISSR markers. D. GRAMAJE[1,*], M. LEON[2], P.W. CROUS[3] and J. ARMENGOL[2]. [1]*Department of Crop Protection, Institute of Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain.* [2]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.* [3]*CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.* *E-mail: dgramaje@ias.csic.es

Cadophora luteo-olivacea is a fungal trunk pathogen of grapevine which has been recently isolated from vines showing decline symptoms in several grape growing regions worldwide. In this study, 80 *C. luteo-olivacea* isolates (65 from Spain and 15 from South Africa) were studied. They were collected in the 1999–2010 period from vines in young vineyards, from planting material showing black vascular streaking and decline symptoms characteristic of Petri disease or from nursery samples and planting material at different stages of the propagation process. These isolates were studied by means of Inter Simple Sequence Repeat (ISSR) analysis. A total of 55 polymorphic ISSR markers were obtained with the four ISSR primers used. The ISSR markers revealed 40 multilocus genotypes (MLGs) in the global population, with MLG13, MLG14, MLG29 and MLG31 being the most frequent. Minimum spanning network analysis showed that the MLGs from South Africa clustered around the most frequent genotype, while the

genotypes from Spain were distributed all across the network. Bayesian and principal component analyses identified two highly differentiated genetic clusters in the Spanish and South African *C. luteo-olivacea* populations, with no intermediate genotypes among these clusters. This suggests several events of introduction, although movement within the Spanish provinces may have occurred repeatedly given the frequent retrieval of the same genotype in distant locations. Our findings provide new insights into the population genetic structure of *C. luteo-olivacea* and highlight the need to produce healthy and quality planting material in grapevine nurseries to avoid its spread throughout different grape growing regions.

The role of *Cadophora* species as trunk pathogens in North American grape-growing regions. D.P. LAWRENCE[1], R. TRAVADON[1,*], S. ROONEY-LATHAM[2], W.D. GUBLER[3], P.E. ROLSHAUSEN[4] and K. BAUMGARTNER[1]. [1]*United States Department of Agriculture - Agricultural Research Service, Crops Pathology and Genetics Research Unit, Davis, CA 95616, USA.* [2]*Plant Pest Diagnostics Branch, California Department of Food & Agriculture, 3294 Meadowview Road, Sacramento, CA 95832-1448, USA.* [3]*Department of Plant Pathology, University of California, Davis, CA 95616, USA.* [4]*Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA. *E-mail: rtravadon@ucdavis.edu*

Cadophora species are recently acknowledged as putative trunk pathogens in independent investigations from California, South Africa, Spain, Uruguay, and Canada. In particular, *Cadophora luteo-olivacea* has been associated with vines affected by Petri disease and Esca, and indeed has been shown to cause the characteristic black streaking in controlled inoculations. In North America, little is known regarding the diversity and relative importance of *Cadophora* species, their geographic distribution, and their ability to degrade grapevine wood. Accordingly, we characterized 41 *Cadophora* isolates recovered from decayed grapevine wood from a broad geographic area in North America, using morphological and molecular analyses, and pathogenicity assays. Morphological characterization (sizes of conidia, hyphae, conidiophores, and conidiogenous cells) identified five groups of isolates, which corresponded to five species as revealed by phylogenetic analyses of ITS, translation elongation factor 1-alpha, and beta-tubulin: *C. luteo-olivacea*, *C. melinii/fastigiata*, *C. malorum*, and two *Cadophora* sp. nov. Restricted to the northeastern US were *C. malorum* and the two *Cadophora* sp. nov., whereas *C. luteo-olivacea* was only recovered from California. *C. melinii/fastigiata* was present both in California and Ontario, Canada. Pathogenicity assays produced lesions significantly longer than those of the non-inoculated control plants after 24 months incubation in the greenhouse, indicating the ability of these *Cadophora* species to degrade grapevine wood. This study showed that *Cadophora* species infecting grapevine in North America are diverse and should help further morphological and molecular identification of this obscure group of fungi.

Identification of *Cylindrocladiella* species isolated from rootstock wood cankers in California. L.J. BETTIGA[1,*], S.T. KOIKE[1], W.D. GUBLER[2] and T. NGUYEN [2]. [1] *University of California Cooperative Extension, 1432 Abbott Street, Salinas, California 93901, USA.* [2] *Department of Plant Pathology, University of California, Davis, California 95616, USA. *E-mail: lbettiga@ucanr.edu*

In late summer 2012, 2-year old grapevines (cv. Chardonnay on 101-14 Mgt) in a vineyard located in the Salinas Valley, California displayed black-foot disease. Internal tissues of roots and rootstock exhibited brown to black streaking and sections. Approximately 2% of vines were affected. Surface disinfested pieces of symptomatic wood, placed on PDA, consistently yielded two types of fungal colonies, one primarily yellow in color, and the other reddish. However, for all isolates the general morphological features were similar and consisted of penicillate conidiophores, cylindrical hyaline conidia, and hastate vesicles. Single-spore cultures of ten yellow and eight red isolates were used for molecular characterization. Based on the morphological and molecular data, yellow isolates were confirmed as *Cylindrocladiella lageniformis* and red isolates were identified as *C. peruviana*. To demonstrate Koch's postulates, 14-day-old colonies of three *C. lageniformis* and four *C. peruviana* isolates were inoculated onto potted plants of cv. Riesling by inserting mycelial agar plugs beneath cuts into the epidermis of shoots. The two *Cylindrocladiella* species were recovered from discolored tissue in all canes of their respective inoculated plants. Analysis of the recovered isolates demonstrated that these matched the morphological and molecular characteristics of the original isolates. Control canes, inoculated with sterile agar plugs, did not develop symptoms and no pathogen was isolated. To our knowledge this is the first documentation of *Cylindrocladiella lageniformis* and *C. peruviana* associated with a grapevine disease in California. These pathogens were also isolated from grapevines in 2014, indicating that the disease may be more broadly distributed.

First molecular characterization of *Phomopsis viticola* and *Diplodia seriata* isolated from Esca-BDA diseased grapevines in Northern Tunisia. A. BEN GHNAYA-CHAKROUN[1,2,3], A. REZGUI[1,2,3], J. VALLANCE[2,3], I. KHAROUBI[1], M. DRIDI[4], M.R. HAJLAOUI[4], P. REY[2,3,*] and N. SADFI-ZOUAOUI[1]. [1] *Laboratoire des Microorgan-*

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Esca and Black Dead Arm (BDA) are two major grapevine trunk diseases (GTDs)diseases, which occur worldwide. Although Esca and BDA have started to cause considerable damage in Tunisian vineyards, knowledge of the symptoms and microflora associated with these GTDs is still incomplete in Tunisia, The objective of this research was to study and characterize the fungal microflora that colonize the wood tissues of Esca-BDA symptomatic and asymptomatic vines. To do this, apparently healthy and necrotic wood tissues were collected from diseased and control plants in eight vineyards in the north of Tunisia, and analyzed using microbiological and molecular approaches. Our results showed that diverse fungal assemblages made up of potentially plant pathogenic and numerous saprophytic fungal species (mainly *Aspergillus* spp. and *Alternaria alternata)* colonize the wood tissues (apparently healthy wood and necrotic) of the sampled vines. The ITS sequencing of the different isolates confirmed the presence of *Phomopsis viticola* and *Diplodia seriata*, two fungi described in the literature as involved in GTDs. The molecular analysis also highlighted that *Alternaria alternata* is the main saprophytic strain colonizing both healthy and diseased wood. A halophilic bacterium (strain J9) was tested as an antagonistic agent against the isolated fungi using *in vitro* confrontations. The results, especially those concerning *D. seriata*, were promising.

Pathogenic fungi and mycoparasites colonizing the functional wood of old vines with no Eutypa dieback or Esca foliar symptoms. E. BRUEZ[1,2], K. BAUMGARTNER[3], S. BASTIEN[1,2], R. TRAVADON[3], L. GUERIN-DUBRANA[1,2] and P. REY[1,2,*]. *[1]Université de Bordeaux, Bordeaux Sciences Agro, UMR1065 SAVE, F-33140 Villenave d'Ornon, France. [2]INRA, UMR1065 Santé et Agroécologie du Vignoble (SAVE), ISVV, F-33140 Villenave d'Ornon, France. [3]United States Department of Agriculture, Agricultural Research Service, University of California, One Shields Avenue, Davis, CA 95616, USA. *E-mail: prey@bordeaux.inra.fr*

In France, grapevine trunk diseases Esca and Eutypa dieback tend to be less severe in vineyards older than 25 years. We compared the fungal microflora colonizing the functional (i.e. non-necrotic) wood of the cultivar 'Baco blanc' (a hybrid grape used in Armagnac production). During the spring season, 42- and 58-year-old vines were sampled; they showed no foliar symptoms of Esca or Eutypa dieback. The methods used for studying the microflora were isolation and subsequent sequencing the rDNA internal transcribed spacer region (ITS), and a molecular fingerprinting method, Single-strand conformation polymorphism analysis (SSCP). Among the 421 cultured strains used for ITS sequencing, 7 plant-pathogenic fungal species, particularly those involved in Esca (42-year-old vines) and Eutypa dieback (58-year-old vines), were identified, including *Phaeomoniella chlamydospora*, *Fomitiporia mediterranea* and *Eutypa lata*. Fungal communities differed according to vine age, based on both cultured-based identification and SSCP profiles. However, communities did not differ significantly among the cordon or the upper, central, or basal parts of the trunk. Regardless of vine age, numerous mycoparasites, such as *Trichoderma* spp. and *Bionectria* sp., and saprobes were also isolated. The absence of foliar symptoms may be associated with an equilibrium resulting from competition between pathogenic and mycoparasitic fungi in the woody tissues.

Molecular and phenotypic characterisation of *Phaeomoniella chlamydospora* isolates from the demarcated wine region of Dão (Portugal). J. SOFIA[1,*], A. PORTUGAL[1], H. PAIVA de CARVALHO[1], N. MESQUITA[1], T. NASCIMENTO[2], M.T. GONÇALVES[1] and C. REGO[2]. *[1]Centre for Functional Ecology, Department of Life Sciences, University of Coimbra. Calçada Martim de Freitas, 3000-446. Coimbra. Portugal. [2]Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal. *E-mail: jorge.sofia@drapc.mamaot.pt*

Phaeomoniella chlamydospora is considered one of the main causal agents of Esca and Petri disease, which are responsible for worldwide grapevine decay, as in the Portuguese demarcated wine region of Dão. In this old and well-known wine region, regional grapevine cultivars are mainly used with a low penetration of alien varieties, and most of the propagation material is produced in local nurseries. In the present work, we used 69 isolates of *Pa. chlamydospora*, obtained from symptomatic Esca and Petri disease grapevines, from different locations across the Dão region. We compared the morphological characteristics, geographic origin and ITS (Internal Transcribed Spacers) sequence variations to determine intraspecific variability and population structure. Furthermore, results will likely allow virulence assessment and prediction.

Current situation of the fungal trunk pathogens of grapevines in Chile. B.A. LATORRE* and G.A. DIAZ. *Departamento de Fruticultura y Enología, Facultad de*

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Trunk diseases in grapevine (*Vitis vinifera*) have been identified as a major problem in the wine and table grapes worldwide, reducing the productivity, quality and longevity of vineyards. The present study examined 694 wood samples from the cordons and trunks of vines with trunk disease symptoms in 67 Chilean vineyards located between Copiapó (27°18′ S) and Los Angeles (37°42′ S) from 2009 to 2012. A total of 1,363 fungal isolates were obtained from diseased cordons and trunks with dark brown streaking, yellowish spongy cankers and brown hard V-shaped cankers. On the basis of morphology and molecular analysis, a total of 11 fungal genera were identified at varying frequencies: *Phaeomoniella chlamydospora* (85%); Botryosphaeriaceae (56%) including *Diplodia mutila, D. seriata, Neofusicoccum parvum* and *Spencermartinsia viticola; Inocutis* sp. (47%); Diatrypaceae *(Cryptovalsa ampelina* and *Eutypella leprosa*) (4.8%); *Seimatosporium botan* (1.7%); *Phomopsis viticola* (0.4%); *Cylindrocarpon* sp. (0.4%); and *Phaeoacremonium aleophilum* (0.2%). All species were pathogenic, inducing dark brown streaking on various aged grapevine wood tissue. In conclusion, several fungal species are associated with grapevine trunk diseases in the Chilean vineyards being *Pa. chlamydospora, D. seriata* and *Inocutis sp*. the most frequent isolated species. These are pathogens that can be found alone or they can coexist in the same plant. There were no evidences of *Eutypa lata* affecting grapevines in Chile.

MYCORRAY: a DNA microarray tool for the early, reliable, simultaneous identification of the grapevine trunk diseases fungal agents. J. WOODHALL[2], G. MARCHI[1], T. CINELLI[1], B. GINETTI[1], D. BOSSIO[1], J. TOMLINSON[2], C. HARRISON[2], N. BOONHAM[2] and L. MUGNAI[1,*]. *[1]Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA) sez. Patologia Vegetale ed Entomologia, P.le delle Cascine, 28, 50144 Firenze, Italy. [2]The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK. *E-mail: laura.mugnai@unifi.it*

Grapevine trunk diseases (GTD) including the Esca complex, Eutypa dieback, Botryosphaeria canker, and Black foot, often involve more than one causal agent and several causal agents may coexist in the same vine. Diagnoses with currently available diagnostic methods only detect one organism per test, making the detection of all GTD causal agents involved impractical and prohibitively expensive. With this purpose, a microarray tool (named MYCORRAY) is under development which aims for early, reliable, simultaneous identification of the 11 most relevant grapevine trunk fungi responsible for the most harmful trunk diseases of grapevines. The MYCORRAY system consists of a disposable Array Tube containing a DNA microarray chip and a cost-effective standalone array reader suitable for use by non-specialists, composed of an optical system and an image processing system. Additionally, a simple and inexpensive methodology for non-lethal sampling and DNA extraction has been developed for optimum detection of GTDs in vine material. Seven DNA extraction methods were compared to ensure maximum fungal DNA extraction and purification coupled with the highest sensitivity and minimum testing costs. Sampling using a drill from four points in the scion and two points on the rootstock that were pooled together gave the best results. Using traditional isolation techniques in combination with a nested PCR approach for pathogen detection, this sampling minimized the number of DNA extractions required. The MYCORRAY system in combination with this sampling protocol is therefore likely to provide a robust, efficient tool to help manage GTDs worldwide.

Epidemiology

Botryosphaeriaceae conidial dissemination in two different locations with Mediterranean semiarid climate in the Valparaíso region of Chile. D. VALENCIA, C. TORRES and X. BESOAIN*. *Facultad de Agronomía, Pontificia Universidad Católica de Valparaíso, Av. Brasil 2950, Valparaíso, Chile. *E-mail: xbesoain@ucv.cl*

The dissemination of Botryosphaeriaceae causing dieback in vineyards has recently received increased attention. Rain and high relative humidity trigger conidial release into the environment which can infect pruning wounds during this time. However, the seasonal abundance of conidia varies among production areas and different latitudes. The goal of this study was to determine the effects of rainfall, relative humidity and temperature on the moment of maximal dispersion of Botryosphaeriaceae conidia in two Mediterranean semiarid temperate climate locations in Valparaíso Region of Chile. To achieve this, five microscope slides covered with liquid Vaseline were placed in four vineyards of two locations: Casablanca (with high marine influence) and Panquehue (with low marine influence) both displaying symptoms of Botryosphaeria dieback. The slides were replaced every week and sent to PUCV Phytopathology Laboratory in order to observe the presence of conidia presence over a one-year time period. In addition, isolations were carried out from plants with Botryosphaeria dieback at each location. Species were identified using morphological and molecular techniques. In Casablanca, a spore "peak" was detected during August, coinciding with the last rainfall recorded. Release started again at the beginning of autumn 2014 and has continued to the current time and

according to winter rain patterns. In Panquehue location, spore release was observed during spring, while in fall-winter release was null or very low. The Botryosphaeriaceae species identified were *Diplodia seriata*, *Dothiorella* sp. and *Neofusicoccum* sp.

Genetic analysis of *Neofusicoccum parvum* and *N. luteum* isolates from nurseries and vineyards indicates different infection sources. R. BILLONES-BAAIJENS[1,2,*], J. BASKARATHEVAN[1,3], M.V. JASPERS[1], E.E. JONES[1], R.H. CRUICKSHANK[1] and H.J. RIDGWAY[1]. [1]*Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln University, Lincoln, New Zealand, 7647.* [2]*National Wine and Grape Industry Centre (NWGIC), Charles Sturt University, Mckeown Drive, Wagga Wagga, NSW 2678 Australia.* [3]*Plant Health and Environment Laboratory, Ministry for Primary Industries, PO Box 2095, Auckland 1140, New Zealand. *E-mail: rbaaijens @csu.edu.au*

Surveys in 2007–08 showed that *Neofusicoccum parvum* and *N. luteum* are the two most prevalent and virulent species found in New Zealand vineyards and nurseries. However, *N. parvum* is more common in vineyards while *N. luteum* dominates in nurseries. Pathogenicity studies also showed that *N. parvum* was more aggressive and produced more severe, darker external lesions on canes than *N. luteum*. This study used genetic data to elucidate population origins of *N. parvum* and *N. luteum* from vineyards and nurseries. Vineyard and nursery isolates of *N. parvum* (n=79) and *N. luteum* (n=64) were genotyped using five universally-primed polymerase chain reaction (UP-PCR) primers. The five primers were able to amplify a total of 51 loci for *N. parvum* (66% polymorphic) and 54 loci for *N. luteum* (44% polymorphic). Phylogenetic analysis using parsimony (PAUP) showed that 92% and 78% of the *N. parvum* and *N. luteum* populations, respectively, were of unique genotypes. The neighbour joining trees showed that *N. parvum* from nurseries clustered separately from the vineyard isolates indicating the two populations were genetically distinct. This supported the initial hypothesis that the nursery infections by this aggressive species were intercepted during the grading process and, therefore, there was less movement of this species to the vineyards. In contrast, the *N. luteum* nursery and vineyard populations showed high genetic similarities. This indicated that the less distinct symptoms caused by this species are not graded out and, therefore, these infections can easily migrate from the nurseries to the vineyards.

Spore release patterns of Petri disease fungi in South African vineyards and rootstock mother blocks. M.A. BALOYI[1], L. MOSTERT[2] and F. HALLEEN[1,2,*]. [1]*Plant Protection Division, ARC Infruitec-Nietvoorbji, Private Bag X5026, Stellenbosch, 7599, South Africa.* [2]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa. *E-mail: halleenf@arc.agric.za*

Phaeomoniella chlamydospora and *Phaeoacremonium* species have been associated with Petri disease in grape growing regions around the world. Spores of these pathogens are aerially dispersed and infect vines through susceptible wounds. However, no information is available about the time when these spores are released in South African vineyards. A study was undertaken in six vineyards and two rootstock mother blocks within the Western Cape Province, over two seasons, from May–November 2012 and March–November 2013. Microscope slides coated with petroleum jelly were affixed to five vines (or rootstock mother plants) in each vineyard. The slides were changed weekly, washed in 5ml sterile water and passed through 5- and 0.45-µm filters. The filters were backwashed and the suspensions plated onto PDA. Colonies resembling Petri disease pathogens were recorded, sub-cultured and identified on the basis of cultural characteristics (*Pa. chlamydospora*), and with species-specific primers or sequencing of the partial betatubulin and actin genes (*Phaeoacremonium* species). Spores of various species were detected within each of the vineyards and inoculum was available whenever pruning or sucker wounds were made in winter and spring. Over the two seasons 13 *Phaeoacremonium* species were detected, namely *Pm. aleophilum*, *Pm. alvesii, Pm. australiense, Pm. viticola, Pm. parasiticum, Pm. scolyti, Pm. sicilianum, Pm. subulatum, Pm. iranianum, Pm inflatipes, Pm. venezuelense*, *Pm. prunicola* and *Pm. griseo-olivacea*, the latter two for the first time in South African vineyards. *Pa. chlamydospora* and *Pm. aleophilum* were the only species detected in all the vineyards. This study confirms the need for effective wound protection strategies.

Studying and modelling the summer development of esca foliar symptoms. P. LECOMTE[1], E. BRUEZ[1,4], J. GERBORE[1,2], P. PIERI[3], L. GUERIN-DUBRANA[1,4], D. BARKA[1], Y. MEZIANI[1], C. BENETREAU[1], M. FERMAUD[1] and P. REY[1,4]. [1]*INRA, Université de Bordeaux, ISVV, UMR1065 SAVE, CS 20032, F-33882, Villenave d'Ornon cedex, France.* [2]*BIOVITIS, F-15400 Saint Etienne de Chomeil.* [3]*INRA, Université de Bordeaux, ISVV, UMR1287 EGFV, F-33140 Villenave d'Ornon, France.* [4]*Université de Bordeaux, Bordeaux Sciences Agro, ISVV, UMR1065 SAVE, CS 20032, F-33882, Villenave d'Ornon cedex, France. *E-mail: lecomte@bordeaux.inra.fr*

A survey carried out in Aquitaine vineyards (France) from 2004 to 2006 showed that the occurrence of esca foliar symptoms had a similar progressive pattern in all the plots surveyed. This pattern corresponded to a

sigmoidal increase in the incidence of vines exhibiting typical symptoms. In general, regardless of the site and year, the leaf-symptomatic vines increased uniformly over time, reaching a maximum incidence by the end of July. To further study this phenomenon, another survey was carried out from 2012 to 2014 in the Bordeaux region. It was based on regular monitoring of vines twice a week. The data confirmed previous results and were used to develop a logistic model aimed at characterizing the period of symptom appearance over time. This period corresponded to the increase in average temperatures and to a putative establishment of water restriction. This model could be used to study the effect of environmental factors, *i.e.* soil, climate and the susceptibility of cultivars. Esca symptoms were mostly associated with longitudinal xylem discolorations located just under the bark on cordons or trunks. The origin of this peculiar symptom is still unclear even though it has been attributed to infections by Botryosphaeriaceae. In the same survey, vines with recent symptoms were identified and investigated. Diverse fungal communities were isolated from discoloured or "apparently healthy wood". The percentages of Botryosphaeriaceae isolated from these samples were not significantly different. Furthermore, their absence in some samples indicated that these fungi cannot be responsible (at least alone) for the xylem discolorations.

Unmanned Aerial Vehicle based remote sensing as a predictive tool for the onset of grapevine leaf stripe disease symptoms. S.F. DI GENNARO[1,2,*], M. BENANCHI[3], A. MATESE[1], J. PRIMICERIO[1], L. GENESIO[1], A. PALLIOTTI[2], O. FACINI[4], S. DI MARCO[4] and L. MUGNAI[3]. *[1]Istituto di Biometeorologia (IBIMET), CNR, Via G. Caproni 8, 50145 Florence, Italy. [2]Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Perugia, Borgo XX Giugno 74, 06128 Perugia, Italy. [3]Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DiSPAA), Sez. Patologia vegetale ed Entomologia, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Florence, Italy. [4] Istituto di Biometeorologia (IBIMET), CNR, Via Gobetti 101, 40129 Bologna, Italy. *E-mail: f.digennaro@ibimet.cnr.it*

Grapevine Leaf Stripe Disease (GLSD) foliar symptoms were proved by several authors to be linked to a drastic alteration of photosynthetic function, as well as an activation of defence responses in affected grapevines, several days before the appearance of the first visible symptoms on leaves. This study suggests a method to investigate the correlation between high-resolution multispectral images acquired by Unmanned Aerial Vehicles (UAV) and GLSD foliar symptoms monitored by ground surveys. This approach previously showed a high correlation between Normalized Difference Vegetation Index (NDVI) acquired by the UAV and GLSD symptoms, and the ability to discriminate symptomatic from asymptomatic plants. High-resolution multispectral images were acquired over 2012 and 2013 in June and July in an experimental vineyard, located in Tuscany (Italy), where vines had been surveyed and mapped since 2003. Each vine was mapped with Leica Differential-GPS, and classified for foliar symptoms appearance and disease severity weekly since the beginning of each season. In 2013, single leaves from the vineyard were analyzed for physiological parameters using a Portable Photosynthesis System (Li-Cor 6400). Remote sensing and ground observation data were analyzed to promptly identify the early stages of symptoms, even before visual detection. The objective of this research was to develop an innovative method aimed primarily at conducting a quantitative and qualitative analysis of the symptom spread, and then to realize a predictive tool of the GLSD symptoms onset.

Seasonal susceptibility of grapevine pruning wounds to artificial infection with *Diplodia seriata* and *Phaeomoniella chlamydospora* in Catalonia, NE Spain. G. ELENA* and J. LUQUE. *IRTA Cabrils, Ctra. de Cabrils km 2, 08348 Cabrils, Spain. *E-mail: georgina.elena@irta.cat*

Dispersal of grapevine trunk diseases in the vineyard relies on the infection of pruning wounds by airborne pathogen spores. The aim of this study was to evaluate the period in which pruning wounds remain susceptible to infection by the pathogenic fungi *Diplodia seriata* and *Phaeomoniella chlamydospora*. An experimental 'Tempranillo' vineyard located in our institution was pruned at two different seasons: *early* in autumn, and *late* in winter. In both seasons, pruning wounds (N=20 per treatment) were artificially inoculated with a conidial suspension of each pathogen separately at different periods after pruning (1 day, and after 1, 2, 4, 8 and 12 weeks). For the control treatment, pruned canes were treated with sterile distilled water. Four months after the inoculations, the percentage of fungal recovery from canes was determined. The experiment was repeated twice in the seasons 201213 and 20132014. The reisolation percentage of *D. seriata* fell below 35% after 2 weeks in the early pruning, while a comparable decrease in the late pruning was not observed until 8 weeks. *Phaeomoniella chlamydospora* showed a similar pattern since the reisolation percentage decreased dramatically after 4 weeks in the early pruning while susceptibility in late pruning only fell below 25% after 8 weeks. The results indicated that pruning wounds remain less susceptible to pathogen infection after an early pruning as compared to a late pruning, thus suggesting that a shift of the traditional late pruning in the area to an early pruning would reduce the risk of pathogen infection in Catalonia.

Grapevine sucker wounds as infection ports for trunk disease pathogens. G. MAKATINI[1], C. MUTAWILA[1], F. HALLEEN[1,2] and L. MOSTERT[1,*]. [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: lmost@sun.ac.za

The susceptibility of sucker wounds to different trunk disease pathogens was assessed from natural and artificial infections. Sucker wounds were sampled from three wine and two table grape vineyards during 2011 and 2012 in the Western Cape province of South Africa. Isolations were made from 161 sucker wounds and the fungal cultures were identified. Sixtytwo percent of wounds were naturally infected by at least one of the trunk pathogens. The most predominant trunk disease pathogens isolated from sucker wounds of field wine and table grape cultivars, respectively were *Phomopsis* (*Po.*) *viticola* (46%; 18%), *Diplodia* (*D.*) *seriata* (30%; 9%) and *Phaeomoniella* (*Ph.*) *chlamydospora* (27%; 5%). Lower incidences of *Phaeoacremonium aleophilum* (18%), *Eutypella* sp. (3%), *Cryptovalsa ampelina* (2%), *Diplodia* sp. (1%) and *Neofusicoccum australe* (1%) were obtained, however, only from wine grapes. Sucker wounds on 1-year-old potted grapevine plants of Chardonnay cultivar were inoculated with spore suspensions of *Eutypa lata*, *N. parvum, Pa. aleophilum*, *Ph. chlamydospora* and *Po. viticola* in the glasshouse. After 4 months all the inoculated pathogens could be re-isolated. Sucker wound susceptibility was further ascertained under field conditions on Cabernet Sauvignon vines by artificial inoculation of the same pathogen species. After 5 months, only *Po. viticola, N. parvum* and *Ph. chlamydospora* could be re-isolated. The duration of susceptibility of field sucker wounds to *Ph. chlamydospora* was assessed for 4 weeks. The wounds remained susceptible for 4 weeks with a decline in susceptibility after one week. This study showed that sucker wounds are susceptible to the major trunk disease pathogens.

Potential role of shears on the infection of grapevine by fungal trunk pathogens. C. AGUSTÍ-BRISACH, M. LEÓN, J. GARCÍA-JIMÉNEZ and J. ARMENGOL*. *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.* *E-mail: jarmengo@eaf.upv.es

Four Spanish vineyards were surveyed during pruning in 2012. In each vineyard, shears were washed with a sterile solution of 0.2% Tween-20 after pruning ten grapevines. Twenty liquid samples were collected from a total of 200 plants. Molecular detection of fungal trunk pathogens in these samples was performed by nested-PCR using specific primers to detect Botryosphaeriaceae spp., *Eutypa lata*, *Cadophora luteo-olivacea*, *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora*. All trunk pathogens, with the exception of *E. lata*, were detected in samples from the four vineyards evaluated by nested-PCR, being *Ca. luteo-olivacea* and *Phaeoacremonium* spp. the most frequent. We found the co-occurrence of 2, 3 or 4 different fungal trunk pathogens in the same sample. *Cadophora luteo-olivacea* and *Phaeoacremonium* spp. were the combination most frequently detected. In addition, fungal isolation from liquid samples in semi-selective culture medium for *Ca. luteo-olivacea*, *Phaeoacremonium* spp. and *Pa. chlamydospora* was also performed, but only *Ca. luteo-olivacea* was recovered from samples collected in 3 out of 4 vineyards evaluated. Shears artificially infested with suspensions of conidia or mycelial fragments of *Diplodia seriata*, *Eutypa lata*, *Ca. luteo-olivacea*, *Pm. aleophilum* and *Pa. chlamydospora*, were used to prune 1-year-old grapevine cuttings of 110 Richter rootstock. Fungal re-isolation from the cuttings four months after pruning confirmed that infested shears were able to infect them through pruning wounds. Our results demonstrate the potential of inoculum present on shears to infect grapevines.

Co-infection by *Neofusicoccum luteum* and *N. parvum* influences direction of lesion expansion but not total lesion size. J. BASKARATHEVAN[1,2], E.E. JONES[2], M.V. JASPERS[2] and H.J. RIDGWAY[2,*]. [1]*Ministry for Primary Industries-Manatū Ahu Matua, PO Box 2095, Auckland 1140, New Zealand.* [2]*Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln University, Lincoln 7647, New Zealand.* *E-mail: Hayley.Ridgway@lincoln.ac.nz

In a national survey of symptomatic vines a total of 238 samples were collected yielding 336 isolates of botryosphaeriaceous species. From this collection 18 lesions were systematically sampled to determine if multiple species were present. Twelve lesions contained multiple species. *Diplodia seriata* was found most frequently in combination with other species (40%) but *Neofusicocum luteum* and *N. parvum* were found most frequently together (20%). To determine whether co-infection by these two *Neofusicoccum* species was synergistic, trunks of 1-year-old Sauvignon blanc vines were co-inoculated with two isolates each of *N. luteum* and *N. parvum*, either together or alone. Each pair of isolates for each species consisted of a weakly virulent and highly virulent isolate, with virulence established in previous experiments. Following inoculation the presence of both species in one lesion was confirmed by PCR. The results showed that in co-inoculated lesions mean total lengths were not larger than the lesion length produced by the most virulent of the isolate pair ($P \leq 0.05$), irrespective of the virulence of the second isolate. However, when the distances from the inoculation points to lesion

edges were analysed, results showed that there were significantly greater lengths below than above inoculation points for all combinations ($P \leq 0.05$). In addition, co-inoculation of two weakly virulent isolates reduced upward movement ($P \leq 0.05$). A decrease in the endophytic movement of *N. luteum* beyond the lesion was also observed in all co-inoculations. Overall, the results demonstrated that there were no synergistic effects of co-inoculation with these two common species.

The genetic structure of Botryosphaeriaceae isolated from vineyards in south eastern Australia as revealed by molecular markers. Y. QIU, C.C. STEEL, G.J. ASH and S. SAVOCCHIA*. *National Wine and Grape Industry Centre, Charles Sturt University, School of Agricultural and Wine Sciences, Locked Bag 588, Wagga Wagga NSW 2678 Australia. *E-mail: ssavocchia@csu.edu.au*

Genetic variation within four species of Botryophaeriaceae isolated from south eastern Australian vineyards (Lower and Upper Hunter Valley and Mudgee) was investigated using a hierarchical sampling and a range of PCR primers. A set of 100 primers (Microsatellite Primer Set #9; Biotechnology laboratory, University of British Columbia, Canada), $(ACC)_5$, R1 (consensus region from a plant intron splice junction) and M13 (core sequence of the M13 minisatellite region) primers were screened with a selection of isolates from all four species. Of these, five of the 100 primer set, $(ACC)_5$, M13 and R1 were selected and resulted in 280 highly reproducible polymorphic bands across 127 *Diplodia seriata*, 23 *Neofusicoccum parvum*, 18 *Botryosphaeria dothidea* and three *Lasiodiplodia theobromae* isolates. Cluster analysis with the integrated locus matrix separated the isolates into four distinguishable groups according to species (cophenetic correlation = 0.97). Within *D. seriata* differentiation by region was evident. Furthermore, for this species there was variation in genotype between vineyards in several instances. Isolates of *N. parvum* showed greater homogeneity between vineyards and regions, while *B. dothidea* showed homogeneity between regions from which they were isolated (Lower and Upper Hunter Valley). The results obtained in this study were effective in determining the genetic variation within the Botryosphaeriaceae species examined and could be used to guide sampling in future surveys and population studies.

Ocurrence of *Phaeomoniella chlamydospora* in *Vitis vinifera* and *V. labrusca* vineyards of the Serra Gaúcha region of the Rio Grande do Sul state in southern Brazil. M.A.K. ALMANÇA[1,*], C. RUSIN[1], G. GIOTTO[1], G.T. MARTINELI[1], M.R. DE OLIVEIRA[1] , F.R. CAVALCANTI[2] and F. HALLEEN[3]. *[1] Rio Grande do Sul Education, Science and Technology Federal Institute, Osvaldo Aranha Avenue, 540, Zip Code 95700-000 Bento Gonçalves, Rio Grande do Sul, Brazil. [2] Embrapa Grape & Wine, Livramento Street, 515, Bento Gonçalves, Rio Grande do Sul, Brazil. [3] Plant Protection Division, ARC Infruitec-Nietvoorbji, Private Bag X5026, Stellenbosch, 7599, South Africa. *E-mail: marcus.almanca@bento.ifrs.edu.br*

Several factors affect the quantity and quality of grapevine production in Brazil. This includes the grapevine decline phenomenon and the various disease complexes associated with it. For example, in Brazil, there is very little knowledge about Esca and Petri disease and the distribution of the pathogens associated with it. The objective of this research was to study *Phaeomoniella chlamydospora* (Pchl) distribution in different cultivars and plant parts from vineyards in the Serra Gaúcha region of the Rio Grande do Sul state in southern Brazil. Grapevines with decline symptoms were collected in 21 vineyards and taken to the laboratory for destructive sampling and analysis. *Vitis vinífera* cultivars included Chardonnay (14 plants), Merlot (1) and Cabernet Sauvignon (7). *Vitis labrusca* cultivars included Isabel (1 plant), Bordô (10) and Niágara (4). Isolations were made from symptoms in spurs, arms, upper part of the trunk, graft union and basal end of the rootstock. Pchl incidences ranged from 2.5 to 27.4% in *V. labrusca* and 4.8 to 8.8% in *V. vinifera*. From the different plant parts Pchl incidences ranged from 7.3 to 13.1%. From the different internal symptoms, Pchl was most frequently isolated from black spotting (17.1 %) and soft and spongy rotted wood (15.4%), followed by brown-red wood (7.8%), black line (7%) and wedge shaped necrosis (6.3%).

Host Pathogen Interaction

Induction of grapevine defence systems using the oomycete *Pythium oligandrum* against a pathogenic fungus involved in Esca. A. YACOUB[1,2,3], J. GERBORE[4], N. MAGNIN[1,2], J. VALLANCE[2,1], D. GRIZARD[4], R. GUYONEAUD[3] and P. REY[2,1,*]. *[1]INRA, UMR1065 Santé et Agroécologie du Vignoble (SAVE), ISVV, F-33140 Villenave d'Ornon, France. [2]Université de Bordeaux, Bordeaux Sciences Agro, ISVV, UMR1065 SAVE, F-33140 Villenave d'Ornon, France. [3]UMR CNRS 5254/IPREM-EEM, IBEAS, Université de Pau et des Pays de l'Adour, 64013Pau, France. [4]BIOVITIS, 15400 Saint Etienne de Chomeil, France. *E-mail: patrice.rey@bordeaux.inra.fr*

The oomycete, *Pythium oligandrum*, has been reported to control several diseases and is able to induce plant defense systems. In order to control grapevine diseases, we isolated *P. oligandrum* strains from the rhizosphere of vines cultivated in 12 vineyards of the Bordeaux region. *Pythium* spp. with echinulated-oospores were frequently isolated from the root system of the sampled vines, with *P. oligandrum* being the most frequently identified species (91% of strains, analyses based on ITS

region). Strains from this oomycete were used to control a fungus involved in esca, *Phaeomoniella chlamydospora*, in a greenhouse assay conducted in 2010, 2011 and 2013. In comparison with control plants infected with the pathogen, necroses were reduced by 50% when *P. oligandrum* colonized the root system of the Cabernet Sauvignon cuttings. Genes involved in various grapevine defense pathways were studied to determine plant responses after inoculation by *P. oligandrum* and/or *P. chlamydospora*. In the trunk, when *P. oligandrum* colonized the roots, infection with *P. chlamydospora* was associated with a quicker and a more intense up-regulation of the 22 studied genes. Interestingly, 7 days post inoculation, genes up-regulations were observed in the biosynthetic pathway of phenylpropanoids (6 genes studied), indoles (2 genes), and cell wall synthesis (3 genes). However, when the plants were infected with *P. chlamydospora* alone, the grapevine defense responses were different: only some specific Pathogenesis-Related proteins were stimulated 14 days post inoculation. Priming is probably induced by *P. oligandrum* and we are currently using *Vitis vinifera* micro-arrays to investigate this finding.

Anatomical differences of grapevine xylem influences tolerance to esca disease. J. POUZOULET and P.E. ROLSHAUSEN*. *Department of Botany and Plant Sciences, University of California, Riverside, CA, 92521, USA. *E-mail: philrols@ucr.edu*

No grapevine cultivar is known to be resistant to *Phaeomoniella chlamydospora* (*Pch*) and *Phaeoacremonium aleophilum (Pal)*, the main causal agents of esca disease. However, the variability of disease incidence and severity amongst grapevine cultivars as observed in the field suggest a degree of tolerance towards these pathogens. The goals of our study were to better understand the mechanism of tolerance to esca disease amongst grapevine cultivars and especially focusing on the xylem morphology and the compartmentalization process of the pathogens. Wood cuttings of 4 commercial grapevine cultivars (i.e, 'Merlot', 'Cabernet Sauvignon', 'Chardonnay' and 'Thompson Seedless') representing a continuum between tolerance and extreme susceptibility were experimentally inoculated with *Pch* and *Pal* and grown in glasshouse. Ten weeks post-inoculation, the colonization of the host vascular system was evaluated by measuring the length of wood discoloration, performing fungal re-isolation, and quantifying fungal DNA through qPCR. For both fungi, the degrees of susceptibility supports previous field and greenhouse studies, and provides evidence that tolerance is related to the ability of the plant host to successfully compartmentalize the fungal pathogens. In addition our results established that vessel diameter is correlated to the degree of susceptibility whereby varieties with small vessels like 'Merlot' were more tolerant to esca pathogens than varieties with large vessels such as 'Thompson seedless'. We discuss how this concept could be use to predict cultivars tolerance, and future prospect for the development of alternative management strategies of esca disease.

Extracellular compounds from *Diplodia seriata* and *Neofussicoccum parvum* induce differential defence gene expression patterns and necrosis in grapevine cells. M. BENARD-GELLON[1,*], C. TARNUS[2], F. MAZET-KIEFFER[1], C. BERTSCH[1] and S. FARINE[1]. *[1]Laboratoire Vigne Biotechnologie et Environnement EA 3991, Université de Haute-Alsace, rue de Herrlisheim 33, BP 68008 Colmar Cedex, France. [2]Laboratoire de Chimie Organique et Bioorganique, École Nationale Supérieure de Chimie, Université de Mulhouse, rue Alfred Werner 3, F-68093 Mulhouse Cedex, France. *E-mail: melanie.gellon@uha.fr*

The different fungi associated with grapevine trunk diseases can be isolated in the necrotic wood but not in the symptomatic leaves. Other factors seem to be responsible for the foliar symptoms and may represent the link between wood and foliar symptoms. We hypothesized that the extracellular compounds produced by the fungi associated with grapevine trunk diseases are responsible for pathogenicity. In our work, we tested the aggressiveness of total extracellular compounds produced by *Diplodia seriata* and *Neofusicoccum parvum* on grapevine cells (cv. Chardonnay). We showed that the total extracellular compounds produced by *N. parvum* induced more necrosis and triggered a different defence gene expression pattern compared to those of *D. seriata*. Additionally we tested the toxicity of purified mellein, a characteristic toxin present in the extracellular compounds of Botryosphaeriaceae. We revealed a delayed necrosis and a lower-level defence gene expression, in our *in vitro* model, with doses one hundred times higher than those found in the liquid fungal cultures. Therefore, the possible function of other types of extracellular compounds, like proteins, was hypothesized. We evaluated the amount, the pattern and the impact of extracellular proteins produced by these fungi. Our results pointed out that *N. parvum* produced more extracellular proteins and in higher concentration when compared to *D. seriata*. Moreover extracellular proteins from *N. parvum* were more aggressive than those from *D. seriata* on necrosis production and clearly induced more grapevine defence genes. Future works to characterize these proteins and their functions should also be investigated in the future.

Visualization of internodal and nodal colonization of *Vitis vinifera* L. by *Phaeoacremonium aleophilum* using a gfp marker. R.J.G. PIERRON[1,2], M. GORFER[3,4], H. BERGER[4], A. JACQUES[1], A. SESSITSCH[4], J. STRAUSS[3,4]

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Esca disease became a major threat for viticulture in recent decades. *Phaeoacremonium aleophilum* is considered a pioneer of the complex pathosystem, which involves several species that cause the grapevine trunk disease esca. The microbial behaviour of this species inside plants remains poorly investigated. We will present evidence of the colonization behaviour of one GFP-tagged derivative of *P. aleophilum* (*gfp7*) after inoculation of grapevine plants in the internodal and nodal regions of the main stems of one year-old rooted cuttings of Cabernet-Sauvignon. Colonization was assessed six weeks post-inoculation using Confocal Laser Scanning Microscopy (CSLM) microscopy. Fungal inoculation after an internodal wounding led to high colonization of the xylem fibers as revealed by GFP-marked mycelium. Parenchymal cells were also colonized by spores and very few mycelia were observed in the lumen of xylem vessel elements six weeks post inoculation. At the nodal level, infection of pruning wounds caused a strong reaction zone in plant tissues and did not allow fungal colonization six weeks post inoculation. Mycelium was detected near the tissues, but with loss of the GFP signal when the fungus approached the plant tissues. These results suggest that some woody tissues can induce defenses against *P. aleophilum*. Furthermore, the investigation of fungal colonization and growth patterns in xylem wood fibers provide key information of the early events of grapevine infection for further studies aimed at developing control tools against esca-associated fungi.

Synthesis of differential physiological perturbations in grapevine organs in response to esca proper and apoplexy. M. MAGNIN-ROBERT[1], A. SPAGNOLO[1], T. D. ALAYI[2], C. CILINDRE[3], L. MERCIER[4], C. SCHAEFFER-REISS[2], A. VAN DORSSELAER[2], C. CLÉMENT[1] and F. FONTAINE[1,*]. [1]*Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire Stress, Défenses et Reproduction des Plantes, BP1039, 51687 Reims Cedex 2, France.* [2]*Université de Strasbourg, IPHC, UMR 7178, Laboratoire de Spectrométrie de Masse Bioorganique, 67087 Strasbourg, France.* [3]*Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire d'Œnologie et Chimie Appliquée, BP 1039, 51687Reims Cedex 2, France.* [4]*Moët & Chandon, 6 rue Croix de Bussy, 51200 Epernay, France. *E-mail: florence.fontaine@univ-reims.fr*

Esca proper poses a significant threat for viticulture along with apoplexy, often occurring on grapevines affected by esca proper. To verify if different responses are activated in leaves, green stems or trunks of apoplectic (A) and esca proper-affected (E) vines, two-dimensional gel electrophoresis coupled to mass spectrometry analysis was used. Moreover, expression of the relative transcripts of targeted proteins was also monitored by qRT-PCR. In leaves, drastic alterations of photosynthetic functions as well as defence response were registered in pre-symptomatic leaves. These alterations were amplified during the symptom development. In green stems, foliar expression of E- and A-affected plants was accompanied by an increase of PR-protein- and oxidative stress-related gene expressions. Moreover, an up-regulation of the phenylpropanoid pathway-related gene expression and an accumulation of phenolic compounds were observed. In trunk, asymptomatic wood was mainly characterized by down-expression of proteins involved in cell growth and defence responses. The proteome of black streaked wood, characterized by extensive presence of GTD agents, revealed over-expression of proteins involved in defense. There was no evidence of strong different response in the trunk wood of A- and E- affected plants. This could mean that, despite the different feature of these external crown symptoms, grapevine responses at trunk wood level are very similar in both cases. These results indicate that leaves, green stems and trunk are able to react before and/or during foliar expression of E and A, but various levels in defence responses are triggered upon onset of their symptoms.

Investigating the pathogenic and beneficial microorganisms that naturally colonize the healthy wood tissues of Esca foliar-symptomatic and asymptomatic vines. E. BRUEZ[1,2], J. VALLANCE[1,2], J. GERBORE[3], P. LECOMTE[1], L. GUERIN-DUBRANA[1,2] and P. REY[1,2,*]. [1]*INRA, UMR1065 Santé et Agroécologie du Vignoble (SAVE), ISVV, F-33140 Villenave d'Ornon, France.* [2]*Université de Bordeaux, Bordeaux Sciences Agro, ISVV, UMR1065 SAVE, F-33140 Villenave d'Ornon, France.* [3]*BIOVITIS, 15400 Saint Etienne de Chomeil, France. *E-mail: prey@bordeaux.inra.fr*

Fungal and bacterial microflora colonizing the wood tissues of grapevines which expressed esca-symptomatic foliar symptoms or not, were characterized and compared. Both trunk and rootstock of 10 year-old vines were sampled. Depending on whether the wood tissues were necrotic or not, the fungal and bacterial communities were different. Observations over a pe-

riod of one year, using a fingerprinting method, Single Strand Conformation Polymorphism (SSCP), and the ITS-DNA sequencing of cultivatable fungi and bacteria, showed that shifts occurred in the fungal and bacterial communities colonizing the "apparently healthy wood tissues". However, whatever the sampling time, spring, summer, autumn or winter, the microbial communities colonizing the healthy tissues of asymptomatic or symptomatic plants were not significantly different. High-throughput sequencing indicated that diverse assemblages of fungi (515 OTUs) and bacteria (222 OTUs) colonise the non-necrotic tissues. Numerous plant protective fungi, such as *Trichoderma* spp., and bacteria, such as *Bacillus* spp. and *Pantoea agglomerans,* have been isolated in these wood tissues. Pathogenic fungi, such as *Botryopshaeria* spp., *Phaeomoniella chlamydospora* were also identified in these non-necrotic tissues. Regarding such microflora, several questions are still the matter of speculation: (i) What is the role of the microbial communities that colonise the non-necrotic wood tissues of the grapevines? (ii) What are the interactions between fungal and bacterial communities? (iii) What are the abiotic factors that have most influence on microbial communities in the wood tissues, leading to a decrease in microbial diversity in the "apparently healthy wood tissues" and to the development of necroses?

Susceptibility of the Vitaceae family to Botryosphaeriaceae: a focus on *Vitis sylvestris*. X. GUAN[1], S. ESSAKI[1], H. LALOUE[1], P. NICK[2], C. BERTSCH[1,*] and J. CHONG[1]. [1]*Laboratoire Vigne Biotechnologies et Environnement EA-3991, Université de Haute-Alsace 33 rue de Herrlisheim 68000 Colmar, France.* [2]*Botanical Institute, Molecular Cell Biology, Karlsruhe Institute of Technology, Kaiserstrasse 2 76128, Germany. *E-mail: christophe.bertsch@uha.fr*

Vitis vinifera cultivars show different levels of susceptibility to grapevine trunk diseases, generally determined by the extent of foliar symptoms appearing in the field. However, the cause of these foliar symptoms and the relation between foliar symptom development and wood necrosis remain only partially understood. In this study, we investigated necrosis symptoms in the Vitaceae family after artificial inoculation of *Neofusicoccum parvum* and *Diplodia seriata* on woody internodes. The resistance level of all the Vitaceae members to *N. parvum* and *D. seriata* ranked into different orders. Moreover, three genotypes from *V. vinifera* ssp. *sylvestris*, the ancestor of *V. vinifera,* were selected based on a lower necrosis development after inoculation with *N. parvum* and *D. seriata*. Expression of defence genes was studied in these *V. vinifera sylvestris* genotypes after inoculation, both in green and necrotic wood. Gene expression was compared between resistant accessions and susceptible ones. In another approach, we studied the response of the *V. vinifera* cytoskeleton to culture filtrate of different Botryosphaeriaceae. The cytoskeleton has indeed been proposed as sensor for processing or decoding stress signals. It is thus of interest to know if *V. vinifera* is able to perceive the metabolites synthesized by fungi associated to BDA. Overall, this study shows that members of the Vitaceae family show different levels of susceptibility to Botryosphaeriaceae and that lower susceptibility is associated to enhanced expression of some defence genes.

Effect of growth stage on sensitivity of grapevine (*Vitis vinifera* L. cv Mourvèdre) to infection by the Botryosphaeria dieback agents *Neofusicoccum parvum* and *Diplodia seriata*. A. SPAGNOLO[1], P. LARIGNON[2], M. MAGNIN-ROBERT[1], A. HOVASSE[3], C. CILINDRE[4], A. VAN DORSSELAER[3], C. CLÉMENT[1], C. SCHAEFFER-REISS[3] and F. FONTAINE[1,*]. [1]*Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire Stress, Défenses et Reproduction des Plantes, BP 1039, Reims (Cedex 2) 51687, France.* [2]*Institut Français de la Vigne et du Vin Pôle Rhône-Méditerranée, France, Domaine de Donadille, Rodilhan 30230, France.* [3]*Université de Strasbourg, IPHC, UMR 7178, Laboratoire de Spectrométrie de Masse Bioorganique, Strasbourg 67087, France.* [4]*Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire d'Œnologie et Chimie Appliquée, BP 1039, 51687Reims Cedex 2, France. *E-mail: florence.fontaine@univ-reims.fr*

Botryosphaeria dieback, esca and Eutypa dieback are trunk diseases which pose important economic problems for vineyards worldwide, and currently, no effective treatment is available to control these diseases. *Neofusicoccum parvum* (Np) and *Diplodia seriata* (Ds) are among the causal agents implicated in Botryosphaeria dieback. Since little information is available on the life cycle of Np and Ds, the goal of this study was to determine the seasonal growth stage when grapevines are most susceptible to infection. Green stems of 16-year-old vines cv. Mourvèdre were artificially infected with *N. parvum* or *D. seriata* at the onset of three different phenological stages [G stage (separated clusters), flowering and veraison]. Highest mean lesion lengths were recorded with inoculation at flowering. Major proteome changes associated with artificial infections during the three different phenological stages were also reported using two dimensional gel electrophoresis (2D)-based analysis. Twenty (G stage), 15 (flowering) and 13 (veraison) differentially expressed protein spots were subjected to nanoLC-MS/MS and a total of 247, 54 and 25 proteins were respectively identified. At flowering, a weaker response to the infection was activated as compared to the other stages, and some defense-related proteins were even down regulated (e.g., superoxide dismutase, major latex-like protein, and pathogenesis related protein 10). Globally, the flowering period seemed to represent the period of highest sensitivity of grapevine to Botryosphaeria dieback agent infection,

possibly being related to the high metabolic activity in the inflorescences.

Plant-based markers of infection for *Neofusicoccum parvum*. K. BAUMGARTNER[1,*], S. CZEMMEL[2], G.R. CRAMER[2], E.R. GALARNEAU[1], R. TRAVADON[1], D.P. LAWRENCE[1], A.J. MCELRONE[1] and D.A. CANTU[3]. [1]*United States Department of Agriculture, Agricultural Research Service, Crops Pathology and Genetics Research Unit, Davis, CA 95616, USA.* [2]*Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV 89557, USA.* [3]*Department of Viticulture and Enology, University of California, Davis, CA 95616, USA. *E-mail: kbaumgartner@ucdavis.edu*

Canopy symptoms of Botryosphaeria dieback do not appear until years after *Neofusicoccum parvum* infects a pruning wound. There are control practices to minimize such infections, but growers tend to wait until symptoms are visible, at which point disease prevention is far less effective. Toward development of an early detection tool that would identify infected plants in nurseries and vineyards, we used RNA-Seq to identify differentially-expressed genes in the leaves of inoculated vs. non-inoculated Cabernet Sauvignon in the greenhouse. Woody stems were examined using light microscopy and high resolution computed tomography (HRCT) to monitor the spread of infection, and its spatial and temporal relationships to wood anatomical changes. The early stage of infection occurred prior to 2 months post-inoculation (MPI), when spread of the pathogen beyond the inoculation site was the farthest. This incubation period was also characterized by the largest stem lesions, the highest levels of fungal colonization and xylem vessels fully-occluded by gels, and the lowest starch content in xylem fibers and rays. Prior to 2 MPI, RNA-Seq and validative qPCR analyses identified eight candidate genes, which were transcriptionally activated by infection, but not by wounding alone. The best candidate genes [a dehydrin, a BURP domain protein, and a peptide similar to abscisic acid-induced wheat plasma membrane polypeptide 19 (AWPM-19)] identified the pathogen's presence with high specificity. Furthermore, expression of the eight candidate genes was not affected by *Planococcus* feeding, powdery or downy mildew infection, or abiotic stresses (heat, UV light), based on screenings of publicly-available, genome-wide expression data.

Variable levels of laccase are secreted by four species of *Ilyonectria* that infect grapevines. B. PATHROSE[1,2], M.O. OUTRAM[2], E.E. JONES[2], M.V. JASPERS[2] and H.J. RIDGWAY[2,*]. [1]*8/38 Cooyong Cres, Toongabbie, NSW, Australia, 2146.* [2]*Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln University, Lincoln, New Zealand, 7647. *E-mail: Hayley.Ridgway@lincoln.ac.nz*

Laccases are a family of enzymes (polyphenol oxidases; PPO-1 and PPO-2) implicated in pathogenesis and degradation of lignin by many phytopathogens, including those that infect grapevines. The aim of this study was to (i) confirm that *Ilyonectria* species pathogenic to grapevines secrete laccase, (ii) to determine whether isolates vary in laccase secretion and (iii) to determine whether the amino acid sequence of laccase (*lcc1*) differs between species. Laccase activity was measured using ABTS (2, 2[1]-azino-bis [3-ethyl-benzthiazoline-6-sulfonic acid]) and DMP (2,6-dimethoxy-phenol). Six isolates of *I. liriodendri* and five isolates of the *I. macrodidyma* complex, including, *I. macrodidyma* (n=3), *I. torrensensis* and *I. novozelandica* were inoculated as agar plugs into minimal liquid media and incubated at 20°C for 7 days. The mycelium free extracellular fluid was assayed for PPO-1 and PPO-2 activity by their oxidation of ABTS and DMP, respectively. The results showed that all isolates produced PPO-1 activity but only seven produced detectable PPO-2 activity. There was isolate variation in both PPO-1 and PPO-2 activity for all species for which >1 isolate was tested (P<0.000). Degenerate PCR was used to amplify the *lcc1* gene from *I. macrodidyma, I. novozelandica, I. torrensensis* and *I. liriodendri*. Six amino acid polymorphisms were identified within isolates of *I. liriodendri* and the *I. macrodidyma* complex. Amino acid polymorphism was not found between isolates of the same species. Thus, variable laccase activity is likely to result from variable amount of enzyme secretion rather than isolate differences in enzyme activity.

Evaluating grapevine germplasm for resistance to Eutypa dieback. R. TRAVADON[1,*], J.E. PREECE[2] and K. BAUMGARTNER[1]. [1]*United States Department of Agriculture - Agricultural Research Service, Crops Pathology and Genetics Research Unit, Davis, CA 95616, USA.* [2]*United States Department of Agriculture - Agricultural Research Service, National Clonal Germplasm Repository, Davis, CA 95616, USA. *E-mail: rtravadon@ucdavis.edu*

Eutypa dieback of grapevine is a trunk disease that impairs vineyard productivity worldwide. In the vineyard, the causal agent *Eutypa lata* infects pruning wounds and forms a canker within months; Eutypa foliar symptoms are observed several years later. Because the wood lesion typically forms first, it is a more common measure of resistance in controlled inoculations. Nonetheless, past research shows a lack of concordance between these two symptoms. We compared three inoculation methods for evaluating resistance among genetically-diverse cultivars originating from the eastern Mediterranean (Black Corinth, Husseine, Thompson Seedless), central Europe (Carignane, Muscat Hamburg, Primi-

tivo), and Western Europe (Merlot, Palomino, Peloursin). We also monitored Eutypa foliar symptoms under field conditions in the USDA National Clonal Germplasm Repository (Winters, California, USA). Using a traditional inoculation assay, rooted, dormant cuttings developed stem lesions and foliar symptoms after 12 months, with Primitivo and Merlot as the most resistant cultivars. The relative resistance of cultivars was similar using inoculations of mist-propagated, rooted, green cuttings, which led to development of stem lesions, but no foliar symptoms after a 4-month incubation period. Inoculations of detached, dormant cuttings produced foliar symptoms within only 5 weeks, but no stem lesions, and confirmed Primitivo and Merlot as the most resistant cultivars. Of the >1,200 *Vitis vinifera* cultivars monitored in the Germplasm Repository, approximately 200 cultivars expressed foliar symptoms in at least one of three growing seasons, including Husseine, Muscat Hamburg, and Thompson Seedless. Our germplasm surveys and fast screening/phenotyping assays will help identify resistant germplasm for future breeding programs.

Searching for resistance to grapevine trunk diseases. M.R. SOSNOWSKI[1,*], M.R. AYRES[1], T.J. WICKS, 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 Australia. *E-mail: mark.sosnowski@sa.gov.au*

Eutypa and botryosphaeria dieback contribute to grapevine decline, reducing productivity and longevity. Vines are infected through pruning wounds by species of Diatrypaceae and Botryosphaeriaceae, which colonise wood, causing dieback and eventual vine death and, in the case of eutypa dieback, stunting of shoots and leaf distortion. Previous reports of varying susceptibility of *Vitis vinifera* cultivars to trunk disease prompted this research to identify potential sources of resistance or tolerance. The germplasm collection in the Barossa Valley, South Australia utilised for this study consisted of 178 mature (31-36 yo) *V. vinifera* winegrape cultivars sourced from around the world. Previous research suggested that the vines were subject to natural infection by eutypa and botryosphaeria dieback pathogens via pruning wounds. Vines were assessed in spring 2012 and 2013 for severity of eutypa dieback foliar symptoms and overall trunk disease symptoms, including presence of dead spurs, cordon die-back and trunk cankers. Severity of symptoms ranged from 0 to 98%, with a number of cultivars observed to have few or no symptoms. Experiments were conducted using a detached cane assay to evaluate; i) a selection of cultivars from the germplasm collection and ii) rootstock genotypes from a breeding program. All cultivars and rootstocks were susceptible to infection by *Eutypa lata* and *Diplodia seriata*, but the rate of growth varied significantly, suggesting possible tolerance or resistance in some accessions. Potted vines will be evaluated to confirm these findings. Clones of Shiraz and Cabernet Sauvignon will also be evaluated to substantiate anecdotal reports of varying susceptibility.

Brown stripe in Botryosphaeria dieback: differential responses of three grapevine cultivars. A. SPAGNOLO[1], M. MAGNIN-ROBERT[1], T. D. ALAYI[2], C. CILINDRE[3], C. SCHAEFFER-REISS[2], A. VAN DORSSELAER[2], C. CLÉMENT[1], P. LARIGNON[4], M. SUERO-RAMIREZ[5], J. CHONG[5], C. BERTSCH[5], E. ABOU-MANSOUR[6] and F. FONTAINE[1,*]. [1]*Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire Stress, Défenses et Reproduction des Plantes, BP 1039, 51687 Reims Cedex 2, France.* [2]*Université de Strasbourg, IPHC, UMR 7178, Laboratoire de Spectrométrie de Masse Bioorganique, 67087 Strasbourg, France.* [3]*Université de Reims Champagne-Ardenne, Laboratoire d'Œnologie et Chimie Appliquée.* [4]*Institut Français de la Vigne et du Vin Pôle Rhône- Méditerranée, France, Domaine de Donadille, 30230 Rodilhan, France.* [5]*Université de Haute-Alsace, UFR PEPS, Laboratoire Vigne, Biotechnologie et Environnement, 33 rue de Herrlisheim, 68008 Colmar cedex, France.* [6]*Plant Biology Department, University of Fribourg, 3 rue Albert Gockel, 1700 Fribourg, Switzerland. *E-mail: florence.fontaine@univ-reims.fr*

Botryosphaeria dieback, esca and Eutypa dieback are three significant grapevine trunk diseases (GTDs) involving several xylem-inhabiting fungi. These GTDs represent a threat for viticulture worldwide due to the decreased production of affected plants and their premature death. Botryosphaeria dieback is characterized by a typical wood discoloration called "brown stripe". Herein, a proteome comparison of the brown striped wood from Botryosphaeria dieback-affected standing vines cultivar 'Chardonnay', 'Gewurztraminer' and 'Mourvèdre' was performed. These three cultivars were selected since cv. 'Chardonnay' is less susceptible than cv. 'Gewurztraminer' and cv. 'Mourvèdre' to Botryospheria dieback and esca disease. The transcript analysis for 15 targeted genes and the quantification of both total phenolics and specific stilbenes were also performed. Several pathogenesis-related proteins and members of the antioxidant system were more abundant in the brown striped wood of the three cultivars, whereas other defence-related proteins were less abundant. Additionally, total phenolics and some specific stilbenes were more accumulated in the brown striped wood.

Strongest differences among the cultivars concerned especially proteins of the primary metabolism, which looked to be particularly impaired in the brown striped wood of 'Chardonnay'. Low abundance of some pro-

teins involved in defence response probably contributes to the insufficient response to avoid the symptom development. The differential susceptibility of the three grapevine cultivars could be linked to the diverse expression of various proteins involved in defense response, stress tolerance and metabolism.

Genomics of Grapevine Trunk Diseases. A. MORALES-CRUZ[1], K.C.H. AMRINE[1], D.P. LAWRENCE[2], R. TRAVADON[2], K. BAUMGARTNER[2], P.E. ROLSHAUSEN[3] and D. CANTU[1,*]. [1]*Department of Viticulture and Enology, University of California Davis, USA.* [2]*United States Department of Agriculture, Agricultural Research Service, Crops Pathology and Genetics Research Unit, Davis, CA 95616, USA.* [3]*Department of Botany and Plant Sciences, University of California Riverside, USA. *E-mail: dacantu@ucdavis.edu*

Grapevine trunk pathogens all cause chronic wood infections, but they vary in their wood-decay mechanisms, especially in terms of enzyme and toxin production. Variability in these mechanisms, which contribute to pathogen virulence, may initiate different host-defense strategies, and this has important implications for research on detection, disease management, and grapevine breeding. In 2013, we released draft genome sequences of pathogens associated with three trunk diseases: Esca (*Togninia minima*), Botryosphaeria dieback (*Neofusicoccum parvum*), and Eutypa dieback (*Eutypa lata*). To provide insights in the transcriptional regulation of putative virulence factors, and to enhance our protein-coding gene prediction pipeline, we used genome-wide transcriptional profiling and comparative analysis of candidate virulence factors, for isolates grown *in vitro* and *in planta*. Protein annotation focused on key families of genes involved in: (i) wood degradation (CAZymes, peroxidases, cytochrome P450s, cellular transporters) and (ii) secondary metabolism, namely toxin production (non-ribosomal peptide synthases, polyketide synthases, terpene synthases). Gene family expansions and contractions, due to gene duplications/losses, were then correlated with isolates or growth conditions to identify unique wood-decay mechanisms and secondary metabolism pathways. Esca pathogens were clearly different in their genomic virulence repertoire from *E. lata* and *N. parvum*. The latter two showed a dramatic expansion of the number of genes related to secondary metabolism, in particular polyketide synthases class I, and secreted glycoside hydrolases.

Grapevine leaf stripe disease: a metabolomic approach by GC-MS identifies affected vines before symptom appearance. L. CALAMAI, A. GORETTI, G. SURICO and L. MUGNAI*. *Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DiSPAA), Sez. Patologia vegetale ed Entomologia, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Florence, Italy. *E-mail: laura.mugnai@unifi.it*

Foliar symptom fluctuation from year to year is a very typical characteristic of grapevine leaf stripe disease (GLSD) - probably more than in any other disease. Several still unpredictable factors induce changes in the normal leaf metabolism that lead to the typical interveinal and marginal discolorations and necrosis. The present research approach used derivatization by metabolomic fingerprinting (MSTFA) and gas chromatography mass spectrometry (GC-MS) analysis of either bleeding sap or leaf extracts from vines collected throughout the growth season. Eighty vines symptomatic in the previous 4 years and 80 asymptomatic vines (cv. Sangiovese) were sampled during the growth season. At the end of that season, the leaves were classified as "future symptomatic" or "future asymptomatic" on the basis of symptom appearance. Bleeding sap and leaf extracts were analysed by GC-MS (after derivatization with MSTFA), and the compounds present were detected and compared. From cane bleeding 88 compounds were separated, and 87 from leaf extracts; these were mostly sugars, acid, phenols and polyphenols, alcohols and polyalcohols. All peak areas were normalized over internal standards on the basis of their respective chemical classes. The normalyzed peak areas were subjected to principal component analysis (PCA) for constructing latent variables to be used for linear discriminant analysis (LDA). Cane bleeding content did distinguish vines that were going to become symptomatic whereas a significant discrimination was found with leaf extracts; about 90% of grapevines could be predicted to become symptomatic 2–3 weeks before symptoms appeared on the basis of their metabolic profiles.

Response of five Portuguese grapevine cultivars to infection by *Phaeomoniella chlamydospora*. J. SOFIA[1,*], M.T. GONÇALVES[1] and C. REGO[2]. [1]*Centre for Functional Ecology, Department of Life Sciences, University of Coimbra. Calçada Martim de Freitas, 3000-446. Coimbra, Portugal.* [2]*Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal. *E-mail: jorge.sofia@drapc.mamaot.pt*

Despite Esca and Petri disease being common and destructive diseases of grapevine, little is known about the response of the most common cultivars in the Portuguese Dão wine region to the causal agents of these diseases. In previous works, *Phaeomoniella chlamydospora* has been found to be one of the major causal agents of both diseases in the Dão wine region. In the present study, plants from five common Dão grapevine cultivars, including Tempranillo, Jaen, Touriga nacional, Alfrocheiro, and Encruzado were infected with *Pa.*

chlamydospora. The infection was performed twice (2012 and 2013) in the field on freshly pruned spurs on standing vines with three different *Pa chlamydospora* isolates from different Portuguese origins and a water control. One year after inoculation (2013 and 2014) previously infected spurs were cut off, and analysed for internal lesions. Lesions, when found, were measured and spur's transversal sections from the middle and bottom lesion as well as from wood below the lesion, were analysed and cultured in order to recover *Pa. chlamydospora*. Also, grapes from the canes grown from the infected spurs were collected and analysed for pH of must and alcohol content. Material infected in 2013 is still being analysed, but results obtained so far indicate that all grapevine cultivars are susceptible to the *Pa. chlamydospora* isolates.

Expression of defence related genes and phenotypes of *Vitis vinifera* cell cultures to *Trichoderma atroviride* and *Eutypa lata* culture filtrates. C. MUTAWILA[1], C. STANDER[2], F. HALLEEN[1,3], M.VIVIER[2] and L. MOSTERT[1,*]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa.* [2]*Institute of Wine Biotechnology, Department of Viticulture and Oenology, University of Stellenbosch, 7600, South Africa.* [3] *Plant Protection Division, ARC Infruitec-Nietvoorbij (The Fruit, Vine and Wine Institute of the Agricultural Research Council), Private Bag X5026, Stellenbosch, South Africa 7599. *E-mail: lmost@sun.ac.za*

Cell suspension cultures of *Vitis vinifera* cv. Dauphine were used in a comparative study of the early response of grapevine to a biological control agent, *Trichoderma atroviride*, used in grapevine pruning wound protection, and the vascular pathogen *Eutypa lata*. The expression of genes coding for enzymes of the phenylpropanoid pathway and pathogenesis related (PR) proteins was profiled over a 48-hour period using quantitative reverse transcriptase PCR. The cell cultures responded to elicitors of both fungi with a hypersensitive-like response that lead to a decrease in cell viability. Similar genes were triggered by both the pathogen and biocontrol agent but the patterns and magnitude of expression was dependent on the specific fungal elicitor. Culture filtrates of both fungi caused up-regulation of phenylalanine ammonia-lyase (PAL), 4 coumaroyl Co-A ligase (CCo-A) and stilbene synthase (STS), and a down regulation of chalcone synthase (CHS) genes. The pathogen filtrate caused a biphasic pattern in the up-regulation of PAL and STS genes which was not observed in cells treated with filtrates of the biocontrol agent. Phenotypic assays showed significantly higher total phenolic content and chitinolytic enzyme activity in the cell cultures treated with the *T. atroviride* filtrate than the pathogen filtrate which caused a higher expression of PAL and chitinase class IV genes. The response of the cell cultures to *T. atroviride* filtrate putatively signifies that the induction of grapevine resistance contributes to wound protection by the biocontrol agent.

Disease Management

Developing effective pruning wound protection against eutypa dieback in grapevine. M.R. AYRES[1,*], T.J. WICKS[1], E.S SCOTT[2] and M.R. SOSNOWSKI[1]. [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 Australia. *E-mail: matthew.ayres@sa.gov.au*

Eutypa dieback is a major fungal disease of grapevines worldwide, causing decline and eventual death of vines. Pruning wounds are infected by spores of the fungus *Eutypa lata* and, in Australia, only two products are registered as pruning wound protectants, both of which must be applied by hand. To optimise control of eutypa dieback, appropriate rates of fungicides need to be determined and efficient methods of spray application developed. Vineyard trials were conducted to evaluate a range of fungicides and alternative products for efficacy as pruning wound protectants. The fungicides tebuconazole, pyrimethanil, pyraclostrobin and fluazinam, representing four chemical activity groups, provided up to 88, 77, 71 and 58% disease control, respectively. Garlic juice and lactoferrin provided 52 and 65% disease control, respectively. Detached cane assays were also conducted and confirmed the efficacy of the above fungicides. Commercial spray equipment was assessed for efficacy in applying pruning wound treatments in the field. There was a positive correlation (R^2=0.5) between spray coverage and disease control. The most effective sprayers were those that best targeted the vine cordons. Recycle and purpose-built sprayers achieved up to 93% disease control, equivalent to that achieved by hand-painting of wounds. Most commercial sprayers are designed to spray foliage and therefore require nozzle adjustment to target the pruning wound zone and high water volumes (>600 L/ha) to achieve maximum wound coverage. This research provides new options for grape-growers to manage eutypa dieback and contributes to the long-term sustainability of the Australian wine industry.

Field evaluation of fungicides against Botryosphaeria canker and Phomopsis cane and leaf spot. C. REGO[1,*], P. REIS[1], A. DIAS[2] and R. CORREIA[3]. [1]*Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal.* [2]*Sustinia, INOVISA, Tapada da Ajuda, 1349-017 Lisboa, Portugal.* [3]*Syngenta Crop Protection, Ribera de Loira 8-10 3ª planta, 28042 Madrid, Spain. *E-mail: crego@isa.utl.pt*

A three year trial was conducted in a 15-year-old vineyard of cv. Aragonez in Alentejo, Portugal, to evaluate the effectiveness of selected products (azoxystrobin (Quadris®), copper oxychloride (Cuprocol®), difenoconazole (Score®), tebuconazole (Horizon®), and two mixtures of Cuprocol + acibenzolarSmethyl (Bion®) and Score + acibenzolarSmethyl (Bion®)) against Botryosphaeria canker and Phomopsis cane and leaf spot. Each year, three spray applications were carried out: after pruning, at growth stages C/D (leaf tip visible/ first leaf separated from shoot tip) and after pruning + growth stages C/D. A total of 18 treatments were applied on the grapevines in a completely randomized design. Incidence and severity were evaluated. In the last year of the trial, the number of dead plants, yield and vigour of plants were recorded. The adoption of the yearly practice of protecting pruning wounds and plants by a full crown spray at phenological stage C/D has demonstrated the capacity to reduce both diseases incidence and severity. Treated vines showed consistently low levels of incidence and severity when compared with control plants. One application of Bion + Cuprocol after pruning followed by one application of Bion + Score at phenological stage C/D was the most efficient treatment. Also, the lowest number of dead plants, the highest yield per plant and the highest percentage value for plant vigour were achieved with the same combination of products/spray application timing. As conclusion, this combination of treatments appears to be a good strategy to control Botryosphaeria canker and Phomopsis cane and leaf spot.

Influence of mustard biofumigation on growth, conidial germination and propagule recovery of *Ilyonectria macrodidyma*-complex species. J.E. BARBOUR[1], H.J. RIDGWAY[1] and E.E. JONES[1,*]. [1] *Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln University, Lincoln, New Zealand, 7647. *E-mail: Eirian.Jones@lincoln.ac.nz*

Black foot caused by *Ilyonectria* spp. is a significant economic problem resulting in the decline and death of vines. Biofumigation using mustard has recently shown potential to reduce this disease. *In vitro* sandwich plate assays and a soil box assay were used to compare the effect of biofumigation using standard brown mustard and a recently released cultivar Caliente 199 to suppress *Ilyonectria macrodidyma, I. novozelandica* and *I. torresensis* isolates associated with black foot disease in New Zealand. Both mustards reduced mycelial growth and conidial germination of all isolates, but overall efficacy of the two mustards varied between experiments and is probably related to plant physiology at harvest. In combination with soil, however, mustard efficacy was reduced. Isolates within a species differed in susceptibility to biofumigation. In addition, the relative effect of biofumigation on mycelial growth versus conidial germination varied for isolates, with *I. macrodidyma* Ack1a the most susceptible isolate with regards to conidial germination but least with regards to mycelial growth. Recovery of mesh bags containing mycelial or conidial inoculum of each species after burial in mustard amended or unamended soil in the box bioassay indicated the rapid conversion of inocula into chlamydospores. Amending soil with either mustard cultivar did not change the overall dynamics of propagule conversion over time, however, it significantly affected the numbers of conidia and chlamydospores recovered from conidial inoculum after different incubation times. Mustard biofumigant crops have potential to be incorporated into an integrated strategy for management of black foot in vineyards and nurseries.

Interaction between arbuscular mycorrhizal fungi and rootstock cultivar on the susceptibility to infection by *Ilyonectria* species. E.E. JONES[1,*], S. HAMMOND[1], C. BLOND[1], D.S. BROWN[1] and H.J. RIDGWAY[1]. [1]*Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln University, Lincoln, New Zealand, 7647. *E-mail: Eirian.Jones@lincoln.ac.nz*

Arbuscular mycorrhizal fungi (AMF) have been shown to increase tolerance of grapevine rootstocks to black foot disease caused by *Ilyonectria* spp. The effect of precolonisation of different rootstocks with two AMF species on the susceptibility to *Ilyonectria* spp. was determined. Three rootstocks (3309C, 5C and Schwarzmann) commonly used in New Zealand colonised with either *Acaulospora laevis, Funneliformis mosseae* or untreated were grown in soil and inoculated with a mixture of *Ilyonectria* spp. isolates representing the species diversity recovered from New Zealand grapevines. After 9 months growth, root and shoot dry weight and trunk base infection by *Ilyonectria* spp., and the catabolic function of the rhizosphere microbial community using MicroResp™ was assessed. Both *A. laevis* and *F. mosseae* increased root dry weight, with no effect on shoot dry weight. Grapevine rootstocks varied in susceptibility with 5C being most susceptible. AMF species altered rootstock susceptibility, with *A. laevis* inoculation of 5C decreasing disease severity and *F. mosseae* having no effect on disease severity of this rootstock. However, *Funneliformis mosseae* inoculation of Schwarzmann decreased disease compared with *A. laevis*. In the absence of the pathogen, the catabolic function of the microbial community in the rhizosphere of 3309C and Schwarzmann differed significantly from that of 5C, but this difference was not apparent following *Ilyonectria* spp. inoculation. AMF inoculation had no effect on the carbon utilisation profile of the rhizosphere microbial community. The results suggested a direct effect of AMF inoculation on rootstock susceptibility rather

than changes in the function of the rhizosphere microbial community.

Control of leaf stripe disease leaf symptoms by specific formulations for foliar nutrition. F. CALZARANO[1,*], V. D'AGOSTINO[1], L. MUGNAI[2], S. SCHIFF[2] and S. DI MARCO[3]. [1]*Facoltà di Bioscienze e Tecnologie Agro-Alimentari e Ambientali, Università degli Studi di Teramo , Via C.R. Lerici, 1, 64023 Mosciano S.A. (TE), Italy.* [2]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DiSPAA), Sez. Patologia vegetale ed Entomologia, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Florence, Italy.* [3]*Istituto di Biometeorologia (IBIMET), CNR, Via Gobetti 101, 40129 Bologna, Italy. *E-mail: fcalzarano@unite.it*

Possible approaches for the control of esca complex diseases including both reduction of wood infections and reduction of foliar symptoms development associated with infections. On this second approach there is no viable solution available at present. Preliminary observations on the influence of grapevine nutrition on the disease, suggested the application of foliar fertilizers based on Ca chloride, Mg nitrate and *Fucales* seaweeds on grapevine leaf stripe disease (GLSD) symptoms. During 2010–2012 growth seasons, 9 treatments were applied in 3 vineyards in the Teramo province. The applications had a 10 day interval from the start of growth to pre- bunch closure. The protocol was planned in order to interfere with the activity of virulence factors and of the plant defence response, at the base of the typical GLSD leaf chlorosis and necrosis. Mixed and single component applications were tested in one cv. Trebbiano D'Abruzzo, and in two cv. Montepulciano d'Abruzzo vineyards. The results were always consistent and showed the highest symptoms reduction with the full components mixture, while lower effects could be obtained with partial mixtures or single components. Quality and quantity of grape production were also evaluated, showing a significant increase in the treated vineyard portion. No phytotoxic effect was ever recorded. Higher *trans*-resveratrol, flavonoids and druse crystals present in the treated and asymptomatic leaves suggest that the treatment enhances the defence response of the vines in general. The positive results obtained can be a promising and suitable base to assess the mechanisms involved in foliar symptoms development.

An economic case for early adoption of preventative practices for management of grapevine trunk diseases. J.D. KAPLAN[1,*], R. TRAVADON[2], M. COOPER[3], V.HILLIS[4], M. LUBELL[4] and K. BAUMGARTNER[2]. [1]*Department of Economics, Sacramento State University, 6000 J Street, Sacramento CA 95819-6082, USA.* [2]*United States Department of Agriculture, Agricultural Research Service, Crops Pathology and Genetics Research Unit, Davis, CA 95616, USA.* [3]*University of California Cooperative Extension, Napa County, 1710 Soscol Avenue, Suite 4, Napa, CA 94559-1315, USA.* [4]*Department of Environmental Science and Policy, University of California Davis, One Shields Avenue, Davis CA 95616. USA. *E-mail:kaplanj@csus.edu*

The trunk diseases Botryosphaeria dieback, Esca, Eutypa dieback, and Phomopsis dieback, significantly decrease yields and vineyard longevity in California. Despite high disease prevalence and substantial yield impacts, most growers routinely wait to adopt preventative practices until vineyards are 8+ years-old and have a 10–40% disease incidence. Grower hesitation may arise in part because the economic benefits from early adoption are difficult to predict. Published experimental trials have demonstrated disease-control efficacy ranging from 28–100%, depending on the disease, of three preventative practices (in order of increasing cost): delayed pruning, 'hand-painting' thiophanate-methyl (TopsinM) onto pruning wounds, and double pruning. We estimated the benefits of adopting these practices in five California regions for a vineyard with a 25-year lifespan, using economic simulations for winegrape production under varying disease-control efficacy levels (25%, 50%, 75%) and vineyard ages (3, 5, 10 years-old). In Northern San Joaquin, Northern California, and Central Coast regions, taking no action results in negative overall returns. In these same three regions, which have relatively low winegrape prices, even lower overall returns are predicted when waiting until year 10 to adopt double pruning (the most expensive practice), and assuming only 25% disease-control efficacy. In contrast, adopting practices in years 3 or 5, and assuming 50–75% disease-control efficacy, translates into positive overall returns in all regions. Further, adopting these practices in years 3, and assuming 75% disease-control efficacy, corresponds to 13 more years of positive returns (>50% increase), thereby increasing profitability to nearly 99% of those from a healthy vineyard.

Effect of the length of the pruned internode on the colonization of grapevine canes by *Diplodia seriata* and *Phaeomoniella chlamydospora*. G. ELENA[1,*] and J. LUQUE[1]. [1]*IRTA Cabrils, Ctra. de Cabrils km 2, E-08348 Cabrils, Spain. *E-mail: georgina.elena@irta.cat*

The objective of this study was to determine the effect of the length of the pruned internode on fungal survival and colonization of canes by the pathogens *Diplodia seriata* and *Phaeomoniella chlamydospora*. In winter (January), 240 canes were pruned in a 'Chardonnay' vineyard leaving two different lengths between the top node and the pruning wound: 2 and 5 cm. Pruning wounds (n=20 per treatment) were either inoculated separately with a conidial suspension of the pathogens

or left untreated. During spring and autumn seasons after inoculations, fungi were recovered from the canes at different sites above and below the top node. Reisolation percentages for each length of internode, site of recovery, season and pathogen were determined. The experiment was repeated twice. In spring, low recovery percentages of *D. seriata* were obtained below the node in 2 cm canes (5%) whereas no fungal recovery was recorded below the node in 5 cm canes. Figures for *P. chlamydospora* recovery were 17% below the node in 2 cm canes and 7% in 5 cm canes. In autumn, recovery of *D. seriata* and *P. chlamydospora* below the node in 2 cm canes was over 35% for both pathogens. In 5 cm canes, the recovery below the top node was lower than 15% for both pathogens in autumn. These results indicated that fungi required more time to colonize the pruned cane when the internode was longer, and this would suggest that leaving a long internode when pruning could make difficult the posterior pathogenic colonization of canes.

Optimizing techniques for evaluating *Eutypa lata* infection in grapevines. G. ELENA[1,*], C. BENETREAU[2], P. LECOMTE[2], M.R. AYRES[3], M.R. SOSNOWSKI[3] and J. LUQUE[1]. *[1]IRTA Cabrils, Ctra. de Cabrils km 2, E-08348 Cabrils, Spain. [2]INRA, University of Bordeaux, Institute of Vine and Wine Sciences, UMR SAVE, CS 20032, 33882 Villenave d'Ornon cedex, France. [3]South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia. *E-mail: georgina.elena@irta.cat*

This study aimed to optimize inoculation, sterilization and incubation procedures for investigating *Eutypa lata* infection of grapevine wounds. Two experiments were conducted to determine the minimum inoculum potential: 1) in a 'Shiraz' vineyard in Australia, and 2) in a greenhouse with 'Cabernet Sauvignon' detached canes in France. In both experiments freshly cut pruning wounds were inoculated with 10, 50, 100, 200, 500 or 1000 ascospores. In the field assay, additional double inoculations of pruning wounds on different days were included: 2×100, 2×250 and 2×500 ascospores. In the field assay, canes were harvested 7 or 11 months after inoculation and in the detached cane assay, two weeks later. In another experiment, three incubation periods were tested for detached canes: two, four and six weeks after inoculation. Four different surface-sterilization techniques were compared for isolating *E. lata* from detached canes: 1) 70% ethanol for 4 min, 2) 2.5% sodium hypochlorite (SH) for 10 min, 3) 70% ethanol with flaming, and 4) same as 3) but with a bark surface disinfection with 0.5% SH for 20 min before inoculation. Infection incidence was determined by isolation onto potato dextrose agar and malt extract agar. Optimal infection (75%) was achieved in field vines inoculated with 200 ascospores per wound and in detached canes with 500 ascospores. Incidence of infection did not differ between harvest dates in the field but was optimal after 4 weeks incubation in detached canes. The most effective sterilization technique was by SH 0.5% for 20 min and flaming with ethanol.

A detached cane assay is an effective tool for evaluating pruning wound infection. M.R. AYRES[1,*], D.C. MUNDY[2], T.J. WICKS[1], E.S SCOTT[3] and M.R. SOSNOWSKI[1]. *[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. [3]School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond SA 5064 Australia. *E-mail: matthew.ayres@sa.gov.au*

Eutypa and botryosphaeria dieback, caused by species of the Diatrypaceae and Botryosphaeriaceae, are major diseases of grapevines, infecting pruning wounds, causing dieback and eventual death of vines. A detached cane assay (DCA) has been adapted to evaluate pruning wound infection. Single node cuttings of Shiraz and Sauvignon Blanc grapevines were placed in polystyrene boards on water. Wounds were inoculated with varying spore doses of trunk pathogens and cuttings removed at different times, sectioned and placed on potato dextrose agar (PDA). In the first experiment, wounds were inoculated with 500–1000 *Eutypa lata* spores, cuttings removed 4–12 weeks later and colonization distance measured. The distance varied between 4 and 9 mm with no significant difference between doses or incubation times. In the second experiment, wounds were inoculated with 10–500 spores of *E. lata* and cuttings removed 4 weeks later. *E. lata* recovery ranged from 20–80% from lowest to highest dose. In the third experiment, *Neofusicoccum luteum, N. parvum* and *E. lata* were each applied to wounds either following treatment with tebuconazole fungicide or not. Each species was recovered from 15–85% of cuttings and fungicide significantly reduced infection by *N. parvum*. In the final experiment, wounds were inoculated with 1000 spores of *Diplodia seriata* or *N. australe* and 4 weeks later, each species was recovered with a distinct yellow pigment produced only by *N. australe* following 3-5 days incubation on PDA. The DCA is currently being used to evaluate pruning wound susceptibility, wound treatments and potential resistance of grapevine to trunk diseases.

Sustainable control of grapevine trunk diseases (COST Action FA1303). F. FONTAINE[1,*] and J. ARMENGOL[2]. *[1]Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire Stress, Défenses et Reproduction des Plantes, BP 1039, 51687 Reims Cedex 2, France.*

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Grapevine trunk diseases (GTDs) are among the most destructive diseases of vineyards worldwide. Fungicides with the potential to control GTDs have been banned and there are no highly effective treatments available. Developing sustainable alternatives to manage GTDs is therefore required. Currently, there is not a coordinated research approach in Europe, even though a strong demand for innovative disease management strategies is given. The goal of this COST Action FA1303 would be to develop a network of European expertise to improve understanding of GTDs by acquiring knowledge on occurrence of pathogens, vine-pathogen interaction, ecology of wood-inhabiting microorganisms, and to develop new management protocols and biocontrol approaches. This COST Action gathers leading multidisciplinary academic researchers and institutes within Europe to propose new recommendations for the management of GTDs and establish Europe as a world leader in GTD research to safeguard vineyards. This knowledge will be promoted in an effort to increase knowledge and awareness of the problem by disseminating information to end-users and authorities in the viticulture sector, and to the general public. Presently, experts from nearly 50 institutions and companies in 20 European (AT, BG, CH, CZ, DE, GR, ES, FR, HR, HU, IL, IT, MT, NL, PL, PT, RO, SE, SI, UK) and near neighbour (Algeria) countries have already responded to join this Action that leads on the period 2013–2017 (http://managtd.eu/).

Examination of different *Trichoderma* isolates as potential biopesticide in laboratory and field experiments. CS. KOVÁCS[1], E. SÁNDOR[1], P. BALLING[2], Z. BIHARI[2] and F. PELES[1]
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The aims of the laboratory experiments were to examine the mycelial growth of 11 *Trichoderma* sp. isolates (TR01-TR11) obtained from asymptomatic plants in the Tokaj wine region, Hungary, and to test their mycoparasitic ability against the pathogen *Diplodia seriata*. The pathogen isolate was obtained from a trunk of a diseased vine in the same vineyard. Mycelial growth rates of the *Trichoderma* isolates were determined at different temperatures (18.5°C, 22.5°C, 25°C, 30°C and 37°C). They showed different mycelial growth rates within the studied temperature range. Some of them (TR01-TR04) grew slower at lower temperatures whereas others (*Trichoderma harzianum* group, TR07-TR10) showed lower rates at highest temperatures. The TR05 and TR06 isolates (*Trichoderma longibrachiatum* group) showed the highest growth rates among all the isolates within the full temperature range, and their growth rates were especially high at 30 and 37°C. All *Trichoderma* isolates overgrew the *Diplodia seriata* mycelium, thus the Biocontrol Index was established at 100% for all *Trichoderma* isolates. The aim of the field experiment was to examine the effect of 10^7 conidia ml^{-1} *Trichoderma* spore suspension of mixed TR04 and TR05 on the pruning wounds on canes. *Diplodia seriata* had earlier been detected from all tested trunks. Three months later only *Trichoderma* species could be isolated from four grapevines, and the symptoms reduced on all but one of the ten tested trunks.

Calculating the economic cost of trunk disease spread. D.C. MUNDY[1,*], V. RAW[1] and A.R.G. McLACHLAN[2]. [1]*The New Zealand Institute for Plant & Food Research Limited, Marlborough Wine Research Centre, PO Box 845, Blenheim 7240, New Zealand.* [2]*The New Zealand Institute for Plant & Food Research Limited, Private Bag 11600, Palmerston North, New Zealand. *E-mail: dion.mundy@plantandfood.co.nz*

Under New Zealand conditions eutypa and botryosphaeria dieback, caused by species of Diatrypaceae and Botryosphaeriaceae, respectively, are major fungal diseases of grapevines. The main point of entry is through infected pruning wounds, causing cane dieback and eventual vine death. Observations of vine death and foliar symptoms from 2005 to 2010 for a mature block of Sauvignon blanc grapes provided information to produce an economic calculator of potential decline in production. Infection of the cane-pruned vines in this block probably occurred because of large mechanical pruning wounds to vines before the start of monitoring. The block has no history of active management such as using pruning wound pastes, so infections may have occurred before or after the large mechanical cuts were made and represents a worst case scenario. The observed rate of vine death was integrated into an interactive spreadsheet to allow growers to estimate the potential cost of replacing or not replacing missing vines in a block projecting forward for a 10-year period. Further development is underway to use the data to enhance the prediction of the spread of symptoms in blocks which are expressing trunk diseases.

Trunk pathogens and wood-colonizing fungi in minimally-pruned versus cordon-pruned vines in southern France. R. TRAVADON[1], P. LECOMTE[2], B.

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The main infection courts for grapevine trunk pathogens are thought to be pruning wounds. Indeed, control practices typically involve modifications to pruning. As such, we expect pruning practices that require fewer/smaller wounds to be associated with fewer pruning-wound infections. In turn, minimal pruning may lead to less wood necroses and a lower diversity of trunk pathogens. To test this hypothesis, we examined wood-colonizing fungi in the trunks of 14-year-old vines (cultivars Mourvèdre and Syrah) that were either cordon pruned or minimally pruned, at an experimental station in southern France (INRA Pech-Rouge). For each cultivar, the trunks of eight minimally-pruned and eight cordon-pruned vines were characterized in terms of proportions of necrotic wood (in longitudinal section) and communities of cultivable fungi (from 64 wood pieces per vine). Cordon-pruned vines had significantly higher proportions of necrotic wood (35%) than minimally-pruned vines (20%). From all 32 vines, 90 fungal taxa were identified following ITS-DNA sequencing of representative isolates, including 29 taxa of known and putative trunk pathogens. Among the 32 vines, the most prevalent trunk pathogens were *Diplodia seriata* (81% of vines), *Phaemoniella chlamydospora* (66%), *Togninia minima* (47%), *Neofusicoccum parvum* (34%), *Diaporthe neotheicola* (25%), *Phomopsis viticola* (19%), *N. australe* (13%), *Eutypa lata* (9%) and *N. luteum* (9%). There were no significant differences in their recovery between cordon-pruned and minimally-pruned vines, suggesting that either infections occurred before minimal pruning was adopted (year 4), or that natural wounds (e.g., crevices in the periderm), in addition to pruning wounds, are alternative infection courts for these pathogens.

Bacteria colonizing Esca-BDA diseased grapevines in the North of Tunisia. A. REZGUI[1,2,3], A. BEN GHNAYA-CHAKROUN[1,2,3], J. VALLANCE[2,3], E. BRUEZ [2,3], M. DRIDI[4], M.R. HAJLAOUI[4], P. REY[2,3,*] and N. SADFI-ZOUAOUI[1]. [1]*Laboratoire des Microorganismes et Biomolécules Actives, Département de Biologie, Faculté des Sciences de Tunis. Campus Universitaire, 2092 Tunis, Tunisie.* [2]*Université de Bordeaux, Bordeaux Sciences Agro, ISVV, UMR1065 SAVE, F-33140 Villenave d'Ornon, France.* [3]*INRA, UMR1065 Santé et Agroécologie du Vignoble (SAVE), ISVV, F-33140 Villenave d'Ornon, France.* [4]*Laboratoire de Protection des végétaux, Institut National de la recherche Agronomique de Tunisie (INRAT) 2049, Ariana Tunisie. *E-mail: prey@bordeaux.inra.fr*

Grapevine trunk diseases (GTDs) such as Esca and Black Dead Arm (BDA) are of major concern for viticulture worldwide. In Tunisia, little is known about these diseases but their incidence is increasing, and no effective treatment currently exists. Our study aimed at comparing the bacterial microflora that colonized the wood tissues of Esca-leaf symptomatic and asymptomatic vines sampled in two regions of Tunisia, *i.e.* Mornag and Bordj el Amri, using microbiological and molecular approaches. Fingerprinting analyses (Single Strand Conformation Polymorphism) showed that specific complex microflora colonize the wood of both the necrotic and apparently healthy wood tissues of the sampled vines, with no differences between the two regions. Nineteen bacterial strains were isolated from necrotic and non-necrotic tissues. Biochemical, physiological and molecular criteria were used to characterized those strains. Sequencing of the 16S rRNA gene led to the identification of four genera: *Bacillus*, *Pantoea*, *Pseudomonas* and *Erwinia*. Community-level physiological profiling (CLPP) showed that the bacteria metabolized the carbon sources differently. The strains were also screened for antibiotics genes (4 fengycines A, B, D and E; 1 bacillicine), phosphates solubilization, pathogenicity on tobacco leaves and *in vitro* confrontations against 4 fungal pathogens (*Neofusicoccum parvum*, *Phaeomoniella chlamydospora*, *Lasiodiplodia pseudotheobromae* and *Schizophyllum commune*). The most interesting strain, a *Bacillus* spp., was selected from those screening tests and it is currently under evaluation in a greenhouse assay in order to protect vines inoculated by *N. parvum* and *P. chlamydospora*.

Greenhouse screening for efficacy of various bacterial strains to control *Neofusicoccum parvum* in grapevine plantlets. R. HAIDAR[1], A. DESCHAMPS[1], J. ROUDET[1], P. REY[1,2,*] and M. FERMAUD[1]. [1]*INRA, UMR1065 Santé et Agroécologie du Vignoble (SAVE), ISVV, CS 20032, 33882 Villenave d'Ornon, France.* [2]*Bordeaux Sciences Agro, Université de Bordeaux, ISVV, UMR1065 SAVE, 33140 Villenave d'Ornon, France. *E-mail: prey@bordeaux.inra.fr*

In France, it has been estimated that Grapevine Trunk Diseases (GTDs) are responsible for *ca* 13% of unproductive vineyards. Wood cankers are among the most frequent GTDs. No appropriate and efficient treatment is currently available against their fungal causal agents. Therefore, detection and use of antagonistic microorganisms as efficient biological control tools to prevent wood infections would be an important alternative practice. Our aim was to carry out a screening *in planta* of 26 bacterial strains for antagonism against

Neofusicoccum parvum. All tested bacterial strains were isolated from grapevine in Bordeaux vineyards (either from grape berry surface or wood tissues) in order to test their antagonism ability in the grapevine wood environment. Under greenhouse conditions, the woody stems of 672 rooted cuttings (Cabernet sauvignon and Ugni blanc) were wounded and co-inoculated with each bacterial strain and one virulent strain of the pathogen (control cuttings were inoculated by the pathogen only). After an approximately three-month incubation period, disease severity was measured visually considering i) the frequency or incidence of external cankers and ii) total internal necrotic lesion length in absolute (mm) and relative (%) values. According to internal lesion length, we detected two bacterial strains of interest belonging to the same undetermined *Pantoea* species. They showed significant antagonistic ability compared with the controls. As further studies, these strains will be investigated to better understand the bacteria-fungi relationships *in planta* with the ultimate goal of an effective biocontrol strategy against one of the major fungal agents of wood diseases in vineyards.

Improved application of *Trichoderma* biocontrol agents. C. MUTAWILA[1], F. HALLEEN[1,2] and L. MOSTERT[1,*]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa.* [2]*Plant Protection Division, ARC Infruitec-Nietvoorbij (The Fruit, Vine and Wine Institute of the Agricultural Research Council), Private Bag X5026, Stellenbosch, South Africa 7599. *E-mail: lmost@sun.ac.za*

The protection of grapevine pruning wounds is essential to prevent infection by aerial inoculum of grapevine trunk disease pathogens. To improve the efficacy of *Trichoderma* biocontrol agents the time of application and the method of application was investigated. The effect of pruning time (early or late) and five timings of application of the biocontrol agent after pruning on pruning wound colonisation by *T. atroviride* and *T. harzianum* were determined. Chenin blanc and Cabernet Sauvignon vineyards were pruned in July (early) and August (late) of 2011 and 2012, and pruning wounds were treated with suspensions of the *Trichoderma* spp. at 0, 6, 24, 48 and 96 hours after pruning. Wound colonisation was depended on the physiological state of the vine at pruning for both cultivars. However, for the 2012 season in Chenin blanc, wound colonisation was similarly high for both pruning times, which was attributed to high rainfall and humidity. Application of the biocontrol agents 6 hours after pruning consistently resulted in high wound colonisation by the *Trichoderma* spp. in both cultivars and pruning times. In both cultivars, pruning wound infection due to natural inoculum was higher in wounds made in late winter than those made earlier. Three different application methods were compared. Two Cabernet Sauvignon vineyards were treated with *T. atroviride* 6 hours after pruning with a paint brush, backpack sprayer or gator (quad bike). The backpack sprayer and painted wounds did not differ significantly and both had significantly higher *T. atroviride* incidences than the gator sprayer.

Preliminary findings on the grapevine yield response to Brassica biofumigation soil treatments. M. WHITELAW-WECKERT[1,*], L. RAHMAN[1], J. CAPPELLO[1] and K. BARTROP[2]. [1]*New South Wales Department of Primary Industries, National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW, 2678, Australia .*[2]*Riverina Wine grapes Marketing Board, 182 Yambil Street, PO Box 385, Griffith NSW 2680. *E-mail: mweckert@csu.edu.au*.

Young vine decline in the Riverina has been associated with *Ilyonectria* spp. (grapevine black-foot disease agents) and Botryosphaeriaceae infections of rootstock cuttings and contaminated nursery soil. *Ilyonectria* soil inoculum builds up with repeated planting. As a recent New Zealand study showed that incorporation into soil of Brassica biofumigation products may reduce this inoculum, we initiated a trial with five replicates of eight under-vine treatments: 1) control; 2) 2t/ha mustard seed meal; 3) 4t/ha mustard seed meal; 4) 4t/ha 'deactivated' mustard seed meal (with negligible glucosinolates); 5) Benomyl fungicide (positive control); 6) inter-row BQ mustard plants slashed, side thrown and buried at flowering; 7) 2t/ha canola seed meal; and 8) 4t/ha rate canola seed meal. The grapevines were 6-year-old Pinot Noir/Ramsey infected with *Ilyonectria* spp. and Botryosphaeriaceae. After one season, three treatments significantly increased the number of bunches produced by diseased grapevines: fungicide (+36%); 4t/ha deactivated mustard seed (+32%); and 4t/ha active mustard seed meal (+26%). Two treatments increased the total yield from diseased grapevine: deactivated and active mustard seed meals (+51% and +61% respectively). We could thus conclude that 4t/ha mustard seed meal, with and without glucosinolates, significantly improved the growth and yield parameters when buried under diseased grapevines. The beneficial effects of deactivated seed meal may be explained by increased rhizosphere beneficial microbial populations but further investigation is required to test this hypothesis. In season two we will replace BQ mustard with Caliente 199 mustard and investigate the efficacy of *Trichoderma* and *Streptomyces* biological control products in soil applications.

Remedial surgery for the management of Botryosphaeria dieback in grapevines. S. SAVOCCHIA[1,*], M. AYRES[2], R. BILLONES-BAAIJENS[1] and M.R. SOSNOWSKI[2]. [1]*National Wine and Grape Industry Centre, Charles*

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Remedial surgery has been demonstrated to control Eutypa dieback in grapevines. In order to ascertain if remedial surgery could also be applied to control Botryosphaeria dieback, field trials were established in two vineyards (cv. Semillon) in the Hunter Valley, New South Wales, Australia. Prior to remedial surgery in October 2013, the severity of dieback was assessed for each grapevine and found to vary from 20–90% and 10–95% for Vineyard 1 (24 year-old grafted vines) and Vineyard 2 (20 year-old own-rooted vines), respectively. Following assessment, 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 was assessed for necrotic staining. The severity of internal staining (wedge and/or central necrosis) varied from 4% (low cut) to 25% (high cut). The incidence of internal staining ranged from 55% (low cut) to 98% (high cut) in Vineyard 1 and 73% (low cut) to 94% (high cut) in Vineyard 2. Botryosphaeriaceae were isolated from all symptomatic wood samples. In Vineyard 1, 15% (low cut) to 64% (high cut) of grapevines produced water-shoots from the scion. In contrast, 46% of grapevines (low cut) and 15% (high cut) produced water-shoots from the rootstock. In Vineyard 2, water-shoot production was 100% (low cut) and 99% (high cut). These trials have demonstrated that Botryosphaeria dieback may be controlled via remedial surgery and grapevines will continue to be monitored for disease recurrence.

Evaluation of Gelseal for control of eutypa dieback. M.R. SOSNOWSKI[1,*], D.C. MUNDY[2] and T. BELAYNEH[3]. [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.* [3]*Omnia Specialties Australia, PO Box 3418 Morwell Vic 3840, Australia. *E-mail: mark.sosnowski@sa.gov.au*

Eutypa dieback (*Eutypa lata*) causes decline and eventual death of grapevines worldwide. Pruning wounds are infected by airborne spores and efficient control can be achieved by applying wound protectants. Gelseal (430 g L^{-1} tebuconazole) and Gelseal Ultra (430 g/L tebuconazole + 15 g L^{-1} boric acid + 17.5 g L^{-1} Octhilinone) were evaluated for control of eutypa dieback. They were applied until runoff with a backpack sprayer at rates between 15 and 60 mL/100 L to freshly pruned wounds of 1-year-old canes of Cabernet Sauvignon in South Australia and Sauvignon Blanc in Marlborough, New Zealand during August 2013. Wounds were inoculated with approximately 500 spores of *E. lata*. Inoculated and uninoculated controls were included. Treated canes were harvested from vines in June 2014 and returned to the laboratory for assessment by isolation onto potato dextrose agar. *E. lata* was recovered from 44 and 35% of the untreated control canes and 4 and 3% of naturally infected canes in Australia and New Zealand, respectively. In Australia, Gelseal significantly reduced the recovery of *E. lata* to between 21 and 4% when applied at 15-60 mL/100 L, providing 51–83% disease control. In comparison, Folicur (430 g L^{-1} tebuconazole) applied to wounds at 30 mL/100 L provided 79% control. In New Zealand, Gelseal Ultra significantly reduced recovery to between 9 and 2% when applied at 30–60 mL/100 L, providing 80–94% disease control. In comparison, Folicur applied at 60 mL/100 L and Greenseal (10 g L^{-1} tebuconazole) painted on wounds provided 80 and 88% disease control, respectively.

New wound infections are really relevant for grapevine leaf stripe disease? The case of *Trichoderma* pruning wound protection. S. DI MARCO[1], F. REGGIORI[2], M. BALEANI[2], M. BENANCHI[2], D. BOSSIO[2], F. OSTI[3] and L. MUGNAI[3,*]. [1]*CNR, IBIMET, Via Gobetti 101, 40129 Bologna, Italy.* [2]*Isagro, via Caldera 21, 20153 Milano, Italy.* [3]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DiSPAA), Sez. Patologia vegetale ed Entomologia, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Florence, Italy. *E-mail: laura.mugnai@unifi.it*

The use of *Trichoderma* products to protect pruning wounds from wood pathogens, has been proposed since many years, first of all in New Zealand. Variable results were obtained with different formulations, in different countries and towards different wood pathogens. In Italy, as in all of Europe, the main grapevine wood disease is certainly the esca complex and grapevine leaf stripe disease in particular. Artificial inoculation trials were carried out during three winters, with a commercial formulation including two strains, *Trichoderma gamsii* and *T. asperellum*, that showed an enhanced ability to colonize wound surface at different temperatures. The commercial formulation showed a variable ability (from 100% protection down to just a reduction in the amount of wood colonised) in avoiding infections by *Phaeomoniella chlamydospora*. The product was also applied in 19 commercial vineyards in 5 different regions and with different cultivars in Italy. Application was carried out with the farm sprayers in March, when temperatures were more suitable for a positive colonization, independently of the pruning time. Treated and untreated

vineyard portions were monitored from three to four years, and leaf stripe disease and apoplexy were monitored at the end of the season. A significant reduction in foliar symptoms expression was obtained usually at the second-third year of application. These results strongly support the idea that, independently from the internal trunk and cordon colonization, the new infections, close to the new bud, have a strong link with foliar symptoms development.

Nursery Propagation

Distribution of Botryosphaeriaceae species and genotypes within a rootstock mother vine indicates multiple inoculum sources. R. BILLONES-BAAIJENS[1,2,*], H.J. RIDGWAY[1], E.E. JONES[1] and M.V. JASPERS[1]. [1]*Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln University, Lincoln, New Zealand, 7647.* [2]*National Wine and Grape Industry Centre (NWGIC), Charles Sturt University, Mckeown Drive, Wagga Wagga, NSW 2678 Australia. *E-mail: rbaaijens@csu.edu.au*

Infected rootstock and scion cuttings by Botryosphaeriaceae fungi have been reported as major sources of infection for young grapevines. To investigate the potential infection pathways of Botryosphaeriaceae species within a rootstock mother vine, a universally-primed polymerase chain reaction (UP-PCR) was conducted. This method differentiated the genotypes within two *Neofusicoccum* species, namely *N. luteum* and *N. parvum*. The first study showed that multiple Botryosphaeriaceae species and genotypes can infect a single mother vine. It further showed that the trunk and shoot isolates of the same species from the same vine were of the same or different genotypes, suggesting multiple infections from different inoculum sources. The second study that investigated the spatial distribution of Botryosphaeriaceae fungi within an entire dormant cane also showed that multiple species and genotypes were distributed along the cane, but most isolates were sited within the bark and less frequently in the wood, suggesting they were latent in surface tissues. Some adjacent wood and bark infections were caused by the same genotypes also suggesting that wood infection may have originated from the bark. The third study further showed that the *Neofusicoccum* isolates recovered by washing the cuttings were of the same or different genotypes from those isolated from adjacent internal tissues, again suggesting multiple sources of external inoculum. These fungi appear to cause latent infections in the bark of dormant cuttings which are used in plant propagation, thus providing an additional infection pathway for a disease that is known to show obvious symptoms only in older vineyards.

Management of *Botryosphaeria* species infection in grapevine propagation materials. R. BILLONES-BAAIJENS[1,2,*], A. ALLARD[1,3], Y. HONG[1,4], E.E. JONES[1], H.J. RIDGWAY[1] and M.V. JASPERS[1]. [1]*Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln, New Zealand.* [2]*National Wine and Grape Industry Centre (NWGIC), Charles Sturt University, Mckeown Drive, Wagga Wagga, Australia.* [3]*Institut National de la Recherche Agronomique UMR 1334, Montpellier, France.* [4]*Department of Conservation Biology, Southwest Forestry University, Kunming City, China. *E-mail: rbaaijens@csu.edu.au*

In New Zealand grapevine propagation nurseries, *Botryosphaeria* spp. were isolated from 23% of grapevine plant materials. The pathogens were widespread in mother vine blocks and infections were often present in the bark, not wood, of cuttings. Studies into control methods began with attempts to wash the *Botryosphaeria* conidia from cuttings, however they were unsuccessful as conidia adhered rapidly (within 5 min) to canes. Hot water treatment (HWT) of rootstock 5C cuttings, previously infected with *Neofusicoccum parvum* and *N. luteum*, at 50°C for 30 min resulted in internal infection incidences of 50% and 100%, respectively. HWT at 53°C for both 30 and 60 min reduced infection incidence for *N. luteum* and *N. parvum* to 0 and 8.5%, respectively, but killed the buds. In naturally infected cuttings, 50°C for 30 min reduced infection incidence from 35% in controls, to 0–15% over all *Botryosphaeria* spp. Shorter periods of HWT, at 55°C for 5 min, designed to kill bark infections, were ineffective. To determine fungicide efficacy for eliminating bark infection, Sauvignon blanc cuttings superficially infected with *N. luteum* were soaked for 30 min in carbendazim, tebuconazole, thiophanate methyl or flusilazole, with and without an organosilicone adjuvant. Results showed that carbendazim with no adjuvant and tebuconazole with 0.5 mL L^{-1} adjuvant eliminated 100% of bark infections. A further experiment that soaked 2,000 cuttings (Sauvignon blanc and Pinot noir) in a carbendazim solution prior to rooting found that all cuttings were free of *Botryosphaeria* spp. infection, compared to 13% natural incidence.

Survival of Botryosphaeriaceae species after hot water treatment. J. LUQUE[1,*], G. ELENA[1], V. DI BELLA[2] and J. ARMENGOL[3]. [1]*IRTA Cabrils, Ctra. de Cabrils km 2, 08348 Cabrils, Spain.* [2]*Università degli Studi di Palermo, Viale delle Scienze, Edificio 4 Ingr. B, 90128 Palermo, Italy.* [3]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia (IAM-UPV), Camino de Vera s/n, 46022 Valencia, Spain. *E-mail: jordi.luque@irta.cat*

The use of hot water treatment (HWT) in the grapevine propagation process has been shown to be a potentially effective tool to control Petri and 'Black Foot'

fungal pathogens. However, effects of HWT on Botryosphaeriaceae species had not been previously studied. Therefore, our objective was to evaluate the survival of eight different species of Botryosphaeriaceae after HWT in two different experiments. First, mycelial plugs contained in Eppendorf tubes with sterile distilled water were subjected to different combinations of temperature (50–54°C) and exposure time (15, 30 and 45 minutes) in a hot water bath. In a second trial, the fungi were inoculated into Richter 110 rootstock canes previously subjected to HWT. Inoculated canes were incubated at 25°C for three weeks to allow for fungal wood colonization and then were subjected to HWT in the range 50–53°C for 30 minutes. Survival of fungi after HWT was assessed in both trials. In addition, growth rates of treated mycelia were compared to untreated controls. Significant differences in survival and growth for all factors (species, temperature and time) and their interactions were observed in the *in vitro* assay. *Diplodia seriata*, *Dothiorella viticola*, *Neofusicoccum luteum* and *N. parvum* were the most susceptible species to temperature while *Lasiodiplodia* sp. and *N. vitifusiforme* were the most tolerant. In the *in planta* experiment, all species sharply reduced their survival after 30 minutes at 51°C. At 50°C, *Lasiodiplodia* sp. was the most tolerant taxon whereas *N. luteum* was the most susceptible. These results demonstrate the feasibility of controlling these pathogens by HWT.

Protection of *Vitis vinifera* L. pruning wounds from *Phaeoacremonium aleophilum* using ozonated water: A solution for integrated esca-associated fungi management in grapevine nurseries? R.J.G. PIERRON[1,2], M. PAGES[3], C. COUDERC[1,3], S. COMPANT[4], F. VIOLLEAU[3] and A. JACQUES[1,*]. [1]*Université de Toulouse, Institut National Polytechnique de Toulouse, Ecole d'Ingénieurs de Purpan, Département des Sciences Agronomiques et Agroalimentaires, Equipe Vins Viticulture et Œnologie, 75 voie du TOEC, BP 57611, F-31076 Toulouse Cedex 03, France.* [2]*Université de Toulouse, LGC UMR 5503 (CNRS/UPS/INPT), Dept BIOSYM, INP-ENSAT, 1 avenue de l'Agrobiopole, 31326 Castanet-Tolosan, France.* [3]*Université de Toulouse, Institut National Polytechnique de Toulouse, Ecole d'Ingénieurs de Purpan, Département Sciences Agronomiques et Agroalimentaires, UPSP/DGER 115, 75 voie du TOEC, BP 57611, F-31076 Toulouse Cedex 03, France.* [4]*Bioresources Unit, Health & Environment Department, AIT Austrian Institute of Technology GmbH, 3430 Tulln, Austria. *E-mail: alban.jacques@purpan.fr*

Ozone is often used as a sanitary agent in the agricultural and food industries. We will present results of our investigation of ozone dissolved into water (for convenience: ozonated water) to control growth of the esca-associated fungus *Phaeoacremonium aleophilum*. We tested the fungicide properties of ozonated water in different contexts. (I) *In vitro*: we exposed *P. aleophilum* spores to several concentrations of ozonated water (2.2, 4.5, or 13.5 g/m^3 of ozone dissolved into water). (II) *In planta*: we tested ozonated water treatments on grapevine cuttings of cv. Cabernet-Sauvignon clone 15. For the latter, *P. aleophilum* DNA was quantified by a quantitative polymerase chain reaction (qPCR) in inoculated pruning wounds treated with ozonated (4.5 g/m^3) or sterile water 4 and 9 weeks post inoculation. Finally effect of ozonated water on plant-defense genes expression was monitored by reverse-transcriptase qPCR (RT-qPCR) 48 hours post treatment. Our results reveal that ozonated water totally suppresses spore germination *in vitro*. In addition, at 9 weeks post inoculation, fungal development was significantly reduced *in planta*. RT-qPCR analysis shows that ozonated water did not induce plant-defense-related genes 48 hours post-treatment. The strong fungicide properties of ozonated water and the absence of gene induction *in planta* make ozonated water a promising candidate for limiting infection by *P. aleophilum*.

Proteome analysis of two *Vitis vinifera* cvs. reveals different responses to hot-water treatment and cold storage and a diverse endophyte population. H. WAITE[1,2,*], M. PADULA[4], M. WHITELAW-WECKERT[1,3] and P. TORLEY[1,2]. [1]*National Wine and Grape Industry Centre.* [2]*School of Agricultural and Wine Sciences.* [3]*New South Wales Department of Primary Industries; Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia.* [4]*School of Medical and Molecular Biosciences, University of Technology, Sydney, Thomas St, Ultimo, NSW 2007, Australia. *E-mail: hwaite@csu.edu.au*

Hot water treatment (HWT; 50°C for 30 min), is currently the most practical and consistently effective control for trunk disease pathogens in grapevine cuttings. However, HWT can reduce strike rate. The effects of HWT on cutting physiology were investigated using two-dimensional gel electrophoresis and tandem mass spectrometry. Expression of stress related proteins was studied in dormant *Vitis vinifera* cv. Pinot Noir [PN] and Cabernet Sauvignon [CS] cuttings 1 and 24 h after HWT, and after 13 weeks cold storage (2°C) in sealed (hypoxic) or unsealed storage bags. Although there was a subset of proteins common to both varieties, the base proteome of CS was much larger than that of PN. At 1 h post-HWT, protein expression was suppressed in CS, but increased in PN. Hypoxic cold storage suppressed protein expression in PN, but increased expression in CS. Relatively high levels of pathogenesis related PR10 in dormant tissue compared to non-dormant tissue may explain the absence of visible symptoms in dormant cuttings infected with trunk disease pathogens. Proteins from microorganisms including bacteria, yeasts and fungi were also found in both varieties suggesting

a diverse population of endophytes. Some of these apparent endophytes are benign, but proteins attributed to known pathogens of plants and mammals were also present. A post HWT recovery period of 24 h is recommended before further processing of cuttings, particularly for CS. The different responses to hypoxic cold storage suggest that manipulating cold storage conditions for different varieties might be a useful means of prolonging storage life.

Grapevine trunk diseases in 2014. How comparable to phylloxera in 1881? R.E. SMART[1,*] and F. FONTAINE[2]. [1]*Smart Viticulture, 31 N. Corner, Newlyn, TR 185 JG, Cornwall, UK.* [2]*Unité de Recherche Vignes et Vins de Champagne, EA 4707, Université de Reims Champagne Ardenne, BP 1039, 51 687 Reims Cedex 2, France. *E-mail: richard@smartvit.com.au*

This contribution will contrast two pestilences of vineyards, one older, one newer. Phylloxera, an insect native to North America began devastating French vineyards around 1863, and is now present in many regions of the world. Phylloxera spreads rapidly in most soils, and death is quick and conspicuous. Man is/has been the principal vector for phylloxera spread. The Bordeaux Phylloxera Congress of 1881 considered two solutions. Grafting to resistant rootstocks from North America was the most successful, and is the basis of the present vine nursery industry. Soil treatment by carbon bisulphide injection, vineyard flooding, and subsequently hybridising the European *Vinifera* vine with American species were all less successful. Grapevine trunk diseases GTD on the other hand are more widespread, perhaps present in all vineyard regions. Generally their presence is insidious, as spread, vine decline and death are slower. Even today GTD are infrequently recognised. Control based on genetic resistance seems remote, and chemical/biological control is not universally proven. Worse, some GTD are being spread much more systematically than phylloxera ever was, because nurserymen unwittingly use GTD infected rootstocks. Paradoxically, it may be the planting of virus-free rootstock mother vines 10-30 years ago, and their subsequent infection by some GTD, which is responsible for the apparent recent rise of GTD in many countries. South Australia, the site of the 9IWGTD, is one of a few areas in the world where phylloxera spread has been contained by quarantine. Which will be the bigger problem in 2050? Phylloxera or GTD? Or maybe LRV3?

"*Fit Vine*". A mobile device application for rapid in-field pre-planting evaluation of grapevines. H. WAITE[1,2,*], M. WHITELAW-WECKERT[1,3] and P. TORLEY[1,2]. [1]*National Wine and Grape Industry Centre.* [2]*School of Agricultural and Wine Sciences.* [3]*New South Wales Department of Primary Industries, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia. *E-mail: hwaite@csu.edu.au*

The leading causes of sporadic failures of newly planted vines and early decline of young vineyards have been traced to trunk disease infections and other defects in planting material. However, these defects often go unnoticed by nurseries and their customers until the vines fail in the vineyard, by which time a great deal of time and money have been lost. Until recently, there has been no objective standard for assessing the quality of grapevines planting material, but this situation has now been remedied with the publication of the Australian Standard for Grapevine Material (AS5588-2013) that describes quality standards for both cuttings and finished vines. To bridge the gap between the Standard and its practical application in nurseries and vineyards, a computer based tool called "*Fit Vine*" has been developed for evaluating the quality, disease status and over all "fitness" of grapevine material prior to planting. The "*Fit Vine*" tool has been developed as an application (app) for mobile devices (iPhones, iPads etc.) and enables nurseries to check the quality of vines before despatch and growers to evaluate vines prior to planting. "*Fit Vine*" is quick and easy to use and requires no special equipment or training. "*Fit Vine*" calculates a risk score for each batch of vines that can be used to determine if the vines are likely to underperform in the vineyard. It also provides an objective basis for negotiation should any disputes arise between nurseries and growers.

The benefits of *Trichoderma atroviride* I-1237 for the protection of grapevines against trunk diseases: from the nursery to the vineyard. E. MOUNIER*, F. CORTES, M. CADIOU and E. PAJOT. *Agrauxine Lesaffre group, 2 rue Henri Becquerel, 49070 Beaucouzé, France. *E-mail: emmanuelle.mounier@agrauxine.fr*

In viticulture severe damage can be caused by grapevine trunk diseases (GTD). The fungi responsible for these diseases are widely present, not only in French vineyards, but also in most plant nurseries. The development of control alternatives is necessary to stop the progression of GTD. The benefits of *Trichoderma atroviride* strain I-1237 to provide protection against GTD have been shown in the laboratory by several public research institutes. Using this strain, Agrauxine conducted trials on young plants in the nursery to demonstrate the efficacy of I-1237 against the infection of *Diplodia seriata, Phaeomoniellachlamydospora* and *Phaeoacremonium aleophilum,* three important GTD fungi. Results showed that dipping young plants in I-1237 treatment decreased the size of the necrosis produced by *D. seriata* and *P.chlamydospora*. Furthermore, a molecular analysis revealed a significant decrease in the quantity of these three pathogens in the wood based on qPCR

studies. Since 2007, a network of 23 trials conducted in French vineyards using the phytopharmaceutical product Esquive® WP (active substance T. atroviride I-1237), has confirmed the efficacy of this strain to protect established grapevine plants against GTD. Two years of application of Esquive® WP by spraying on pruning wounds significantly reduced, by an average of 50%, the expression of foliar symptoms of Esca, BDA and Eutypiose dieback. Furthermore, the rate of plant mortality due to GTD sharply decreased on treated plots. Agrauxine contributes, through these studies and with the development of a product line against GTD, to the protection of grapevines from the nursery to the vineyard.

Efficacy of treatments against *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* during nursery propagation. Á. KUN[1,*] and L. KOCSIS[2]. [1]*Government Office of Baranya County, Plant Protection and Soil Conservation Directorate, H-7634 Kodó dűlő 1, Pécs, Hungary.* [2]*University of Pannonia, Georgikon Department, Doctoral School of Horticultural Sciences, Deák F. u. 16. H-8360 Keszthely Hungary. *E-mail: kuna@nebih.gov.hu, 28kuna@gmail.com*

Young dieback pathogens, *Phaeomoniella chlamydospora* (Pch) and *Phaeoacremonium aleophilum* (Pal) cause serious phytopathological problems in Hungarian vineyards and are mostly spread with grapevine propagation materials. For disinfestation of the scion and rootstock propagation material, 8-oxichinolin sulphate was permitted for use until the end of the century. Since then nursery owners have had no effective registered plant protection treatments against Pch and Pal. Four points were identified in the grafting and propagation process where infections could occur and treatment applications of thiram and thiophanate-methyl were made as follows: 1) at hydration, material was soaked in treatment for 30 h before cold storage, 2) at pre-grafting hydration material was soaked for 1 hour, 3) at stratification, sawdust was moistened with the treatments and 4) before planting into nursery, basal part of the grafts were soaked for 24 hours. During the propagation procedure grafting sites (omega grafting) were artificially inoculated with Pch or Pal, and wax applied 24 h later. Controls included untreated propagation material which was either inoculated with Pch or Pal, or uninoculated. Propagation material was assessed by reisolation to determine efficacy of treatments on pathogen survival. The natural infection of Pch and Pal were 6.5% compared with up to 71% in the artificially inoculated treatments. Thiophanate-methyl and thiram reduced infection by 39 and 28 %, respectively, compared with inoculated controls.

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