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ABSTRACTS

Abstracts of oral and poster presentations given at the 7th International Workshop on Grapevine Trunk Diseases, Santa Cruz, Chile, 17–21 January 2010

The 7th International Workshop on Grapevine Trunk Diseases, was held in Santa Cruz, Chile, on January 17–21 2010. Santa Cruz is located in the Colchagua valley, a major wine-producing area in central Chile. The meeting was attended by 120 participants, and 80 papers were presented either as oral or poster presentations in four sessions: Pathogen Identification and Characterization, Disease Detection and Losses, Host-Pathogen Interactions and Disease Management.

Pathogen Identification and Characterization

Basidiomycetes and other fungi associated with esca diseased grapevines in South Africa. C. White[1], F. Halleen[2], M. Fischer[3] and L. Mostert[1]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bax X1, Matieland, 7602, South Africa.* [2]*Plant Protection Division, ARC Infruitec-Nietvoorbji, Private Bag X5026, Stellenbosch, 7599, South Africa.* [3]*Julius-Kühn-Institut (Bundesforschungsanstalt für Kulturpflanzen), Geilweilerhof, Germany.*
E-mail: halleenf@arc.agric.za

Esca is a relatively unknown disease in South Africa and only a few incidences have been reported in the past. The aim of this study was to determine the identity of the fungi associated with the typical internal wood symptoms. Additionally, the role of the basidiomycetes was investigated with toxin and enzyme analyses. Vines showing the presence of foliar and/or internal symptoms were collected from various regions in the Limpopo, Western and Northern Cape provinces. Esca diseased vines were found in all of the grapevine production areas sampled from and on all of the cultivars collected including wine, table and raisin grapes. The internal symptoms were similar to those found in Europe and included: white rot, black and brown wood streaking; brown necrosis within white rot; sectorial brown necrosis and central brown/ red/ black margin. The fungal species were determined with the internal transcribed spacers and the 5.8S rRNA gene for the basidiomycetes and *Phomopsis* isolates, the partial beta-tubulin gene for *Phaeoacremonium* isolates and the partial translation elongation 1-alpha gene for the Botryosphaeriaceae isolates. The basidiomycetes belonged to eight new species in the genera *Fomitiporella, Fomitiporia, Inonotus, Inocutis, Phellinus* and two species in a possible unknown genus. *Phaeomoniella chlamydospora* and six species of *Phaeoacremonium* including *Pm. aleophilum, Pm. alvesii, Pm. parasiticum, Pm. iranianum, Pm. mortoniae* and *Pm. sicilianum* were also isolated of which the latter three are reported for the first time in South Africa. The other taxa that were also found include *Phomopsis viticola, Phomopsis sp. 1, Diaporthe ambigua, Diplodia seriata, Neofusicoccum parvum* and *Neofusicoccum australe*. Extractions from liquid broth cultures were tested for the presence of 4-hydroxybenzaldehyde. All of the basidiomycete isolates produced this toxin. Some of the

basidiomycete isolates were able to produce lignin peroxidase, and the majority of the isolates were able to produce manganese peroxidase, laccase and pectin lyase. All the basidiomycete isolates were able to produce cellulase. The enzyme tests showed that the basidiomycetes are able to degrade cellulose and lignin.

Characterization of *Cadophora luteo-olivacea* and *Cadophora melinii* isolates obtained from grapevine nurseries and plants in Spain. D. Gramaje[1], L. Mostert[2] and J. Armengol[1]. [1]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022, Valencia, Spain.* [2]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa.*
E-mail: dagrape@upvnet.upv.es

In the present study fifty-eight *Cadophora luteo-olivacea* and three *C. melinii* isolates were obtained from young grapevines as well as environmental samples taken from grapevine nurseries in Spain. The isolates were studied by means of phenotypical characterization, DNA analyses and pathogenicity tests. The morphological characters that were observed included conidiophores, phialides and conidia. The colony characters and pigment production on MEA, PDA and OA were also noted. The phenotypic data were subjected to cluster analysis. Partial sequences of the nuclear ribosomal internal transcribed spacers and 5.8S ribosomal gene (ITS), beta-tubulin (BT) and elongation factor (EF) were analysed. Pathogenicity tests were conducted in 1-year-old grapevine cuttings of four different rootstocks (140 Ruggeri, 161-49 Couderc, 1103 Paulsen and 110 Richter) using four *C. luteo-olivacea* isolates and one *C. melinii* isolate. According to the phenotypical analysis, *C. luteo-olivacea* isolates were separated into four groups. Very little sequence variation (up to 3 nucleotides) were observed for *C. luteo-olivacea* among the ITS, BT and EF sequences and no significant groups were found with phylogenetic analyses of the separate gene areas. In the pathogenicity trial the *Cadophora luteo-olivacea* isolates, however, not the *C. melinii* isolate, caused lesions in the xylem of grapevine rootstocks that were significantly longer than the controls. These results confirm *C. luteo-olivacea* as a potential pathogen in grapevine nurseries and young vineyards.

Two new *Phaeoacremonium* species identified on grapevines in Iran and Spain. D. Gramaje[1], J. Armengol[1], H. Mohammadi[2], Z. Banihashemi[2] and L. Mostert[3]. [1]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.* [2]*Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz, Iran.* [3]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa.*
E-mail: dagrape@upvnet.upv.es

The genus *Phaeoacremonium* is an ecologically important taxon which includes species associated with two declining diseases on grapevine (*Vitis vinifera* L.) namely Petri disease and esca. In this study, eight *Phaeoacremonium* isolates obtained from grapevines in Iran and Spain were studied using morphological and cultural characteristics as well as phylogenetic analyses of the combined actin and β-tubulin gene sequences. Two new species were found and described. *Phaeoacremonium cinereum* was isolated from a young vine (6-year-old) in Spain and from older vines (25–30-year-old) in Iran and can be identified by its distinct grey colored colonies on malt extract agar, an optimum growth temperature of 25°C and subulate type III phialides. *Phaeoacremonium hispanicum* was isolated only once from a young vine (5-year-old) in Spain and can be identified by the common occurrence of percurrent rejuvenating phialides, an optimum growth temperature of 20°C, and predominant type II phialides. This study brings the total number of *Phaeoacremonium* species isolated from grapevines to 25.

Study of *Phaeoacremonium* species associated with trunk diseases of grapevines in Uruguay. E. Abreo, S. Lupo, M. Martínez and L. Bettucci. *Laboratorio de Micología, Facultad de Ciencias-Facultad de Ingeniería, Universidad de la República. Zip Code 11300, Montevideo, Uruguay.*
E-mail: eabreo@fing.edu.uy

Symptoms corresponding to esca, Petri and dead arm diseases have been described in Uruguay. *Phaeoacremonium* spp. were commonly associated with Petri and esca diseases, with *P. aleophilum* considered the most prevalent species. However, other members of this genus have been found able to produce the same symptoms as *P. aleophilum*. Samples of symptomatic plants were collected and taken to the laboratory. The bark was removed, and the cleared trunk surface was sterilized. Chips were obtained from inner tissues, plated onto PDA Petri dishes, and incubated at 25°C. Species were identified based on the macro and micromorphological characteristics of the isolates, and sequencing of the ITS rDNA, β-tubulin and actin genes. Isolates were characterized by their growth pattern at 25°C and 37°C, and colony morphology on MEA and PDA. Growth at 37°C made possible the differentiation of isolates, with 24 of them showing growth at this temperature, and three showing no growth. Among those that grew, four colony morphologies were observed on MEA: (a) white colonies with yellow reverse; (b) white colonies with dark center on reverse; (c) brown colonies with clear growth mar-

gin and dark center on reverse; (d) brown colonies with dark brown diffusible pigments and brown reverse. Phylogenetic analysis of the ITS region did not allow for a correct identification of species, but the analysis of the β-tubulin region allowed for the differentiation of 26 *P. aleophilum* isolates and one *P. australiense* isolate. Combined phylogenetic analysis of β-tubulin and Actin sequences showed a further differentiation of the "a" morphological group, which was separated from the *P. aleophilum* clade.

Distribution and occurrence of fungi associated with grapevine trunk diseases in Northeastern American vineyards. P.E. Rolshausen[1], W. Wilcox[2] and K. Baumgartner[3]. [1]*Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA.* [2]*Department of Plant Pathology, Cornell University, Geneva, NY 14456, USA.* [3]*USDA-ARS, University of California, One Shields Avenue, Davis, CA, 95616, USA.*
E-mail: philrols@ucr.edu

Concord grapes (*Vitis labruscana*) have been traditionally the main grapes grown for juice production in northeastern America. Wine grape production is a relatively new developing industry in these regions. However, cold climatic conditions and a short growing season necessitate the planting of inter-specific hybrids (North American *Vitis* species × *V. vinifera*) although *V. vinifera* can be planted in some locations. The viticultural practices are quite specific in these cold climate regions and very different from the temperate/Mediterranean regions. The objectives of our study are to identify the causal agents of trunk diseases in northeastern American vineyards (which ones?), to map their distribution and to quantify their occurrence. We surveyed a total of 50 vineyards representing 30 varieties in 11 U.S. states (VA, MD, NJ, NY, CT, RI, MA, NH, VT, OH, and MI) and two provinces in Canada (Quebec and Ontario). Four species of *Eutypa* were recovered, two of which were not identified before. *Eutypa lata* was found only in Rhode Island. Of the three species in the Botryosphaeriaceae identified, *Neofusicoccum parvum* was the most common. Species of *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum*, *Cadophora* sp., and *Phomopsis* spp. were ubiquitous across states. *Cytopsora* sp. was only recovered in the northern regions surveyed. Several species of *Cylindrocarpon* were recovered from Quebec. Basidiomycetous fungi were also isolated. Overall, the main disease found across these states is Esca. Our results suggest that the fungal communities associated with trunk diseases in northeastern American vineyards are composed of taxa similar to those of temperate/Mediterranean regions and of unique taxa not previously reported.

Pathogenicity of fungi less frequently associated with trunk diseases in grapevines. F. Navarrete, E. Abreo, L. Betucci and S. Lupo. *Laboratorio de Micología, Facultad de Ciencias-Facultad de Ingeniería, Universidad de la República. Zip Code 11300, Montevideo, Uruguay.*
E-mail: kissloff@gmail.com

In Uruguay, there are 8000 ha of *Vitis vinifera.* A recent survey has identified several pathogenic and non pathogenic fungi associated with affected tissues from grapevines showing symptoms of trunk diseases. *Eutypella vitis, Botryosphaeria* spp. and an unidentified Coelomycete were isolated from typical necrotic tissues of dead arms of grapevines. In young plants suffering from Petri disease, *Cadophora luteo-olivacea* and *C. melinii* were commonly isolated from affected tissues in addition to *Phaeomoniella clamydospora* and *Phaeoacremonium* spp. Pathogencity of the Coelomycete, *C. luteo-olivacea* and *C. melinii* was evaluated on canes and plantlets of Tannat and Cabernet Sauvignon grafted on rootstock 3309 and SO4. Wounds made on canes were inoculated with a mycelium covered disc of agar obtained from actively growing colonies on PDA. Plantlets of each cultivar were inoculated on scion and rootstock as described before. Control canes and plants were inoculated with sterile agar discs. Size of lesions was measured, and segments of xylem were transferred to PDA to recover the inoculated fungi. The Coelomycete was identified as *Greeneria uvicola* based on micromorphological characteristics and partial DNA sequence of LSU. Only *G. uvicola* was able to produce discoloration in both canes and plantlets. This fungus showed high percentage of recovery (70–100 %) in canes and lower recovery in plantlets (20–60%). *Cadophora* spp. produced discoloration in 20% of plantlets and a low percentage of recovery (29–43%) only in canes. These results point at *G. uvicola* as part of the complex of fungi causing dead arm of grapevines in Uruguay.

Botryosphaeriaceae species associated with grapevine decline in Mexico. O. Candolfi-Arballo[1], C. Valenzuela-Solano[2], W.D. Gubler[3] and R. Hernández-Martínez[1]. [1]*Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, BC, 22860, México.* [2]*INIFAP, Campo Experimental Costa de Ensenada, Ensenada, BC, 22800, México.* [3]*Department of Plant Pathology, University of California, Davis, CA 95616, USA.*
E-mail: ruhernan@cicese.mx

In recent years an increasing number of Botryosphaeriaceae species belonging to different anamorphic genera had been found associated with grapevine in all growing regions worldwide. To determine if *Botryosphaeria*

anamorphs were playing a role in grapevine decline, a survey conducted from 2007–2009 in vineyards of Baja California, the main wine producing area of Mexico, was done. Four Botryosphaeriaceae species: *Diplodia seriata, Neofusicoccum vitifusiforme, Diplodia corticola,* and *Neofusicoccum australe* were isolated and identified based on their morphological characteristics and internal transcribed spacer region (ITS-5.8S-ITS2) sequence data. *Diplodia seriata* was the most common species found in the vineyards and it was mainly isolated from wedge-shaped sectors in the wood and dead arms, while *N. vitifusiforme, D. corticola* and *N. australe* were mainly associated with black streaks and brown-red wood. A pathogenicity test done using green grapevine shoots of cv. Merlot showed that all species were able to produce symptomatic infections; however lesions were smaller than those produced by *Lasiodiplodia theobromae* used as positive control. To our knowledge, this is the first report of *N. vitifusiforme, D. corticola* and *N. australe* occurring on grapevine in Mexico.

Botryosphaeriaceae associated with bunch rot of grapes in South Eastern Australia. N. Wunderlich[1], G. Ash[1], C. Steel[1], H. Rahman[2] and S. Savocchia[1]. [1]*National Wine and Grape Industry Centre, School of Agricultural and Wine Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia.* [2]*Industry & Investment New South Wales, Private Mail Bag, Wagga Wagga, NSW 2650, Australia.*
E-mail: nwunderlich@csu.edu.au

Species of fungi belonging to the Botryosphaeriaceae are responsible for the grapevine trunk disease Botryosphaeria canker. While the epidemiology and distribution of Botryosphaeria canker in the wood of grapevines and other hosts has been widely researched, little is known about the epidemiology of these pathogens on grape berries. Frequent isolations of Botryosphaeriaceae have recently been reported from grape bunches, raising the question as to whether these fungi can be considered as primary bunch rot pathogens. A survey was conducted over two consecutive seasons (2007–2009) in two vineyards of the lower Hunter Valley with a known history of Botryosphaeria canker. Samples were taken from various parts of the grapevine at different phenological stages, as well as from the trunk of each grapevine. Fungi conforming to seven species of the Botryosphaeriaceae: *Diplodia seriata, Neofusicoccum parvum, N. luteum, Dothiorella viticola, Lasiodiplodia theobromae, D. mutila* and *Botryosphaeria dothidea* were isolated. The largest number of isolates originated from dormant buds and wood followed by berries at harvest. A small number of isolates was found on flowers and pea-sized berries. Isolates originating from different tissues were found to be pathogenic on berries at harvest and one-year-old canes of Shiraz and Chardonnay. In addition, dormant buds of glasshouse and field grown Chardonnay were inoculated with various Botryosphaeriaceae to investigate into the affect of *Botryosphaeria* infection on budburst and shoot development.

The status of grapevine canker dieback in the United States. J.R. Úrbez-Torres[1], F. Peduto[1], P. Adams[2], K. Striegler[3] and W.D. Gubler[1]. [1]*Department of Plant Pathology, University of California, Davis, CA 95616, USA.* [2]*Texas AgriLife Extension, Texas A&M University System, Fredericksburg, TX 78624, USA.* [3]*Institute of Continental Climate Viticulture & Enology, University of Missouri, Columbia, MO 65211, USA.*
E-mail: jrurbez@ucdavis.edu

Grapevine cankers and consequent dieback are one of the primary factors limiting vineyard longevity and productivity, causing significant economic losses to the grapevine industry. Grapevine cankers have been longley observed in American vineyards and have been commonly attributed to the diatrypaceous fungus *Eutypa lata*. However, studies conducted during the past years throughout different grape-growing regions showed that several species in the Botryosphaeriaceae family are not only associated with grapevine cankers but constituted the most prevalent fungi isolated from cankered wood in the United States. To date, ten different Botryosphaeriaceae spp. have been identified throughout the United States and have been shown to be pathogenic on several *Vitis vinifera* cultivars. In addition, field surveys showed different fungal species including, *Phomopsis viticola*, *Eutypella vitis*, *Diatrypella* sp., *Diatrype* sp., *Pestalotiopsis* spp., *Truncatella* spp., and *Paraconiothyrum* sp. to be associated with grapevines cankers in different areas in the United States as well. Although virulence varied depending on the fungal species, Koch's postulates confirmed all these species to be pathogenic in both *Vitis vinifera* and hybrid grapevine cultivars. Moreover, this study also confirmed for first time the pathogenicity of Botryosphaeriaceae spp. on different hybrid cultivars as well. Therefore, it is now clear that *E. lata* is not the sole cause of cankers and that many other fungi are associated with grapevine cankers contributing to the dieback of grapevines and adding much more complexity to the grapevine canker problem worldwide.

Effect of temperature and time on spore germination in Botryosphaeriaceae spp. associated with Botryosphaeria canker disease of grapevines. J.R. Úrbez-Torres, E. Bruez, J. Hurtado and W.D. Gubler. *Department of Plant Pathology, University of California, Davis, CA 95616, USA.*
E-mail: jrurbez@ucdavis.edu

Ten different Botryosphaeriaceae spp. in the genera *Botryosphaeria*, *Lasiodiplodia*, *Diplodia*, *Dothiorella*, *Spencermartinsia*, and *Neofusicoccum* have been reported to cause Botryosphaeria canker disease of grapevines in different grape-growing areas in the United States and Mexico. Previous studies hypothesized that geographical distribution of Botryosphaeriaceae spp. in the United States and Mexico could be a consequence of temperature differences. To test this hypothesis, the effect of 8 different temperatures (5, 10, 15, 20, 25, 30, 35, and 40°C) on Botryosphaeriaceae spore germination at 5 different incubation periods (2, 4, 6, 12, and 24h) was studied. Twenty different isolates from 8 different Botryosphaeriaceae spp. representing all genera associated with Botryosphaeria canker disease of grapevines in the United States and Mexico were examined in this study. Spore germination occurred from 10 to 35°C after at least 24h incubation period in all the species. However, both temperature and time required for at least 50% of spore germination varied depending on the Botryosphaeriaceae species. For example, *Lasiodiplodia theobromae*, a prevalent species of warm and dry grape-growing areas in the United States and Mexico, reached over 80% spore germination at 40°C in 4h. On the other hand, species known to be more prevalent in cooler areas such as *Dothiorella iberica* and *Spencermartinsia viticola*, reached over 70 and 80% spore germination at 15°C in 12h, respectively. The effect of temperature observed on spore germination could help to explain the current distribution of Botryosphaeriaceae spp. associated with Botryosphaeria canker disease of grapevines in the United States and Mexico.

Dispersal of Botryosphaeriaceae species conidia from infected grapevines in rain water. N.T. Amponsah, E.E. Jones, H.J. Ridgway and M.V. Jaspers. *Department of Ecology, Faculty of Agricukture and Life Sciences, P. O. Box 84, Lincoln University, New Zealand.*
E-mail: Nicholas.Amponsah@lincoln.ac.nz

The mechanisms for dispersal of *Botryosphaeria* conidia, which can infect grapevines through wounds, were investigated to determine their seasonal prevalence in a New Zealand vineyard and to further develop control strategies. Airborne dispersal was investigated in April 2008 in four vineyards using Vaseline®-coated slides which were collected and replaced weekly. Inspection with a light microscope for characteristic *Neofusicoccum* and *Diplodia* conidia, distinguishable by their morphology and colour, found no conidia characteristic of the Botryosphaeriaceae although spores of other fungi were collected. Waterborne conidia were trapped by collecting rainwater that ran off the vines during May 2008–April 2009. The continuously collected rainwater suspensions were examined for characteristic conidia after every significant rainfall event. The rainwater suspensions yielded conidia of the Botryosphaeriaceae, with 59.8% of the conidia caught being *Neofusicoccum* spp. and 40.2% being *Diplodia* spp. These conidia were present throughout the entire year, but were most abundant during December, January and February when summer temperatures were high and when routine trimming of mature canes provided the wounds needed for infection. Subsequent PCR of rDNA followed by DNA sequencing confirmed the conidia as belonging to *D. mutila*, *N. luteum*, *N. australe, N. parvum* and *N. ribis*.

Use of endogenous molecular markers to measure rain water splash dispersal of *Neofusicoccum* species in the vineyard. J. Baskarathevan, M.V. Jaspers, E.E. Jones and H.J. Ridgway. *Faculty of Agriculture and Life Sciences, Lincoln University, P.O. Box 84, Lincoln 7647, New Zealand.*
E-mail: jeyaseelan.baskarathevan@lincolnuni.ac.nz

Decline and dieback of grapevines is commonly caused by several species of the Botryosphaeriaceae. Among these, *Neofusicoccum* species are predominantly found in New Zealand vineyards and cause considerable losses. Infection by these species occurs mainly through wounds and the dispersal mechanisms are reported to be through rain water splash. The aim of this study was to measure distance of rain water splash dispersal of *Neofusicoccum* species conidia in the vineyard using endogenous molecular markers. Pycnidia of *N. luteum* and *N. parvum* were produced on grapevine green shoots *in-vitro* and used in the field as a source of inoculum. Shoots that were oozing conidia from the pycnidia were attached to field grapevines prior to heavy rainfall. The water collection cups were placed at 50 cm intervals from the inoculum point up to 3 m distance in four perpendicular directions. The experiment was replicated three times across the vineyard. Rain water samples were collected after each rainfall event (four times during two weeks). The water samples were examined microscopically and also used for molecular detection of Botryosphaeriaceae species using specific primers. The microscopic examination confirmed the presence of conidia from both species in the water samples. The molecular tools confirmed these conidia as belonging to the marker strains. Spore movement was detected up to 2 m from the inoculum source in a single rainfall event. The dispersal was influenced by wind direction, with downwind movement being much greater than upwind movement.

Diatrypaceae associated with grapevine canker diseases in South Australia and New South Wales. F.P. Trouillas[1], W.M. Pitt[2], M. Sosnowski[3], F. Peduto[1], R. Huang[2], A. Loschiavo[3], S. Savocchia[2], C. Steel[2], E.

Scott[4] and W.D Gubler[1]. [1]*Department of Plant Pathology, University of California, One Shields Avenue, Davis, CA 95616, USA.* [2]*National Wine and Grape Industry Centre, School of Agricultural and Wine Sciences, Charles Sturt University, Locked Bag 588,Wagga Wagga, NSW 2678, Australia.* [3]*South Australian Research and Development Institute, GPO Box 397, Adelaide, SA 5001, Australia.* [4]*School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond, SA, 5064 Australia.*
E-mail: flotrouillas@ucdavis.edu

Diatrypaceae are common pyrenomycetous fungi occurring on a wide range of woody angiosperms around the world. *Eutypa lata*, the causal agent of Eutypa dieback of grapevine, is the most common diatrypaceous fungus associated with grapevine cankers and it is found in all major grape growing regions worldwide. Recent studies in California have shown that additional Diatrypaceae can infect grapevine wood and possibly cause canker diseases similar to Eutypa dieback. During 2007–08, a number of surveys for fungi associated with canker diseases in grapevine were conducted in South Australia and New South Wales. In many instances, fungal colonies displaying morphological characteristics typical of Diatrypaceae were isolated from grapevine cankers. Fruiting bodies of Diatrypaceae were also found on dead grapevine wood. Morphological studies and phylogenetic analyses of the complete sequence of the ITS region of the rDNA and partial β-tubulin gene identified *Cryptovalsa ampelina*, *Diatrypella* sp., as well as two species of *Eutypella* from grapevine cankers, in addition to *E. lata*. Similar species occurred on other agricultural host plants and ornamentals adjacent to vineyards. Surveys also documented *Eutypa leptoplaca* on *Fraxinus*, *Populus* and *Schinus* spp., however it was not isolated from grapevine. Pathogenicity studies of Diatrypaceae were conducted by drilling a 3-mm-diameter hole into the wood of potted Cabernet Sauvignon grapevines and inserting an agar plug with fresh mycelium obtained from the margin of 8-day-old colonies. Plants were maintained in a shade house for 10 months before being assessed for lesion development and fungal recovery. Preliminary results for pathogenicity are discussed.

Intercontinental genetic structure of the fungal grapevine pathogen *Eutypa lata*. R. Travadon[1], K. Baumgartner[2], P.E. Rolshausen[3], F.P. Trouillas[1], W.D. Gubler[1], M. Sosnowski[4], P. Lecomte[5], F. Halleen[6] and J-P. Peros[7]. [1]*Department of Plant Pathology, University of California, One Shields Avenue, Davis, CA 95616, USA.* [2]*USDA-ARS, 363 Hutchison Hall, University of California, One Shields Avenue, Davis, CA 95616, USA.* [3]*Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA.* [4]*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia.* [5]*ISVV, UMR Santé Végétale, 33883 Villenave d'Ornon Cedex, France.* [6]*ARC Infruitec-Nietvoorbij, P. Bag X5026, Stellenbosch 7599, South Africa.* [7]*INRA, UMR DIAPC, 34060 Montpellier Cedex, France.*
E-mail: rtravadon@ucdavis.edu

The ascomycete fungus *Eutypa lata*, causal agent of Eutypa dieback of grapevine (*Vitis vinifera*), impacts all vineyard production systems worldwide. Our objectives were to characterize the population structure of *E. lata* at different geographical scales to identify migration patterns through ascospore dispersal, centers of diversity and new introductions areas. We genotyped 145 isolates from four vineyards, in each of two locations (California, South Australia), using nine polymorphic microsatellite loci. Results showed high levels of gene (H=0.56 to 0.62) and genotypic (G=0.85 to 1) diversity among the eight vineyards. There was no significant linkage disequilibrium among loci (P<0.01). We found that 93% of genetic variance was found within vineyards (P<0.01) and 3% of variance could be attributed to differences between California and South Australia (P<0.05). Three pair-wise comparisons revealed significant genetic differentiation between populations from these two continental regions (F_{ST}=0.03-0.10, P<0.05). When isolates were pooled by continental regions, genetic differentiation between the two populations was low, but significant (F_{ST}=0.03; P<0.05). These preliminary findings suggest that gene flow prevents genetic differentiation at the regional scale within continents, spanning distances up to 410 km, whereas continental populations are somewhat isolated. An additional collection of 156 isolates from Europe and South Africa, representing 12 vineyards populations, was recently obtained, and are currently being genotyped using the same nine microsatellite loci.

Identity of *Phomopsis* species recovered from wood cankers in eastern North American vineyards. K. Baumgartner[1], R. Travadon[2], W. Wilcox[3] and P.E. Rolshausen[4]. [1]*USDA-ARS, Department of Plant Pathology, University of California, One Shields Avenue, Davis, CA 95616, USA.* [2]*Department of Plant Pathology, University of California, One Shields Avenue, Davis, CA 95616, USA.* [3]*Department of Plant Pathology and Plant Microbe Biology, Cornell University, Geneva, NY, USA.* [4]*Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA.*
E-mail: kbaumgartner@ucdavis.edu

Phomopsis cane and leaf spot is relatively common in eastern North American *Vitis labruscana* vineyards, from which *P. viticola* is consistently recovered from

green shoot and berry lesions. *Vitis vinifera* vineyards, and associated training and pruning practices, are becoming more common. As such, pruning wounds accumulate on individual vines, thereby increasing the potential for infection by trunk pathogens. Fifteen *Phomopsis* species have been identified from grape (from green shoot lesions and wood cankers), but primarily from Mediterranean regions. In this cold-climate, to characterize the species community of *Phomopsis* grape pathogens, we surveyed vineyards in the northeastern US (CT, MA, MD, MI, NH, NJ, NY, OH, RI, VA, VT) and Quebec and Ontario, Canada, primarily for general symptoms of trunk disease (wood cankers), but also for typical symptoms of Phomopsis cane and leaf spot (green shoot lesions). Species-level identification of collections with morphological characteristics of *Phomopsis* was based on phylogenetic analyses of five nuclear loci: rDNA internal transcribed spacer region, translational elongation factor subunit 1-alpha, calmodulin, beta-tubulin, and RNA polymerase II subunit B. Individual and combined phylogenies showed that all isolates from green shoot lesions were *P. viticola*. A few isolates recovered from wood cankers were identified as *P. viticola*, but the majority were the following other species: *Diaporthe phaseolorum*, *P. vaccinii*, *P. amygdali*, and several unknown *Phomopsis* species. Therefore, Phomopsis cane and leaf spot controls may not be effective against pruning wound infection by this species complex.

Association of *Seimatosporium* spp. in symptomatic grapevine wine varieties in California vineyards. Z. Morales, P. Uribe, L. Merrill, R. Stern, E. Taniguchi, H.G. Stanghellini and J. Monis. *Eurofins STA Laboratories, 7240 Holsclaw Rd. Gilroy, CA 95020, USA.*
E-mail: juditmonis@eurofinsus.com

Seimatosporium spp. was isolated from three different symptomatic wine grape varieties in California. The symptoms observed in the affected vines differed in each variety sampled. Cabernet Franc exhibited "blotchy red leaf" symptoms, which could have been confused with the presence of virus infection, and streaking in the wood. Sauvignon Blanc displayed wedge shaped necrosis and dead arm symptoms. While the Syrah variety displayed the most severe symptoms which included cracked trunk with extensive necrosis, pitting, dead arm, severe decline, and mortality. In all varieties, except Syrah, the growers were able to control the disease using cultural practices. Specific ELISA and RT-PCR were performed to detect viruses associated with decline and/or leafroll disease. All tests ruled out the presence of known viruses in the symptomatic vines. However, *Seimatosporium* spp. was the only significant fungus isolated and identified using taxonomic reference guides. The nucleic acid extracted from the *Seimatosporium* cultures was subjected to sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA. The sequence data confirmed the presence of *Seimatosporium* spp. This is the first report of *Seimatosporium* spp. in grapevines grown in California. However, *Seimatosporium* spp. and other species of appendaged *Coelomycetes* were reported previously to affect grapevines in Australia. Future work will require pathogenicity assays to determine differential susceptibility among cultivated grapevine varieties.

On the taxonomy of *Sorosphaera viticola*. S. Neuhauser[1], L. Huber[2] and M. Kirchmair[1]. [1]*Institute of Microbiology, Faculty of Biology, University Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Austria.* [2]*Geisenheim Research Centre, Department of Grapevine Breeding and Grafting, Eibinger Weg 1, 65366 Geisenheim, Germany.*
E-mail: Sigrid.Neuhauser@uibk.ac.at

Sorosphaera viticola (Plasmodiophorida, Phytomyxea) is a soil-borne, endophytic parasite of grapevine. Plasmodiophorids have long been treated as basal group of fungi and are now placed in the protozoan phylum *Cercozoa*. A molecular phylogeny based on an 18S rDNA dataset split the plasmodiophorids into several clades. A clade tentatively named as "*Polymyxa* clade" was supported by high posterior probabilities in bayesian inference. The clade comprises *Polymyxa graminis*, *P. betae*, *S. veronicae, Ligniera junci,* and *S. viticola*. *Sorosphaeara viticola* was repeatedly found to form a distinct and well supported sub-clade together with *S. veronicae* and *P. betae*. *Sorosphaera veronicae* is characterised by forming galls on the shoots of *Veronica* spp. so one can speculate that *S. viticola* may not infect only the roots of *Vitis* spp. but also stem tissues. This is supported by previous observations of *S. viticola* resting spores in the root stem. Moreover, *Polymyxa*-species are well known as vectors for plant viruses of cereals (*P. graminis*) and of sugar beet (*P. betae*). Therefore, the potential role of *S. viticola* as vector for plant viruses needs to be assessed in the future. During a survey on the global distribution of *S. viticola,* root samples from Ukraine and Uruguay were positively tested for plasmodiophorids using specific PCR primers. But sequences of the 18S rDNA region were forming a distinct cluster with close affiliation to *Ligniera junci*. It seems likely that *S. viticola* is not the only plasmodiophorid associated with grapevine.

Grapevine wood pathogens from New Zealand; the recognition of a bacterium in the disease complex. I. C. Harvey[1], P.D. Turpin[1] and M. Braithwaite[2].

[1]*PLANTwise Services Ltd, P.O. Box 181, Lincoln 7640.* [2]*Bio-Protection Research Centre, P.O. Box 84, Lincoln University, Canterbury, New Zealand.*
E-mail: harveyi@plantwise.co.nz

A possible new bacterial pathogen of grapevine wood has recently been characterised from the Marlborough region of New Zealand. Up until recently, this bacterium appears to have been largely overlooked in the hundreds of samples of vines that have been processed at PLANTwise laboratory at Lincoln over the past 10 years. Most vines received have been less than 3-years-old and were all grafted plants from nurseries or newly established vineyards. A variety of fungi has been isolated from these vines with *Botryosphaeria* spp. being the most prevalent. Although bacteria have regularly been isolated either by themselves or in combination with these fungi, they have not been suspected as a major contributor to the die-back or lack of vigour symptoms observed in establishing vineyards and nurseries, being viewed as either natural endophytes of wood or cultural contaminants. Recently, however, two vineyards submitted samples with die-back symptoms and unusual internal wood staining from which bacteria only were consistently isolated but no fungi. The isolated bacterium was characterised as a *Paenibacillus* sp. which has never previously been reported as being associated with grapevines. The isolate was inoculated back into fresh green shoots and one year wood of greenhouse grown vines and consistently re-isolated back into pure culture from lesions that developed within the tissue. Short term inoculations of the bacterium into 1 and 2 year wood at an old vineyard in Lincoln had mixed results. Methods of detection and control are being investigated.

Evidence for sympatric genetic differentiation and recombination in French populations of the grapevine fungal pathogen *Phaeomoniella chlamydospora*. G. Comont[1], F. Delmotte[1], P. Larignon[2] and M.F. Corio-Costet[1]. [1]*INRA, UMR, Santé Végétale, Centre de Bordeaux, ISVV, BP 81, F-33883 Villenave d'Ornon, France.* [2]*IFV Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France.*
E-mail: gwenaelle.comont@bordeaux.inra.fr

Phaeomoniella chlamydospora (Chaetothyriales, Herpotrichiellaceae) is one of the main causal agents of Petri disease and esca on grapevines. We used AFLP markers to study the population genetic structure of 74 isolates collected at different spatial scales: 56 isolates originated from vines with esca disease sampled from four French vineyards (Poitou-Charentes, Aquitaine, Languedoc-Roussillon, Alsace); 18 isolates were collected from a single plot (Aquitaine vineyard). A high level of haplotypic diversity was observed, with a total of 72 single multilocus haplotypes identified among the 74 isolates analyzed. Clustering analyses revealed a genetic admixture, with the presence of two genetically well differentiated and sympatric clusters of isolates. The level of differentiation between both clusters is high (F_{ST}=0.23) and significant at 13 out of the 21 locus analyzed. The presence of hybrid isolates indicating shared ancestry suggests that recombinations between isolates from different clusters have occurred. Although the teleomorph of *P. chlamydospora* has not yet been found in natural conditions, our results provided evidence for recurrent recombination through sexual reproduction, indicating that *P. chlamydospora* may not be a strictly asexual fungus. Finally, the low level of spatial genetic differentiation in this study is consistent with the spread of this fungus through the transport of infected plant material by human activities.

Influence of culture media and temperature on mycelial growth of *Phaeoacremonium angustius*. R. Gava, A.F. Urben and L.R. Garrido. *Phytopathology Laboratory, Embrapa Grape and Wine. Zip Code 95700000, Bento Gonçalves, Brazil.*
E-mail: garrido@cnpuv.embrapa.br

Several species of *Phaeoacremonium* have been associated with young grapevine decline in major production regions of California, South Africa, Australia, Europe and now in Brazil. Infection of *Phaeoacremonium* spp. causes darkening of xylem vessels with production of tyloses and dark gummy masses resulting in occlusion of xylem vessels. The fungus isolated from these samples produced colonies that rarely exceed 15 to 20 mm diameter after several weeks growth at 20°C. In this study the effects of culture media and temperature on mycelial growth of *Phaeaocremonium angustius* CNPUV 533 were evaluated. The isolate is maintained in the collection of the Phytopathology Laboratory of Embrapa Grape and Wine. Mycelial growth was evaluated on six media and three temperatures. Agar plugs with 5 mm diameter from the margin of young cultures were placed at center of plates containing the culture media, with three replicate plates of each culture medium under each temperature. Plates were incubated at 20 and 25°C under intermittent light (12 h), for 4 weeks. Two culture media were incubated at 30°C. Colony diameter was measured weekly along two axes perpendicular to each other and the average of the two dimensions was recorded as the radial colony diameter. The culture media and temperature influenced the growth of *P. angustius*. The fungus grew on all six media tested; however, peptone dextrose agar and Cantino PYG agar were most fa-

vorable for radial growth at 25°C and potato dextrose agar at 30°C. The mycelial growth was slow at 20°C on all media tested.

Molecular characterization of *Phaeoacremonium aloeophilum* isolated from grapevines in Castilla y León (Spain). L. Martín and M.T. Martín. *Departemento Viticultura, ITACYL, Ctra. de Burgos Km 119, 47071 Valladolid, Spain.*
E-mail: marvilte@itacyl.es

Genetic diversity among isolates of *Phaeoacremonium aleophilum,* one of the major causal organisms of grapevine decline in Castilla y Leon, was determined using random amplified polymorphic DNA (RAPD), six multigene nucleotide sequences, and amplified fragment length polymorphisms (AFLP) techniques. RAPD markers were used to determine the degree of genetic variation of thirty-six *Pm. aleophilum* isolates mainly from grapevines in Castilla y León, Spain. Reference *Phaeoacremonium scolyti*, *Phaeoacremonium viticola* and *Phaeoacremonium parasiticum* were also included. Seventy-six random amplified polymorphic DNA markers were observed generated by twelve primers. A dendrogram depicting RAPD patterns of genetic variation among 36 isolates of *Pm. aleophilum* showed 3 main groups. Moreover, thirty *Pm. aleophilum* collected from different production areas and grapevine varieties were tested by nucleotide sequences amplified by PCR from six well known genomic regions. Isolates were subjected to PCR to amplify the nuclear 5.8S ribosomal RNA gene and its flanking ITS regions (539bp) showing 99.6% homology. Two fragments of the β-tubulin gene (614 bp and 525 bp) were amplified, 7 nucleotides variation was found including a punctual deletion of two nucleotides. A partial 5'end of the translation elongation factor 1-alpha-gene EF (384 bp) gave 4 nucleotides variation. A fragment of 262 bp from the actine gene was sequenced and 3 nucleotides variation grouped the 30 isolates studied in 4 groups. Within the six genes studies amplicon obtained from calmodulin partial gene (479 bp), was the most informative showing 14 nucleotides variation. Phylogenetic analyses of combined DNA sequence data revealed the presence of a total of thirty nucleotide differences among thirty isolates of *Pm. aleophilum* and revealed the presence of three groups which are in concordance with results from RAPD analysis. In addition, from 17 selected *Pm. aleophilum* isolates AFLP analysis were performed. Five primer combinations were used producing 372 scorable markers, of which 81.7% were polymorphic. A dendrogram depicting AFLP patterns of genetic variation among the selected isolates showed the same 3 groups that were found with RAPD and multigene nucleotide sequences.

Survey of grapevine weeds as potential hosts of black foot and Petri disease pathogens. C. Agustí-Brisach, D. Gramaje, 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: caragbri@upvnet.upv.es

Weeds were sampled in grapevine mother fields and commercial vineyards in Spain in 2009 and evaluated as potential hosts of black foot and Petri disease pathogens. Weed plants were carefully dug out from the soil to keep the root system intact and taken back to the laboratory for immediate processing. Weed plant samples were nonsymptomatic and roots showed some discolorations or necrotic lesions. For *Cylindrocarpon* isolation, root sections were cut from necrotic areas, washed under running tap water, surface-disinfested for 1 min in a 1.5% sodium hypochlorite solution and washed twice with sterile distilled water. Small root pieces were plated on PDAS. For Petri disease pathogens, isolations were made from sections (10 cm long) that were cut from the basal stem and disinfected as previously described. Small pieces of internal xylem tissues were plated on MEAS. Plates were incubated for 10–15 days at 25°C in the dark. Emerging colonies were transferred to potato dextrose agar (PDA) for sporulation and morphological identification. Identity of *Cylindrocarpon* species was confirmed by a multiplex PCR system using a set of three pair of specific primers (Mac1/MaPa2, Lir1/Lir2 and Pau1/MaPa2). Identification of Petri disease pathogens and *Cadophora luteo-olivacea* was confirmed by analyses of DNA sequences of the β-tubulin and ITS genes, respectively. *C. macrodidymum* was isolated from roots of several weed species in different locations. *Phaeomoniella chlamydospora* and *C. luteo-olivacea* were isolated only once from xylem tissue of *Convolvulus arvensis* and *Bidens subalternans*, respectively.

Study of the genetic variability of Brazilian populations of *Cylindrocarpon* spp., causal agent of grapevine black foot. A. Russi, R. Nalin, G. Dequigiovanni, R. Gava, V. Quecini, L.R. Garrido and P. Ritschel. *Molecular Biology Laboratory, Embrapa Grape and Wine. Zip Code 95700000, Bento Gonçalves, Brazil.*
E-mail: patricia@cnpuv.embrapa.br

Two fungal species have been historically associated with grapevine black foot, *Cylindrocarpon destructans* and *C. macrodidymum*. Analyses of DNA sequences showed that *C. destructans* is represented by a complex of several species and, recently, grapevine isolates associated with black foot have been reidentified as *C. liriodendri*. *C. destructans* was identified in Southern

Brazil, but little is known about the fungus variability. The purpose of this work was to evaluate the genetic diversity of *Cylindrocarpon* in Southern Brazil. Twenty-four isolates were obtained from 12 localities and eight wine grapevine plants showing symptoms of black foot. DNA was extracted and PCR amplified. The study was initiated with an investigative RAPD analysis to probe the variability of the fungus using 140 fragments produced by 32 arbitrary primers. Samples were then analyzed via ITS region amplification using primers ITS4 and ITS5, followed by digestion with seven restriction enzymes. NTSYS software package was used to estimate similarity between accessions as well as classification by the clustering algorithm UPGMA. Finally, selected ITS fragments were sequenced and compared to *C. destructans* and *C. liriodrendri* sequences deposited at GenBank using ClustalX algorithm. Trees were obtained by TreeV32. RAPD dendrogram revealed a range of similarity (DICE coefficient) from 31% to 96%. Similarity based on ITS analysis varied from 16% to 100% and eleven patterns of enzyme digestion were observed. One representant of each pattern was sequenced. Sequences of Brazilian *Cylindrocarpon* clustered with *C. liriodrendri* isolate CBS117526 collected from *V. vinifera* in Portugal.

Identification of Botryosphaeriaceae spp. associated with *Vitis vinifera* arm dieback. A. Morales[1], X. Besoain[1] and B. Latorre[2]. *[1]Facultad de Agronomía, Pontificia Universidad Católica de Valparaíso, Casilla 4-D, Quillota, Chile. [2]Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Casilla 306-22, Santiago, Chile.*
E-mail: andrea.morales.t@gmail.com

In Chile, table grapes for fresh market reach an area of 55,119 ha, being the most important crop in the national fruit industry. Preliminary studies have described *Botryosphaeria dothidea* and *B. obtusa*, causing canker and arm dieback in table grape vineyards. In this context, our work had the aim to determine the prevalence of arm dieback disease and its damage and to determine which Botryosphaeriaceae species are present in table grape vineyards located in the Valparaíso and Metropolitan regions, where quadrants of 100 plants were randomly selected in each vineyard. A damage index was calculated using a scale varying from 0 (= healthy plant) to 4 (= plant with four disease arms). In the different surveyed vineyards, chlorosis, necrosis and leaf deformation were observed as well as intermodal shortened, arm dieback, vascular discoloration and cankers with a "V"-shape. The prevalence of the disease ranged from 22 to 69% in vineyards aged 11 to 22 years. Damage index were used to build a potential curve was obtained between the plant age and the damage index. On the other hand, samples collected from 22 different vineyards were examined by isolations and three Botryosphaeriaceae species were identified: *Diplodia seriata* (=*B. obtusa*), *D. mutila* (=*B. stevensii*) and *Dothiorella viticola* (= *B. viticola*). These species were identified on the basis of either their morphological characteristics or on the comparison of their internal transcript region (ITS1-5,8S-ITS4) with sequences provided by gene bank (Genbank NCBI).

Identification and distribution of the Botryosphaeriaceae associated with grapevine decline in south-eastern Australia. W.M. Pitt, R. Huang, C.C. Steel, M. A. Whitelaw-Weckert and S. Savocchia. *National Wine and Grape Industry Centre, School of Agricultural and Wine Sciences, Charles Sturt University, Wagga Wagga, NSW 2678, Australia.*
E-mail: wpitt@csu.edu.au

Botryosphaeriaceae species are recognised as important pathogens of grapevines both in Australia and overseas. The identity, prevalence, and distribution of Botryosphaeriaceae species in vineyards throughout the major winegrowing regions of New South Wales (NSW) and South Australia (SA) was determined to provide a foundation for improved disease prevention and management. Field surveys from 91 vineyards across NSW and SA resulted in the collection of 2239 diseased wood samples and subsequent isolation of 1258 Botryosphaeriaceae isolates. Morphological identification along with phylogenetic analysis of ribosomal DNA internal transcribed spacer regions (ITS1-5.8S-ITS2) and partial sequences of the translation elongation factor 1-α gene (EF1-α) showed that eight Botryosphaeriaceae species including *Diplodia seriata*, *Diplodia mutila, Lasiodiplodia theobromae*, *Neofusicoccum parvum, Neofusicoccum australe, Botryosphaeria dothidea, Dothiorella viticola*, and *Dothiorella iberica* occur on grapevines in south-eastern Australia. The prevalence of individual species varied according to geography and climate. Species of *Diplodia* and *Dothiorella*, characterised by thick-walled, pigmented conidia were the most prevalent and were distributed widely throughout both NSW and SA. Species with hyaline conidia, such as *Neofusicoccum* and *Fusicoccum*, were isolated less frequently and displayed more limited geographic ranges, whilst only a single isolate of *Lasiodiplodia* was recovered, this being from the northern most region of NSW. Other trunk disease fungi isolated included *Phomopsis viticola, Phaeomoniella chlamydospora, Eutypa lata* and other diatrypaceous species. We have established a sound base for control strategies based on the prevailing species in Australian viticultural regions.

Observation of *Neofusicoccum parvum* decline in French young vineyards. P. Larignon *Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France.*
E-mail: philippe.larignon@vignevin.com

In one viticultural area in French vineyards (Côtes-de-Provence), some important declines were observed in young plantations. These plants between 3- and 6-years-old were characterized by dieback of shoots accompanied by leaf drop, shrivelling and drying of fruit clusters. In the most serious cases, grafts had died and from the rootstock, it was possible to observe new vegetation. In the wood of such grapevines, were observed some brown stripes and brown necroses in wedge shape which were especially developed well in the graft-union. Isolations made on 27 vines of different cultivars (Cinsault, Grenache, Syrah, Sauvignon) collected between 2007 and 2009 in 8 vineyards in "Les Côteaux de la Sainte-Victoire" revealed especially the presence of *Neofusicoccum parvum* (92% of examined plants). The fungus was isolated from the graft union, scion, and rootstock and especially occurred in the brown stripe and the brown sectorial necrosis. Other fungi were found but in low frequencies in these plants: *Acremonium* sp. (11%), *Alternaria* sp. (14.8%) *Aspergillus* sp. (3.7%), *Botryosphaeria dothidea* (3.7%), *Chaetomium* sp. (7.4%), *Clonostachys rosea* (3.7%), *Diplodia seriata* (3.7%), *Eutypa lata* (7.4%), *Fusarium* sp. (11%), *Phaeoacremonium aleophilum* (11%), *Phaeomoniella chlamydospora* (22%), *Phomopsis* sp. (11%). Pathogenicity tests made on Sauvignon cuttings inoculated during the callusing showed that *N. parvum* caused the decline of the vegetation and important necroses in the xylem four months after the inoculation. From these studies, it was found that *N. parvum* should probably be responsible for this decline.

Identification of Botryosphaeriaceae anamorphs associated with grapevines in Brazil. R. Gava, M. Menegotto, A. F. Urben and L.R. Garrido. *Phytopathology Laboratory, Embrapa Grape and Wine. Zip Code 95700000, Bento Gonçalves, Brazil.*
E-mail: garrido@cnpuv.embrapa.br

Several species of Botryosphaeriaceae have been isolated frequently from grapevines showing decline, dieback symptoms and brown-wood streaking. A major problem of the grape and wine industry concerns the correct identification of the Botryosphaeriaceae species causing disease on vines since the diversity of anamorph in this family leads to some taxonomic difficulties. In this study, 42 isolates of anamorphic Botryosphaeriaceae associated with grapevines were found in four states of the South and Southeast of Brazil, and were distinguished after their anamorph characteristics such as colony and conidial morphology. Samples were obtained from 29 cultivars of wine and table grapes. Small pieces of cankered tissues were placed onto potato dextrose agar (PDA) and cultures were incubated at room temperature on 24-h light cycle until sporulation. Squashed mounts of pycnidia were prepared in lactophenol. Morphological observations and measurements of conidial dimensions were made under a light microscope. *Diplodia* sp. was the most frequently isolated species from cankers, found in thirty-three samples. Four isolates were identified as *Neofusicoccum luteum*, one isolate as *Neofusicoccum aesculi* and four isolates as *Lasiodiplodia theobromae*. This study shows the presence and diversity of Botryosphaeriaceae taxa on grapevine in Brazil. All the Botryosphaeriaceae species found in this study have already been reported in other worldwide grape-growing areas.

Genetic variability of Botryosphaeriaceae associated with grapevine in Brazil using RAPD and polimorphisms of rRNA-ITS region. R. Nalin, A. Russi, G. Dequigiovanni, R. Gava, V. Quecini, L.R. Garrido and P. Ritschel. *Molecular Biology Laboratory, Embrapa Grape and Wine. Zip Code 95700000, Bento Gonçalves, Brazil.*
E-mail: patricia@cnpuv.embrapa.br

In Brazil, little is known about genetic diversity of Botryosphaeriaceae causing grapevine decline. The purpose of this work was to evaluate the genetic variability of Botryosphaeriaceae isolates from South and Southeast Brazil, in order to provide support to morphological identification of species. Forty-four Botryosphaeriaceae isolates from the collection of Embrapa Grape and Wine obtained from 29 cultivars of wine and table grapes showing dieback symptoms were used in this study. DNA was extracted and molecular analysis proceeded by PCR amplification of fragments. Initially, an exploratory RAPD analysis was carried out to investigate isolates variability. For this analysis, 110 fragments from 24 arbitrary primers were studied. Subsequently, the ITS region was amplified using primers ITS4 and ITS5, and the fragments were digested with nine restriction enzymes. NTSYS software package was used to estimate similarity between accessions as well as classification by the clustering algorithm UPGMA. The dendrogram resulting from RAPD analysis showed a range of similarity (DICE coefficient), between 45% and 100%. ITS similarity varied from 24% to 100%. On both analyses, two clades were observed. The former put together three anamorph species related to Botryosphaeriaceae, *Sphaeropsis viticola Fusicoccum luteum* and *Fusicoccum aesculi*. *Lasiodiplodia* genus clustered with the second group. In order to provide further insight on the molecular characteriza-

tion of the fungal species, ITS products will be sequenced and compared to fragments deposited in GenBank. Both analyses confirm Botryosphaeriaceae variability in Brazil and provide support to the morphological study.

Molecular and cultural approaches to characterize the microflora that colonizes wood tissues from vines affected by esca. J. Gerbore, J. Vallance, T. Novoa, P. Lecomte, L. Guerin-Dubrana and P. Rey. *UMR 1065 Santé Végétale INRA-ENITAB, Institut des Sciences de la Vigne et du Vin, Ave. E. Bourleaux, BP 81, 33883, Villenave d'Ornon cedex, France.*
E-mail: prey@bordeaux.inra.fr

The knowledge about the microflora associated with esca decline is still incomplete. Two approaches were developed to characterize the microorganisms (mainly fungi) that colonize the grapevine wood. First, isolations on malt-agar media were done from *i)* apparently healthy and necrotic tissues (e.g. brown stripes) collected from vines showing recent (a few days) and 1-month-old leaf symptoms and *ii)* external apparently healthy tissues of asymptomatic vines. Whatever the cultivar investigated, i.e. Cabernet Sauvignon, Merlot, Cabernet Franc, it was pointed out that an abundant and diverse fungal community colonized the wood of vines either *i)* from altered wood tissues as well as healthy ones or *ii)* from vines showing esca symptoms or asymptomatic ones. In recent years, molecular tools have indicated that conventional plating only reveals a small proportion of the microbial populations. Molecular fingerprinting technique such as PCR-SSCP (Single Strand Conformation Polymorphism) analyses based on partial rDNA amplification of genes extracted from a microbial community is potentially useful for elucidating the microbial diversity of these populations. In the second approach, PCR-SSCP results also pointed out that a large diversity of fungi colonized the grapevine wood and that bacterial communities colonized these wood tissues too. The pathogenic, neutral or protective potential of several wood-colonizing microorganisms of diseased or healthy vines still remains to be determined.

Studies of Eutypa dieback of grapevine in Serbia. S. Gajić[1], V. Trkulja[2], M. Ivanovic[1] and T. Vasić[3]. *[1]Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Serbia. [2]Agricultural Institute of Republic of Srpska, Knjaza Milosa 17, 78000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina. [3]Institute for forage crops, Trg Kosturnice 50, 37000 Kruševac, Serbia.*
E-mail: *gajicsanja43@gmail.com*

Eutypa lata causes a serious disease of grapevine called Eutypa dieback (eutypiose) that may lead to significant reduction of yield. Eutypa dieback develops slowly and has highly variable symptom expression making vineyard diagnosis difficult. The first noticeable symptoms in spring are stunted shoots with small, chlorotic, cupped leaves. In the wood, infection results in a brown lesion which appears as wedges in cross sections of diseased wood. In this study four isolates of *E. lata* from Serbia (VL17, VL27, VL29 and VL30) were used along with two isolates previously identified in France (8F and B.X1.10), as controls. Isolates were analysed by PCR with ITS1/ITS4 primers using methods described by Rolshausen *et al*. (2004). Morphological characteristics of the isolates were studied using light microscopy. Pathogenicity of isolates was determined using the methods of Sosnowski *et al*. (2005) by inoculating stems of rooted cuttings with mycelial agar plugs using varieties which commonly express Eutypa dieback symptoms in Serbia: Riesling Italien white, Tamnjanika, Prokupac, Drenak, Cardinal, Rkaciteli and Cabernet Sauvignon. Foliar symptoms were assessed eight months after inoculation. All isolates were confirmed as *E. lata* based on PCR analysis. The growth of cultures on agar was similar between isolates and mycelium filled Petri dishes after 7 days. Cultures are initially white and cottony, cream-coloured on the reverse side, with no fruiting structures. After 2 weeks, some cultures develop a grey pigment, and the reverse side may become almost black. Pathogenicity of isolates was similar but some differences in symptom expression were observed between varieties. Further study of this disease is in progress, with the aim of better recognition and finding adequate measures to control it.

Disease Detection and Losses

A rapid diagnostic of grapevine trunk diseases causal agents with a specific 4-plex-Q-PCR assay. J. Pouzoulet[1], L.G. Morello[2], N. Mailhac[1] and M. Lummerzheim[1]. *[1]Laboratoire d'Agrophysiologie, Ecole d'Ingénieusr de PURPAN, 75 Voie du TOEC, 31076 Toulouse Cedex 3. [2]Laboratorio de Microbiologia, Universidade Estadual de Londrina, PR, Brazil.*
E-mail: nathalie.mailhac@purpan.fr

Petri disease on young grapevines and Esca on older vines are caused by *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* in association with some other fungi. A number of Botryosphaeriaceae species, amongst which *D. seriata,* have been isolated and found to be associated with dieback of grapevines worldwide and have been identified as causal agents of black Dead Arm (BDA). *Eutypa lata* causes Eutypa dieback which affects vineyards all over the world. In fact, numerous fungal pathogens are supposed to be present on canes surface. They take advantage of wounds made along the process to infect plant material during soaking and stratification

at the nursery step, and during vineyard management. These cryptogamic ascomycetes have in common a slow growth and induce extremely complex and variable symptom expression on *Vitis vinifera*, making diseases identification using traditional isolation methods problematic. In the goal to propose a quick detection and quantification tool to appreciate this contamination, we developed specific primers of four of the main causal fungi associated to grapevine trunk diseases (*P. chlamydospora, P. aleophilum, D. seriata,* and *E. lata)*. In this work, we discuss about preliminary developmental steps, tests of primers specificity and results obtained on a mixture of pure fungal DNA than on healthy and infected young vines in nurseries and fields. The results are compared with the ones obtained through the microbiological method.

A quantitative PCR method for detecting two *Cylindrocarpon* species in soil. C.M Probst, M.V. Jaspers, E.E. Jones and H.J. Ridgway. *Ecology Department, Agriculture and Life Sciences Faculty, Lincoln University, P.O. Box 84, Lincoln, 7647 New Zealand.*
E-mail: Marlene.Jaspers@Lincoln.ac.nz

Black foot of grapevines, caused by several species of *Cylindrocarpon* and related genera, is soil-borne, and so this research aimed to quantify its soil infestation levels. A quantitative PCR (qPCR) using species specific primers for the β-tubulin gene was developed to test large (~10g) soil samples for presence of two *Cylindrocarpon* species, *C. macrodidymum* and *C. liriodendri*. Vineyard soil in 5 L pots was infested with each species to final concentrations of 10^5 conidia per gram of soil, using conidium suspensions ($10^6 \cdot mL^{-1}$) prepared from three isolates per species. After 0, 1, 2, 3 and 6 weeks, soil samples (2×15 g) were taken from each pot for DNA extraction. The extracted DNA was amplified by qPCR using SYBR green chemistry and a *Rox* internal standard on an ABI Prism 7700 sequence detector. Results showed that the species specific primers could detect as little as 3 pg of the specific DNA in soil, which was equivalent to 30 *Cylindrocarpon* macroconidia or 3 conidia per g of soil. The time-course assay showed 100% recovery at time 0, but after 1 week in soil, the quantity of DNA detected was equivalent to about 10% of the conidium nuclei, and this remained the same in subsequent samples. Parallel experiments, on effects of the soil environment on conidia and mycelium, provided an explanation for the apparent loss of DNA.

Botryosphaeriaceae infection in New Zealand grapevine nursery plant materials. R. Billones, E.E. Jones, H.J. Ridgway and M.V. Jaspers. *Lincoln University, P.O. Box 84, Lincoln University, Canterbury, New Zealand.*
E-mail: billoner@lincoln.ac.nz

This study tested different grapevine nursery plant materials for presence of Botryosphaeriaceae species. Tissue samples (0.5 cm.) were cut from surface-sterilised grapevine samples [apparently healthy grafted plants, failed grafted plants (or Grade 2 plants), scion and rootstock cuttings] collected from nine grapevine nurseries around New Zealand for isolation onto potato dextrose agar plates. The *Botryosphaeria*-like isolates were identified by conidial characteristics and molecular methods. Twenty-three percent of the propagation materials and plants received had Botryosphaeriaceae infection. The majority of the isolates were identified as *Neofusicoccum luteum* (59%) and *N. parvum* (20%). Other species isolated were *Neofusicoccum australe*, *Botryosphaeria dothidea*, *Diplodia seriata*, *D. mutila* and four isolates that still need further molecular identification. The majority of Botryosphaeriaceae species (49%) were isolated from 1 cm above or below the graft unions of failed/Grade 2 and Grade 1 plants, but also in the scions (10%) and the rootstocks (3%) of the grafted plants. The scion and rootstock cuttings collected from the mothervines had 17% and 21% of the Botryosphaeriaceae infections, respectively, mostly from the middle and basal parts of the cuttings. The distribution of Botryosphaeriaceae species on different parts of the plants was statistically significant using a Pearson Chi-square test ($P<0.05$). These results show that Botryosphaeriaceae species are present in grafted plants in New Zealand, providing a primary source of vineyard infection. In the propagation nurseries, the sources of the infection are likely to be the infected canes, from infected mothervines and contamination of propagation tools or materials.

Development of crop loss models for Eutypa dieback in 'Concord' grapevines to aid grower decision making. S. Jordan, A. Jarosz and A. Schilder. *Department of Plant Pathology, 107 Center for Integrated Plant Systems, Michigan State University, East Lansing, Michigan, USA.*
E-mail: schilder@msu.edu

The effect of Eutypa dieback on the yield of 'Concord' grapevines in Michigan was studied and crop loss models were developed. Data from selected vines (n=421) were collected for 3 years in three commercial vineyards with natural *Eutypa lata* infections. There was a significant relationship between the disease severity rating and the number of shoots and clusters. Yield loss can partially be explained by a reduction in both. Yield loss models were developed for individual vineyards by year. A cumulative model and a reduced model (not including the number of shoots as an independent variable) were developed for each measure of disease severity. When using the percent symptomatic shoots as the measure of disease severity, the goodness of fit (R^2) for the indi-

vidual vineyard models ranged from 0.50 to 0.86 while the R^2 was 0.66 for the cumulative model was and 0.60 for the reduced model. When using a disease severity scale (0 to 4, where 0 = healthy and 4 = almost dead), the goodness of fit for the individual vineyard models ranged from 0.59 to 0.90 while R^2 was 0.67 for the cumulative model and 0.58 for the reduced model. The models developed in this study were combined with economic data to produce cost-benefit-analysis tools to aid producers in making management decisions.

Sampling strategies for PCR based detection of *Roesleria subterranean*. S. Neuhauser[1], R. Held[1], L. Huber[2] and M. Kirchmair[1]. [1]*Institute of Microbiologoy, Faculty of Biology, University Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Austria.* [2]*Geisenheim Research Centre, Department of Grapevine Breeding and Grafting, Eibinger Weg 1, 65366 Geisenheim, Germany.*
E-mail: Sigrid.Neuhauser@uibk.ac.at

Roesleria subterranea is a hypogeous fungal pathogen of grapevine and has recently been reported to cause severe losses in vineyards in Germany, the USA, Hungary, Austria and Canada. A PCR based standard operating protocol (SOP) for the detection of this pathogen was drafted and assessed in a German vineyard with known infestation. Samples were taken three times during the vegetation period (23 May 2008, 14 Jul 2008, 6 Oct 2008). To facilitate a rapid sampling soil samples were randomly taken 10–20 cm beside the grapevine-trunk underneath the row from the upper 20 cm soil horizon with a spade at the trail vineyard (2 ha, n = 40). To identify the best sampling strategy all 40 samples were processed (i) individually and (ii) pooled to one sample. *Roesleria subterranea* could be detected in 42.5–47.5% of the individual soil samples and 25–53.3% of the root samples; it could not be detected in the pooled soil samples, although 30 sub-samples were processed at each sampling point. When the results of individual soil samples were plotted onto growth assessment data, it became evident that positive samples mainly came from the margin of areas with diseased vines. Our data indicate that pooling soil samples leads to wrong-negative results because of dilution effects. More small individual samples taken at the right places at the edge of an infested area result in a more reliable detection of fungal pathogens in soils.

Fungal trunk pathogens associated with declining Syrah vines in grapevine nurseries and young vineyard. D. Gramaje[1], R.M. Muñoz[2], M.L. Lerma[2], J. García-Jiménez[1] and J. Armengol[1]. [1]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022, Valencia, Spain.* [2]*Servicio de Diagnóstico y Asistencia Fitosanitaria, Instituto Técnico Agronómico Provincial de Albacete, Avda. Gregorio Arcos s/n, 02080, Albacete, Spain.*
E-mail: dagrape@upvnet.upv.es

"Syrah decline" has been increasingly observed and reported in many vineyards worldwide. In recent years, an increase of samples of *Vitis vinifera* cv. Syrah showing symptoms of general decline has also been noted in Spain. Sixty-two samples of Syrah grafted grapevines with such symptoms were obtained from grapevine nurseries and young vineyards between 2007 and 2009 and subjected to fungal isolation. Species identification was performed by means of morphological and molecular methods. Several species of *Phaeoacremonium,* Botryosphaeriaceae and *Cylindrocarpon*, as well as *Phaeomoniella chlamydospora* and *Cadophora luteo-olivacea* were isolated from symptomatic Syrah vines. This study demonstrates that fungal pathogens should be considered as a potential factor associated with 'Syrah decline'.

Molecular detection of *Cylindrocarpon* and *Campylocarpon* species associated with black foot disease of grapevines in South Africa. L. Mostert[1], S. Safodien[2], P.W. Crous[3], P.H. Fourie[1,4] and F. Halleen[2]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bax X1, Matieland, 7602, South Africa.* [2]*ARC Infruitec-Nietvoorbji, Private Bag X5026, Stellenbosch, 7599, South Africa.* [3]*Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, Netherlands.* [4]*Citrus Research International P.O. Box 28, Nelspruit, 1200, South Africa.*
E-mail: lmost@sun.ac.za

Black foot of grapevines is a serious disease that can cause substantial losses especially in newly established vineyards. In South African vineyards, black foot is caused by two *Cylindrocarpon* species (*Cy. liriodendri* and *Cy. macrodidymum*) and two *Campylocarpon* species (*Ca. fasciculare* and *Ca. pseudofasciculare*). Diseased vines are more often infected with *Cylindrocarpon* species. The identification of these species on phenotypic characters is difficult and isolations from plant material take longer than molecular detection methods. Therefore species-specific primers were developed from the beta-tubulin nuclear gene area to detect the different species from soil and grapevine root material. Primers were designed for all four taxa: *Cy. liriodendri* (CyliF and CyliR), *Cy. macrodidymum* (CymaF and CymaR), *Ca. fasciculare* (CafaF and CafaR) and *Ca. pseudofasciculare* (CapsF and CapsR). The PCR product sizes were respectively 192, 304, 350 and 339 bases long. A touch-down PCR program starting at 66°C included the different annealing temperatures of the primer sets and allowed that different reactions can be done on the same

PCR block. The primers were specific and did not amplify 12 different fungi associated with the soil, grapevine roots or rootstocks. To increase the sensitivity of the PCR a nested PCR was developed by using the universal primers T1 and T2 for the first round of amplification. The optimisation of the nested PCR is currently underway. Soil and root samples of vines showing black foot symptoms will be screened with the nested PCR.

Development of species-specific primers for the identification of *Cylindrocarpon liriodendri*, *C. macrodidymum* and *C. pauciseptatum* from grapevine. S. Alaniz[1,2], J. Armengol[2], J. García-Jiménez[2], P. Abad-Campos[2] and M. León[2]. [1]*Departamento de Protección Vegetal, Facultad de Agronomía, Universidad de la República, Garzón 780, CO 12900, Montevideo, Uruguay.* [2]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.*
E-mail: salaniz@fagro.edu.uy

Black foot disease is a severe disease of grapevines caused by C*ylindrocarpon liriodendri* and *C. macrodidymum*. Recently, a third species, *C. pauciseptatum,* has been isolated from roots of grapevine showing decline symptoms. To date, the reliable identification of isolates of these species through phenotypical characteristics has not been possible. The PCR-based method developed in this study, allows a quick and easy detection of *Cylindrocarpon* spp. associated with grapevine. Three primer pairs annealing to variable, partly species-specific sites of the ITS regions amplified species-specific PCR fragments of different sizes in *C. liriodendri* (253 bp), *C. macrodidymum* (387 bp) and *C. pauciseptatum* (117 bp) in a multiplex assay. They did not generate any PCR product in other fungal trunk pathogens or contaminants commonly associated with grapevines list the ones you tested. When universal fungal ITS primers were used in a nested multiplex PCR, the three primer pairs also detected *C. liriodendri, C. macrodidymum* and *C. pauciseptatum* in total DNA extracted from roots of inoculated grapevines. The designed methods developed in this work can be used for a rapid diagnosis of these fungi from pure culture or infected grapevines plants.

Host-Pathogen Interactions

Phytotoxic polysaccharides produced by two fungi involved in grapevine trunk diseases, *Phaeomoniella chlamydospora* and *Neofusicoccum parvum*. A. Cimmino[1], A. Andolfi[1], M. Masi[1], L. Mugnai[2], G. Surico[2], T. Cinelli[2], C. Comparini[2], J. Luque[3], A. Motta[1] and A. Evidente[1]. [1]*Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Università di Napoli Federico II, 80055 Portici, Italy.* [2]*Dipartimento di Biotecnologie-Patologia Vegetale, Università di Firenze, 50144 Firenze, Italy.* [3]*Institut de Recerca i Tecnologia Agroalimentàries (IRTA), 08348 Cabrils, Barcelona, Spain.*
E-mail: alessio.cimmino@unina.it

Phaeomoniella chlamydospora is a tracheomycotic pathogen that is associated with all the syndromes in the esca complex (brown wood streaking of the rootstock, Petri disease, young esca), one of the most important grapevine trunk diseases worldwide. In recent years an increasing number of species of Botryosphaeriaceae has also been associated with grapevine decline, with several species causing dieback, cankers, and characteristic wedge-shaped necrosis in arms and trunks. In order to understand the development of decline symptoms and colonization by fungi in the wood investigations have been carried out on the activity of metabolites produced by the pathogens involved. In preliminary chemical investigations *P. chlamydospora*, *Phaeoacremonium aleophilum* as well as several Botryosphaeriaceae species (including *Neofusicoccum parvum*) have been shown to produce lipophilic low molecular weight compounds (scytalone, isosclerone, melleins, etc.) and high-molecular weight phytotoxins, the latter appearing to be exo-polysaccharides (EPSs). In this communication the chemical and biological characterization of the EPSs produced by *P. chlamydospora* and *N. parvum* are reported, including the evaluation of their phytotoxic activity on host and non-host plantlets.

Interaction between *Cylindrocarpon* and glyphosate in young vine decline. M. A. Whitelaw-Weckert. *National Wine and Grape Industry Centre, Wagga Wagga, NSW, Australia.*
E-mail: mweckert@csu.edu.au

Young Vine Decline (YVD) has been a serious problem for many new vineyard plantings in Australia over the past decade. Affected vines either die soon after planting or they survive but suffer from poor vigour and low yields for many years. We have consistently isolated plant pathogenic fungi *Cylindrocarpon*, *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp. and *Botryosphaeria* spp. from the roots and trunks of the declining young grapevines. It appears that we are the first to regularly isolate Botryosphaeriaceae species from the roots and lower trunk of grapevines, indicating that the fungus may not originate from pruning wound infections but possibly from the soil or inside the roots or wood when planted. Pot experiments showed that under water stress, glyphosate applied to the soil at 0.36 kg a.i. ha^{-1} increases the

severity of the disease symptoms caused by *Cylindrocarpon*. Shoot growth was decreased by 89% early in the season compared with diseased vines without glyphosate. As glyphosate is commonly used in Australian viticulture, we will need to investigate this interaction further.

Infection and disease progression of *Neofusicoccum luteum* in grapevine plants. N.T. Amponsah, E.E. Jones, H.J. Ridgway and M.V. Jaspers. *Department of Ecology, Faculty of Agriculture and Life Sciences, P. O. Box 84, Lincoln University, New Zealand.*
E-mail: Nicholas.Amponsah@lincoln.ac.nz

Monitoring of disease progression by microscopy is an important component of the study of host-pathogen interactions. The aim of this research was to investigate infection processes and the progression of *Neofusicoccum luteum* infection of grapevine leaves and shoots. Conidial suspensions (10^4 conidia mL^{-1}) were inoculated onto wounded or non-wounded leaves and shoots of 18-month-old potted Pinot Noir vines growing in a shade house. The tissues remained attached, or were detached and incubated under high relaive humidity (RH) after inoculation. Scanning electron microscope (SEM) observation and pathogen re-isolation from the leaves at 24 to 72 h after inoculation and with the shoots at monthly intervals for 4 months. Results showed no infection in any non-wounded shoots or leaves. In wounded attached shoots and leaves, SEM at 24 h after inoculation, showed that conidia had germinated and germ tubes penetrated into the tissue. However, on the detached wounded shoots and leaves, the 24 h SEM studies showed more rapid pathogen development, with networks of mycelium present on plant surfaces. These differences may have been associated with presence of inhibitory plant responses which were more active in attached than detached tissues. Further SEM of the shoots at 3 months after inoculation showed hyphae growing through the vessels. Detection of pathogen movement by re-isolation at 1 cm intervals below and above the inoculation points showed pathogen progression was greater in the upward than the downward direction, possibly because it followed nutrient flow in xylem vessels.

Transformation of *Eutypa lata* and *Eutypella vitis* by restriction enzyme-mediated integration. S. Jordan, F. Trail and A. Schilder. *Department of Plant Pathology, 107 Center for Integrated Plant Systems, Michigan State University, East Lansing, Michigan, USA.*
E-mail: schilder@msu.edu

The role of phytotoxins in Eutypa dieback disease development is unknown. To enable the study of the role of phytotoxins as either pathogenicity or virulence factors, putative toxin-deficient mutants were generated. *Eutypa lata* isolate E30, a highly virulent, phytotoxin-producing isolate, was chosen for transformation. A restriction enzyme-mediated integration (REMI) protocol was developed using gGFP, a plasmid which expresses green fluorescent protein (GFP) in the fungus. Transformation efficiencies of ~15 transformants per μg of plasmid DNA were obtained, a tenfold increase from previous methods. Transformation was also successful with *Eutypella vitis*. Using fluorescence confocal microscopy, visualization of GFP-expressing transformants of *E. lata* and *El. vitis* was possible in inoculated Concord grapevine wood, confirming the role of *El. vitis* as a pathogen of grapevine. This is the first successful genetic transformation of *El. vitis*. Through REMI transformation, 2184 transformants were generated and screened for toxin production. Twenty two possible toxin-deficient candidates were identified. Southern analysis indicated single, random insertion events for all 22 candidates. Putative toxin-deficient transformants await further characterization.

Characterization of the fungal communities colonizing the internal and external wood of symptomatic and asymptomatic vines affected by esca. P. Lecomte, V. Mayet, G. Darrieutort, D. Blancard, F. Boiffard, J.M. Liminana and P. Rey. *UMR 1065 Santé Végétale INRA-ENITAB, Institut des Sciences de la Vigne et du Vin, Ave. E. Bourleaux, BP 81, 33883, Villenave d'Ornon cedex, France.*
E-mail: lecomte@bordeaux.inra.fr

In order to further characterize the microflora that colonize the various necrosis that may occur into the wood of grapevines, samples from symptomatic and asymptomatic adult vines were taken in late summer 2006–2009. Different cultivars were used. All vines showed inner necrosis into the wood. All symptomatic vines, showing leaf damages, including light or severe wilting, exhibited longitudinal stripes as previously described by Lecomte *et al.* (2006, 2009). Different types of wood chips were collected from: necrotic-tissues, tissues at their frontiers; longitudinal stripes (under the bark) and apparently healthy-tissues. Assays done from the inner wood showed that a large community of fungi colonized either the discoloured tissues or their frontiers. Interestingly, apparently healthy zones also allowed isolations of fungi whatever was the disease status. Fungi already described as wood pathogens of grapevine were frequently isolated from the five tissues. Botryosphaeriaceous fungi were the ones most often isolated. Assays carried out with tissues collected from longitudinal stripes allowed the identification of various fungi among which Botryosphaeriaceae were also predominant. Interestingly, *P. chlamydospora* and *P. aleophilum* were also identified from these wood

external zones. Whole results indicated that the grapevine wood, either necrotic or apparently healthy collected from either symptomatic or asymptomatic vines, can be colonized by numerous wood-inhabiting fungi and other micro-organisms. Bacteria and yeasts were also detected but not identified. The putative role of this microflora in the development of the esca decline is discussed.

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Lecomte, P., G. Darrieutort, A. Defives, G. Louvet, J.-M. Liminana and D. Blancard 2006. Observations of black Dead Arm symptoms in Bordeaux vineyards: evolution of foliar symptoms, localisation of longitudinal necroses, questions, hypotheses. *Integrated Protection in viticulture - OILB/wprs Bulletin* 29(11), 93–94.

Endophytes from healthy and esca diseased vines. M. Kirchmair[1], S. Trenkwalder[1], L. Huber[2] and S. Neuhauser[1]. [1]*Institute of Microbiology, Faculty of Biology, University Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Austria.* [2]*Geisenheim Research Centre, Department of Grapevine Breeding and Grafting, Eibinger Weg 1, 65366 Geisenheim, Germany.*
E-mail: martin.kirchmair@uibk.ac.at

Pathogenic fungi associated with esca symptoms share their habitat with other endophytic microorganisms. It was hypothesised that the endophytic community of plants differs with their sanitary status. The aim of this study was (i) to investigate the endophytic community of healthy and esca diseased grapevines using cultivation based techniques and (ii) to explore the ecological role of these endophytes. Xylem sap was plated onto nutrient media and fragments of surface sterilised grapevine shoots were directly plated. The isolated endophytes were identified morphologically and by DNA sequence analysis. Twenty seven hyphomycete taxa, 14 different yeast taxa and 35 different bacterial taxa were identified. Frequent hyphomycete isolates were *Alternaria* spp., *Cladosporium* spp. and *Penicillium* spp. Dominant yeast endophytes were *Aureobasidium pullulans*, *Cryptococcus magnus* and *Rhodotorula* cf. *glutinis*. The most abundant endophytic bacteria belonged to the genera *Curtobacterium*, *Frigoribacterium*, *Pantoea*, *Pseudomonas* and *Bacillus*. No differences were observed between the fungal communities of healthy and esca diseased vines, but differences in bacterial populations could be detected. Many of the hyphomycete and bacterial isolates obtained during this study are well known endophytes of grapevine and other host plants, but most of the yeasts were formerly known as epiphytes only.

No physiological alteration in grapevine leaves before the expression of the chronic form of esca disease. P. Letousey[1], F. Baillieul[1], M. Boulay[2], C. Clement[1] and F. Fontaine[1]. [1]*Laboratoire de Stress, Défenses et Reproduction des Plantes - UFR Sciences - Moulin de la Housse - BP 1039 - 51687 Reims, France.* [2]*Moët et Chandon - 6 rue Croix de Bussy - 51200 Epernay, France.*
E-mail: florence.fontaine@univ-reims.fr

Development of alternative methods to control esca requires better knowledge of the disease, including characterization of changes in grapevine physiology due to esca. Physiological and molecular approaches were used to determine if the plant physiology is affected before and during the expression of the chronic form of esca. This study was investigated in French Moët & Chandon vineyard on Chardonnay cultivar. Different leaf samples were analyzed: (i) pre-symptomatic leaves without symptoms on visually healthy plants, (ii) pre-symptomatic leaves without symptoms on diseased plants and (iii) symptomatic leaves. Control samples were leaves harvested from vines which have not developed any visual leaf symptoms since 2001. Our results showed no alteration of photosynthetic mechanisms by chronic esca before the expression of foliar symptoms. Fourteen and 7 days prior to any visible symptoms, net photosynthetic activity (Pn), stomatal conductance and activity of photosystem II of leaves were similar to controls. When chronic symptoms appeared on the plants but not on the leaves studied, three leaf types were proposed according to the intensity of the Pn: <50% Pn, 50-75% Pn and >75% Pn. Analysis by quantitative RT-PCR showed a strong repression of genes encoding Rubisco and thus explained in part the reduction in Pn activity. In addition, grapevine activated the expression of some stress-related genes encoding especially enzymes of phenylpropanoid pathway and chitinases. On symptomatic leaves, Pn decreased drastically and was correlated to both a degradation of chlorophyll and repression of genes encoding Rubisco. Stress-related genes were induced more in yellow than in green parts of symptomatic leaves. These results indicate that grapevine physiology is altered during the visual expression of leaf symptoms but no change is observed earlier.

Recent advances in the identification of the toxins from *Neofusiccocum parvum*. E. Abou-Mansour[1], J-L. Débieux, P. Larignon[2] and C. Rego[3]. [1]*Biology Department, University of Fribourg, rue Albert Gockel 3, 1700 Fribourg, Switzerland.* [2]*Institut Français de la Vigne et du Vin, Unité Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France.* [3]*Institut Supérieur d'Agronomie, Tapada da Ajuda, 1349-017 Lisbone, Portugal.*
E-mail: eliane.abou-mansour@unifr.ch

Neofusicoccum parvum (Botryosphaeriaceae, Ascomycetes) is a plant pathogenic fungus with world-wide distribution on a wide range of woody hosts. Recently, an increasing number of species of Botryosphaeriaceae have been associated with grapevine decline worldwide. Thirteen strains of *N. parvum* isolated from vines showing decline symptoms in Portugal and France were screened for phytotoxic activity. Further chemical studies of fermentation extracts of an *N. parvum* isolate Bourgogne 2-116 from a nursery plant led to the identification of a new compound along with mellein, *cis* hydroxymellein and *trans* hydroxymellein. We report here the fermentation conditions, the structural identification and the biological activity of isolated compounds on a grape leaf disc assay.

Screening phytolaexins in canes of Swiss *Vitis vinifera* cultivars as an indicator of grapevine wood disease resistance. E. Abou-Mansour[1], D. Poggiali, P. Voirin[2] and C. Arnold[3]. [1]*Biology Department, University of Fribourg, rue Albert Gockel 3, 1700 Fribourg, Switzerland.* [2]*Ecole d'ingénieurs et d'architectes de Fribourg, Bd de Pérolles 80, 1705 Fribourg, Switzerland.* [3]*Institute of Biology, University of Neuchâtel, rue Emile Argand 11, 2009 Neuchâtel, Switzerland.*
E-mail: eliane.abou-mansour@unifr.ch

Phytoalexins have been shown to possess biological activity against a wide range of pathogens and can be considered as markers for plant resistance. Phytoalexins from Vitaceae are constituted of a restricted group of molecules belonging to the stilbenes family, the skeleton of which is based on trans-resveratrol structure. Several simple stilbenes such as *trans*-pterostilbene (dimethylated resveratrol), *trans* and *cis* piceid a 3-O-α-D-glucoside of resveratrol, and oligomers of resveratrol as viniferins have also been found in grapevine as a result of infection or stress. The major compound appears to be ε-viniferin, a cyclic dehydromer of resveratrol. Grapevine wood diseases such as eutypa dieback, esca, and black dead arm, are destructive diseases affecting vineyards all over the world, caused by one or several fungal xylotrophs respectively. The main research findings concern pathogen identification, reproduction of symptoms through inoculation with various fungi, the influence of the environment on the incidence of the disease. There is no emphasis on the influence of the wood constituent such as phytoalexins on fungal growth or disease development within the bark. The aim of this study was to screen the wood of *V. vinifera* cultivars for the presence of phytoalexins in order to establish a correlation between the abundance of phytoalexins and the resistance of the cultivar to wood pathogens. Twenty Swiss red and twenty eight white cultivars were selected for this study. Phytoalexins were extracted from basal cane of healthy plants. One step extraction allowed the extraction of 87% of the phytoalexins. Samples were analysed by reverse-phase chromatography with diode array detection. Quantification of phytoalexins was performed by adding an internal standard and identification by comparison of retention and UV spectra with the standards. Standards were initially isolated and identified by MS and RMN analysis from grape stem bark. We report the quantification of *trans* resveratrol, *trans* ε-viniferin and *trans* vitisin A. Finally a hypothesis of the intrinsic genetic factors of *V. vinifera* in relation of phytoalexin biosynthesis and resistance and/or tolerance against pathogen is proposed.

Effects of water stress and inoculation with *Eutypa lata* and *Neofusicoccum parvum* on young grapevine plants. J. Luque[1], S. Martos[2] and F. García-Figuéres[3]. [1]*Dep. Protecció Vegetal, IRTA Cabrils; Ctra. de Cabrils km 2, E-08348 Cabrils, Spain.* [2]*Unitat Fisiologia Vegetal, Dep. Biologia Animal, Biologia Vegetal i Ecologia. Fac. Ciències, Universitat Autònoma de Barcelona; Campus de Bellaterra, E-08193 Bellaterra, Spain.* [3]*Laboratori de Sanitat Vegetal; Via Circulació Nord, Tram VI, Carrer 3, Zona Franca, E-08040 Barcelona, Spain.*
E-mail: jordi.luque@irta.cat

The combined effects of water stress and artificial inoculations with either *Eutypa lata* or *Neofusicoccum parvum* on the growth and water relationships of young grapevine plants were studied. Two independent experiments were conducted on one-year-old potted vines of cv. 'Tempranillo' grafted onto 110R rootstock plants during the period June–October in 2007 (*E. lata*) and 2008 (*N. parvum*). For each experiment, four groups of 40 plants were used following these combinations: 1) water stress with inoculation, 2) water stress without inoculation, 3) sufficient irrigation with inoculation, 4) sufficient irrigation without inoculation. Irrigation of water-stressed plants was done in variable periods (mostly every 5–7 days) after estimating plant water loss periodically. A Linear Variable Differential Transformer (LVDT) was installed on the shoot of eight plants in each treatment group to monitor daily microvariations of the shoot diameter. The remaining 32 plants of each treatment were used to measure stomatic conductance and leaf water potential on five randomly selected plants per group and monitoring date (every 7–14 days). Inoculations were performed with mycelial plugs placed into wounds made on the stem of the rootstock. Non-inoculated plants were treated similarly with sterile water agar plugs. At the end of the experiments, the length

of vascular necrosis in each plant was measured. For both pathogens, water stress increased the length of necroses in inoculated plants, but neither this effect nor the interaction 'stress × pathogen' were statistically significant. Fungal inoculations and water stress reduced significantly leaf water potential, but again the interaction of both factors was not significant. Water stress and the inoculation with *N. parvum* reduced significantly stomatic conductance, whilst inoculation with *E. lata* resulted in a non-significant increase of this parameter.

Endophytic fungi associated with *Vitis vinifera* in the Madrid Region (central Spain). V. González and M. L. Tello. *Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Autovía A-2, Km. 38,2. Alcalá de Henares, Spain.*
E-mail: vicente.gonzalez.garcia@madrid.org

At present, there is no data available on the composition and role of fungal endophytic communities associated with grapevines in Spain. Studies dealing with the characterization of existing relationships between the endophytic mycoflora and pathogenic taxa within *V. vinifera* are also scarce. Interestingly, some of the most important diseases of this plant species are caused by fungi that could remain inside plant tissues as saprophytes, coexisting with endophytic species in the same stand, and finally unleashing pathogenic processes, as in the case of the so-called "young vine decline" or Petri disease. This study is focused on the characterization of species diversity of endophytic fungi associated with the several varieties and culture types of grapevine existing in the Madrid region (central Spain). Based on these surveys, new biocontrol agents of fungal origin will be screened, in order to prevent and control grapevine diseases, especially those associated with young vines produced in nurseries. During 2008–2009, up to 500 fungal isolates representing 50-60 taxa were isolated from five localities in central Spain . Differences regarding type of cultivar, sampling period, as well as plant parts analyzed were detected in terms of composition and relative abundance of species. Some of the more commonly isolated strains represented taxa from genera *Alternaria, Cladosporium, Fusarium, Gibberella, Phoma, Nectria, Epicoccum, Botryotinia, Aureobasidium, Penicillium, Trichoderma* and *Acremonium*. Among the pathogenic agents associated with grapevine, *Botrytis* spp. and *Phomopsis viticola* were some of the most frequently isolated taxa. Furthermore, we have isolated several fungal strains belonging to genera *Acremonium, Phoma* (*P. glomerata*) and *Chaetomium* that have shown promising antagonistic activity *in vitro*.

Effect of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* on grapevine rootstock cuttings in Chile. G. Díaz, J. Auger and M. Esterio. *Laboratorio de Fitopatología Frutal y Molecular, Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile. 8820808, Santiago, Chile.*
E-mail: jauger@uchile.cl

Chile is a major exporter of table grape (*Vitis vinifera* L.) and one of the main exporters of quality wines from the new world, which has experienced significant growth in recent years. The rapid development of the grape growing area has increased the demand for grafted grapevines, which has led to a loss in quality of propagation material. In Chile, studies have not yet been carried out to assess the effect of the causal agent of Petri disease in the loss of the quality of grapevine cuttings. The objective of this study was to analyze the effect of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* on quality parameters in grapevine rootstock cuttings. Cuttings of five grapevine rootstocks were wounded and immediately inoculated with different conidial suspensions (approximately 5×10^3 conidia mL^{-1}) of *Pa. chlamydospora*, *Pm. aleophilum* or a combination of both. These fungi affected all quality parameters of root and callus formation in each of the five rootstocks. Among all roostocks used in this study, 1103P and 101-14 MG were the least susceptible to the infection caused by *Pa. chlamydospora* and *Pm. aleophilum*, but the rootstock SO4 was the most susceptible.

Pathogenicity and virulence of Botryosphaeriaceae associated with arm dieback of *Vitis vinifera* in Chile. A. Morales[1], X. Besoaín[1] and B. Latorre[2]. *[1]Facultad de Agronomía, Pontificia Universidad Católica de Valparaíso, Casilla 4-D, Quillota, Chile. [2]Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Casilla 306-22, Santiago, Chile.*
E-mail: xbesoain@ucv.cl

Arm dieback and canker diseases affecting grapevine have been broadly studied worldwide. Botryosphaeriaceae spp. have been identified as important pathogens associated with some of these diseases, especially arm dieback, although some differences have been detected in the virulence of this genus. The objectives of this study were: i) to evaluate the varietal susceptibility of grapevine to *Diplodia seriata, D. mutila* and to *B. dothidea* on excised vine shoots and ii) to examine the development of these fungal species on Flame Seedless plant tissue of different age. Excised plant shoots taken from Thompson Seedless, Red Globe and Flame Seedless plants, were inoculated by making a v-shaped cut through the vascular tissue, and inserting an 8

mm-diameter mycelium plug using the three fungal species.,Inoculated vines were maintained for 35 days in a humid chamber at 23°C. The results indicated there was no interaction between cultivar and species and there were no significant differences between the table grape cultivars. Considering the fungal species, all were pathogenic compared to the control, and *B. dothidea* produced significantly greater lesion length to the other species. Tests of pathogenicity on Flame Seedless plant tissues were studied in a vineyard located in San Felipe in the Valparaíso region. In this vineyard, three types of plant tissue were inoculated: >5-year-old wood, 2-year-old wood and canes from the current season. Results obtained from the length of canker development observed 137 days after inoculation, showed that there was no interaction between the inoculated species and the age of the investigated tissues. Nevertheless, when analyzing the fungal species factor, *D. mutila* was significantly more virulent than *B. dothidea*, while *D. seriata* showed an intermediate virulence

Bot canker disease of grapevines in California.
J.R. Úrbez-Torres and W.D. Gubler. *Department of Plant Pathology, University of California, Davis, CA 95616, USA.*
E-mail: jrurbez@ucdavis.edu

Bot canker disease of grapevines in California is caused by ten different Botryosphaeriaceae spp. belonging to six different genera. All ten species were confirmed to infect both young and mature grapevine tissue causing cankers, vascular discoloration, and/or otherwise dark streaking of the wood. However, virulence was observed to depend on the Botryosphaeriaceae spp. Pathogenicity tests showed *Lasiodiplodia theobromae* and *Neofusicoccum parvum* to be the most virulent species, whereas *Dothiorella iberica* and *Spencermartinsia viticola* were the least virulent. Biological studies were conducted to characterize the different Botryosphaeriaceae spp. and to understand their geographical distribution in California. Botryosphaeriaceae spp. are wood pathogens infecting mainly through pruning wounds. Therefore, knowledge of low risk infection periods and pruning wound susceptibility are critical in deciding appropriate timing for pruning and wound treatment. Epidemiological studies throughout the state revealed that Botryosphaeriaceae spore discharge occurred from the first rainfall through to the last spring rains in Californian vineyards. However, the highest numbers of spores were trapped following rain events during the winter months, which correlate with the grapevine pruning season in California. In addition to rainfall, spore release was also confirmed to be triggered by overhead sprinkler irrigation. Pruning wound susceptibility studies showed that pruning wounds can be susceptible to Botryosphaeriaceae infection for up to 13 weeks however, susceptibility decreased with age of pruning wound. Successful disease management practices have been developed against Bot canker which includes pruning wound treatments with thiophanate-methyl as well as double-pruning, a practical technique to achieve late pruning in large vineyard acreages.

Steps in understanding esca foliar symptoms and the role of toxic metabolites.
L. Cantalupo[1], L. Calamai[2], A. Gessler[3], H.H. Kassemeyer[2], L. Mugnai[1] and G. Surico[1]. *[1]Dipartimento di Biotecnologie-Patologia Vegetale, Università di Firenze, 50144 Firenze, Italy. [2]Zentrum für Biosystemanalyse (ZBSI) Albert-Ludwigs-Universität Freiburg, Habsburgerstr 49, 79104 Freiburg Germany. [1]Dipartimento di Scienza del Suolo e Nutrizione della Pianta, Università degli Studi di Firenze, Piazzale delle Cascine 18, 50144 Firenze, Italy. [3]Staatliches Weinbauinstitut, Merzhauser Str. 119, D-79100 Freiburg, Germany.*
E-mail: laura.mugnai@unifi.it

One of the most intriguing aspects of esca is the mechanism causing the foliar symptoms. Stress factors and toxic metabolites produced by the main causal organisms are thought to have an important role. In particular the role of toxic metabolites produced by *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* was investigated, studying the translocation and accumulation of scytalone and isosclerone in symptomatic leaves, as they are claimed to cause (or help to cause) photosynthesis disfunctioning, leaf discolouration with pigment production and necrosis. Specifically, the pattern of production, degradation and accumulation of scytalone (and isosclerone) in vine tissue were studied in a vineyard cv. Cabernet Sauvignon located in Tuscany and in a vineyard cv. Mueller Thurgau located in southern Germany. Levels of scytalone and isosclerone in the leaves (cv. Cabernet Sauvignon) and/or in the xylem sap (cv. Cabernet Sauvignon and cv. Mueller Thurgau) were analysed by LC/MS/MS in 2 laboratories in Italy and Germany, with limits of detection (LOD) of 50 ppb and 0.2 ppb, respectively. Scytalone was found only in trace concentrations (10–37 ppb) in 3 of 38 symptomatic leaves sampled, while isosclerone was never detected. In the spring sap of symptomatic vines no more than 0.1 ppb of scytalone was found. Production of scytalone by *P. aleophilum* grown under various stress conditions was also tested. The results obtained confirm that scytalone is linked to necrosis and the darkening of infected wood, and enters the biosynthetic pathway of melanin, but they challenge recent hypotheses on the role of scytalone (and isosclerone) in foliar symptom expression, as the toxin concentrations measured did not confirm the concentrations reported in the literature. Data reported up to now in the literature were obtained using differ-

ent, and much less sensitive, analytical techniques from the ones used in the present research, and on specimens of different cultivars and of different environments.

Identification of climatic factors favourable to the expression of black dead arm symptoms in a French vineyard. P. Larignon. *Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France.*
E-mail: philippe.larignon@vignevin.com

The aim of our study was to identify the climatic conditions favourable to expression of black dead arm symptoms. Observations were carried out between May and September from 2003–2008 on a vineyard in the South-East of the France Sauvignon blanc vines were grafted on SO4 rootstock in 1989, cordon trained and have not been treated with sodium arsenite since 2000. Weekly observations showed that symptoms appeared at flowering (late May, early June) and incidence increased until late August or early September. Symptoms appeared following increased temperature (max temperature >30°C). Incidence of symptoms increased by 4–7.5% each year (R^2=0.99). The percentage of plants with symptoms fluctuated from year to year (4.88, 8.04, 13.35, 11.68, 25.75 and 19.16% during the years 2003–2008, respectively). Comparison between symptoms and climatic data showed that there was a high correlation between the incidence of symptoms and rain in May (R^2=0.91) or rain occurring one month before the apparition of first symptoms (R^2=0.81). There was a high correlation between the average of potential evapotranspiration (ETP) for the period from expression of first symptoms to 80% symptom expression and the incidence of symptoms (R^2=0.83). Years of high incidence (2007 and 2008) were characterized by a rainy spring at least one month before the apparition of symptoms (180 and 105 mm rain in May, respectively) and by a low average ETP (5.44 and 6.78, respectively). On the contrary, years of low expression (2003) were characterized by a dry spring (22 mm) and a high average of ETP (7.64). This information may help to predict the incidence of black dead arm.

The effect of water and temperature stress on eutypa dieback. M.R. Sosnowski[1], A.P. Loschiavo[1,2], T.W. Wicks[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 dieback is caused by the fungus *Eutypa lata* which invades wood tissue causing dieback, stunted shoots, and the gradual death of grapevines. Yearly variation of foliar symptoms is thought to be due to environmental conditions. The effect of temperature and water stress on symptom expression in inoculated vines was evaluated. Potted grapevines were grown in controlled environment rooms at 14, 22 and 30°C for 7 months. Within each room, soil was maintained at low, medium and high water content. Foliar symptoms tended to be most severe on inoculated vines subjected to the upper and lower extremes of both soil water content and temperature. The most severe symptoms developed on vines grown at 30°C with high soil water content. Conversely, growth of *E. lata* from the inoculation site, assessed by isolation, appeared to be less for vines kept in extreme conditions, suggesting fungal growth may be impeded under stressful conditions. The effect of deficit irrigation on wound infection by *E. lata* was investigated in field experiments in the Barossa Valley and Riverland wine regions of South Australia. Control vines were irrigated according to annual allocation whereas deficit-irrigated vines received 0–60% of the volume applied to controls. The frequency of recovery of *E. lata* from wounds increased from 74 to 95% in vines under severe water deficit in the Riverland, yet it remained unchanged in the Barossa Valley. As the Riverland is drier and warmer than the Barossa Valley, these results suggest that vines under severe stress are more susceptible to infection by *E. lata*.

Screening for the production of exocellular enzymes by Botryosphaeriaceae species from an Italian vineyard (Tuscany) and nursery stock material. A. Spagnolo[1], G. Marchi[1], L. Mugnai[1], A.J.L. Phillips[2] and G. Surico[1]. *[1]Dipartimento di Biotecnologie Agrarie – Sezione di Patologia vegetale, Università degli Studi di Firenze, piazzale delle Cascine 28, 50144 Firenze, Italy. [2]Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.*
E-mail: alessandro.spagnolo@unifi.it

The association of species of Botryosphaeriaceae with grapevine trunk diseases has been documented in many grape growing areas around the world and the pathogenic potential of these fungi has been well documented. The purpose of the present study was to verify the production of exocellular enzymes, some of which considered important virulence factors, in a selection of 11 isolates recovered from a Tuscan vineyard and two lots of Italian nursery stock material in 2007 and 2008. The isolates (eight *Neofusicoccum parvum*, one *Botryosphaeria dothidea* and two *Diplodia seriata*) were representative of the different phylogenetic groups revealed in a phylogenetic analysis based on nucleotide

sequences of the ITS1-5,8S-ITS2 (ITS r-DNA) region and part of the translation elongation factor 1-α (EF1-α) gene. The production of polygalacturonase, xylanase, laccase, Mn peroxidase, lignin peroxidase, lipase, β-glucosidase, endo-1,4-β-glucanase, the complete cellulasic activity (exo-cellobiohydrolase + β-glucosidase + endo-1,4-β-glucanase) and tannic acid conversion activity were determined in plate assays by measuring colony growth and checking for changes in the appearance of the substrate. The results indicate that the isolates considered in this study were able to produce most of the abovementioned enzymes, since only on three occasions did all the isolates give a negative result for xylanase, laccase and complete cellulase activity. Enzymatic activity was not associated with any particular species nor with any particular phylogenetic group. In fact, only the results of the tannic acid conversion assay were related to the phylogenetic position, being positive only for the three *N. parvum* isolates belonging to the same phylogenetic group within this species. The capability of some strains to produce one enzyme but not another suggests that they may interact with one another or with other organisms in the colonization of grapevine wood.

Search of physiological and molecular markers for grapevine tolerance to fungi-induced wood decay diseases. C. Valtaud[1], P. Lecomte[2], J.P. Goutouly[3], G. Comont[2], S. La Camera[4], A. Destrac[3], J.M. Liminana[2], T. Robert[3], S. Gambier[2], G. Ferrari[1], L. Lurton[1], M.F. Corio-Costet[2], S. Delrot[3], P. Coutos-Thévenot[4] and E. Gomes[2]. *[1]Bureau National Interprofessionnel du Cognac, Station Viticole, 69 rue Bellefonds 16100 Cognac, France. [2]UMR 1065 Santé Végétale INRA-ENITA, Institut National de la Recherche Agronomique, Centre de recherche de Bordeaux, BP81, Ave. E. Bourleaux, 33883 Villenave d'Ornon, Cedex, France. [3]UMR INRA-Université de Bordeaux-ENITA 1287 Ecophysiologie et Génomique Fonctionnelle de la Vigne, Institut des Sciences de La Vigne et du Vin, 210 chemin de Leysotte, CS 50008, 33882 Villenave d'Ornon cedex, France. [4]FRE CNRS-Université de Poitiers 3091 Physiologie Moléculaire du Transport des Sucres chez les Végétaux, Bâtiment Botanique, 40 Avenue du Recteur Pineau, 86022 Poitiers Cedex, France.*
E-mail: eric.gomes@bordeaux.inra.fr

A possible strategy to cope with grapevine wood-decay diseases involves the selection of new cultivars or clones more tolerant to fungal pathogens responsible for these pathologies. Such a selection implies to find simple and relevant selection criterions, linked to physiological and/or molecular responses of the plant to fungal infection; and correlated with wood-decay disease tolerance. In order to point out such criterions, a better understanding of grapevine/wood-decay causing agents interaction is needed. Therefore, a three year project, starting in January 2010 will compare the molecular and physiological responses of four grapevine genotypes ('Ugni blanc', 'Cabernet sauvignon', 'Merlot' and *Muscadinia)* to the infection of the ascomycete *Eutypa lata*, the causal agent of Eutypa dieback. For each genotype, artificially infected and non-infected batches of wood cuttings will be compared for: (i) wood necrosis progression, (ii) foliar symptom expression, (iii) physiological parameters (photosynthesis, transpiration rates, gas exchanges) and (iv) gene expression using genome-wide Combimatrix® microarrays both in foliage and wood. Correlation analysis of the measured physiological parameters, specific gene expression, necrotic progression and symptom expression in the different genotypes will help to identify potential tolerance markers. Furthermore, preliminary work with three Botryosphaeriaceae species will also be performed, but on a smaller scale (necrosis analysis, symptom expression, candidate gene expression by RT-qPCR) in order to investigate whether genotypes more tolerant to *E. lata* are also more tolerant to the Botryosphaeriaceae.

Development of rapid bioassays for the screening of plant grapevine material with regard to Eutypa dieback. V. Jung[1], C. Valtaud[1], C. Hevin[3], J.M. Liminana[2], S.Gambier[2], E. Gomes[3], S. Delrot[3], G. Ferrari[1] and P. Lecomte[2]. *[1]Bureau National Interprofessionnel du Cognac, 69 rue de Bellefonds, BP18, 16101, Cognac cedex, France. [2]UMR 1065, Santé Végétale INRA-ENITAB, Institut des Sciences de la Vigne et du Vin, Ave E. Bourleaux, BP 81, 33883, Villenave d'Ornon cedex, France. [3]UMR 1287, Ecophysiologie et Génomique Fonctionnelle de la Vigne, Institut des Sciences de la Vigne et du Vin, 210 Chemin de Leysotte, CS 50008, 33882 Villenave d'Ornon cedex, France.*
E-mail: lecomte@bordeaux.inra.fr

Eutypa dieback, caused by the fungus *Eutypa lata*, is one of the most destructive trunk decline disease that affects grapevine. This disease is particularly severe on the susceptible variety 'Ugni Blanc' which is the most cultivated in the Cognac vineyard. In order to quickly reveal potential differences of susceptibility between clones or selections, reproducible methods should be used. Two approaches were tentatively investigated. One approach was based on the use of vitro-plantlets in lab to get infected tissues as previously described (Camps, 2008). Results showed that the length of necrosis obtained after 1 month-incubation varied between 1-3 mm and were not enough developed to allow a reliable screening whatever the cultivar, the technique or the isolate. The herbaceous nature of the grapevine tissues was supposed to be a difficulty for the lesion devel-

opment. The second approach was based on the method reported by Péros and Berger (1994) using cuttings to reproduce typical leaf symptoms (necrosis and dwarfing). Some trials were carried out with different clones or varieties showing very low levels of leaf expression and indicating that the success of such procedure may be influenced by several factors (inoculation technique, quantity of mycelium, nitrogen supply). We concluded that there is a need for a highly reliable bioassay which can enable a rapid and a more significant induction of symptoms for providing a reproducible tool to the breeders.

Camps C., 2008. *Transcriptomic study of the grapevine response* (Vitis vinifera *cv. Cabernet Sauvignon*) *to the vascular ascomycete fungus* Eutypa lata, *causing Eutypa dieback*. PhD, University of Poitiers, Poitiers, France, 129 pp.

Péros J.P. and G. Berger, 1994. A rapid method to assess the aggressiveness of *Eutypa lata* isolates and the suscepeptibility of grapevine cultivars to Eutypa dieback. *Agronomie* 14, 513–523.

Disease Management

Control strategies for trunk diseases of grapevine. R. Herche and W.D. Gubler. *Department of Plant Pathology, University of California, Davis, One Shields Avenue, Davis, CA, 95616, USA.*
E-mail: wdgubler@ucdavis.edu

There are no known controls for canker diseases of grapevine. These diseases can be prevented, however, by protecting dormant pruning wounds from airborne inoculum. We found that a single fungicide spray application after pruning reduced canker pathogen incidence in 159/160 cases and canker pathogen severity in 16/16 cases. Thiophanate-methyl was observed to be most effective against *Lasiodiplodia theobromae* and myclobutanil was found to most effective against *Eutypa lata*. Both fungicides were effective against *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora*. Pruning wounds were found to be most susceptible to canker pathogens in December and least susceptible in March, and late or double pruning was shown to augment fungicide effectiveness in reducing canker disease incidence. Pruning wound susceptibility to canker pathogens was found to decrease significantly three weeks after pruning. Implementation of these results is discussed.

Controlling eutypa dieback by remedial surgery. M.R. Sosnowski[1], T.W. Wicks[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 dieback is a disease of grapevine caused by the fungus *Eutypa lata,* which infects through wounds and gradually colonises wood tissue, causing stunted shoots with cupped, chlorotic and necrotic leaves, dieback of cordons and eventually death of vines. Once infection is established control is difficult as shown by the lack of success using fungicides, biocontrol agents and nutrients. Twelve long-term trials were established in South Australia to evaluate two methods of remedial surgery to control eutypa dieback; i) low-cut, which involves removing the trunk 30 cm above ground and training a watershoot to replace the canopy and ii) high-cut, where trunks are cut at the crown and the lowest shoot is trained to form a new canopy, followed by removal of excess trunk. Between 42 and 100% of vines produced watershoots, generally reaching a plateau within 3 years. More shoots developed on high-cut vines than on low-cut vines, with considerable variation among cultivars. Recurrence of stunted shoots ranged from 0 to 11% up to 9 years after surgery on low-cut vines, compared with 9 to 54% on high-cut vines. On high-cut vines, the lowest watershoot suitable for reworking emerged from anywhere between the ground and the crown and recurrence of stunting increased with height above ground. The frequency of stunting in high-cut vines was probably due to infected tissue remaining in the trunk. Findings support the need to remove all *E. lata*-infected tissue from diseased vines to restore long-term productivity to vineyards.

Effects of a copper oxychloride formulation against esca and associated fungi. S. Di Marco[1], F. Osti[1] and L. Mugnai[2]. [1]*IBIMET-CNR,Via Gobetti 101, 40139 Bologna, Italy.* [2]*DIBA. Università degli Studi, P.le delle Cascine 28, 50144, Firenze, Italy.*
E-mail: s.dimarco@ibimet .cnr.it

The research here reported deals with the activity of treatments against pathogens associated with esca on young vines, and their efficacy for the reduction of foliar symptom expression. Copper oxychloride plus gentian violet (Labimethyl®) is a peptide complex copper formulation that appears to be able to enter the plant tissue. Laboratory trials were carried out as following: 1) the growth rate inhibition of the formulate was tested *in vitro* on *Phaeomoniella chlamydospora* (Pch), *Phaeoacremonium aleophilum* (Pal) and *Fomitiporia mediterranea* (Fmed); 2) the Pch and Pal colonies were grown in liquid medium containing the formulate and cultural filtrates were analyzed for the presence of the phytotoxin scytalone; 3) the amount of copper penetrated

inside the trunk of treated potted vines was analyzed by Atomic Absorption Spectrophotometer. Further trials were conducted on potted vines inoculated with Pch, and in vineyards naturally esca infected. The formulate was sprayed onto the trunk of potted vines or in the vineyard soon after grape harvesting, at the end of pruning, and at 6–7 cm shoot length as recommended in the experimental plan proposed by the manufacturer. The formulate was effective *in vitro* for Pch, Pal and Fmed giving EC_{50} values of 0.4, 3.31 and 5.53 mg copper L^{-1} respectively; scytalone was not found in the culture filtrate of Pch and Pal grown in the presence of the formulate (detection sensitivity 2 ppm); copper was found inside the trunk of potted vines. Treatments on inoculated potted plants did not give significant reductions of the necrosis length caused by Pch, while in the vineyard a reduction of foliar symptom expression was assessed in treated plots.

***In vivo* growth of reporter gene transformed *Trichoderma harzianum*, *Phaeomoniella chlamydospora* and *Eutypa lata* on the grapevine pruning wound and histology of the wood response to infection.** C. Mutawila[1], F. Halleen[2], P.H. Fourie[1,3] 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.*
[3]*Citrus Research International, P.O. Box 28, Nelspruit, 1200, South Africa.*
E-mail: lmost@sun.ac.za

Protection of grapevine pruning wounds with *Trichoderma* spp. can prevent infection by trunk disease pathogens however, their relative growth and interaction are not well understood. Green fluorescent protein (GFP) and red fluorescent protein (DsRed) labeled *Trichoderma harzianum* (GFP and DsRed), was dual inoculated with *Phaeomoniella chlamydospora* (DsRed) or *Eutypa lata* (GFP) onto fresh pruning wounds of 1-year-old Cabernet Sauvignon and Sauvignon Blanc shoots. The inoculated fungi were isolated from varying depths within the shoots at 30-day isolation intervals for a period of 90 days. *Trichoderma* suppressed pathogens and grew deeper in the presence of the pathogens than when singly inoculated and indication of pathogen recognition and competitive response. *Trichoderma* was isolated to a maximum depth of 20 mm and 30 mm in *E. lata* and *P. chlamydospora* after 90 days dual inoculated canes as compared to 10 mm when it was the only fungus inoculated in Sauvignon Blanc. *Eutypa lata* was isolated at 10 mm depth in singly inoculated canes but was completely eliminated from Sauvignon blanc in dual inoculated canes after 90 days. Growth of *P. chlamydospora* was suppressed by *Trichoderma* in Sauvignon Blanc, while in Cabernet Sauvignon it was isolated at up to 15 mm in both single and dual inoculations, but at reduced frequency in the latter. Extensive growth of *P. chlamydospora* (DsRed) hyphae was observed in the xylem vessels of wood sections, often associated with gum or gel formation. Blockage of vessels and thickening of vessel walls was observed in *P. chlamydospora* and *E. lata* inoculated shoots. This was attributed to the formation of tyloses and the deposition of phenolic compounds in infected vessels, a host response to pathogenesis.

Effectiveness of *Trichoderma* pruning wound protectants against *Eutypa lata*. P. Larignon. *Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France.*
E-mail: philippe.larignon@vignevin.com

Four strains of *Trichoderma atroviride* and two strains of *Trichoderma harzianum* were assessed as wound protectants against *Eutypa lata*. The *Trichoderma* treatments (10^7 conidia per fresh pruning wound on 1-year-old canes) were followed after 1 day with 1500 ascospores of *E. lata* during a rainy period. Isolation from pruning wounds found *Trichoderma* strains in 87–100% of samples irrespective of the isolation date. Presence or absence of *E. lata* was determined by isolation after 5 weeks. Isolation along the cane after 3, 7 and 11 months, found that *E. lata* had penetrated up to 40 mm. *E. lata* incidence in *Trichoderma* treated wounds was similar to the inoculated control after 3 months (85%), 7 months (67%) and 11 months (80%), except for the strain T10 after 3 mo (45%). The *E. lata* disease severity (percentage of pieces of wood infected) was similar between the wound treatments after 3 months (30–40%), 7 months (18–25%) and 11 months (35–49%) and to the inoculated control after 3 months (39%), 7 months (21%) and11 months (35%), except for the strain T10 after 3 months (8%), 7 months (5%) and 11 months (25%). Some *Trichoderma* strains (T4, T7 and T10) delayed *E. lata* colonization in tissues adjacent to the wound for 7 months after application, but this delay was not noticeable at 11 months. In winter 2006–2007, pruning wounds were collected 10 months (October) after the inoculation (December). Each strain of *Trichoderma* was isolated from all the pruning wounds 10 months after their protection, and *E. lata* was occurred between 90 and 100% of pruning wounds while it was found in 70 % of untreated pruning wounds. Clearly the *Trichoderma* strains tested were not effective in preventing the development of *E. lata* in the wounded wood.

Factors affecting pathogenicity tests and fungicide assays on dormant grapevine canes in the field. A. Muruamendiaraz[1],_F.J. Legorburu[1] and J. Luque[2]. [1]*NEIKER-Tecnalia, Basque Institute for Agri-*

culture Research and Development, Apdo 46, E-01080 VITORIA / GASTEIZ, Álava, Spain. [2]*IRTA-Institut de Recerca i Tecnologia Agroalimentàries, Ctra. de Cabrils Km 2, 08348 Cabrils, Barcelona, Spain.*
E-mail: jlegorburu@neiker.net

Pathogenicity tests are central to phytopathological work. For grapevine trunk diseases, it is customary to inoculate the prospect pathogens on dormant canes, either in the field or the glasshouse, and 1) to measure the necrosis obtained, and 2) to record the re-isolation frequencies. However, results obtained when chemicals are evaluated for necrosis development and re-isolation frequencies differ from one laboratory to another. In order to address this variability, a factorial experiment was set up, in which several fungi were inoculated, as mycelium, on dormant canes of grapevine in the field. This was done in the winters 2007–2008 and 2008–2009 in two different wine regions in Spain, Rioja Alavesa and Penedés. In addition, different fungicides were applied. Necrosis length results were greatly influenced by the factors 'Year' and 'Wine Region'. This indicates a high environmental influence on this variable and a poor reproducibility. On average, *Phaeomoniella chlamydospora* and *Eutypa lata* induced longer necroses than did *Diplodia seriata*. Among fungicides, only methyl thiophanate reduced the necrosis, while Cubiet (a copper-sulfate formulation) and tetraconazole made it longer. In contrast, the frequency of re-isolation was much more a consistent variable. 'Year' and 'Wine Region' did not affect the re-isolation frequency statistically, while differences among 'Fungi' and 'Fungicides' were significant. *Diplodia seriata* could be re-isolated much more frequently than *E. lata* and *P. chlamydospora*. In addition, *D. seriata* was only, though partially, controlled by methyl thiophanate. This fungicide also controlled *E. lata*, while Cubiet only controlled *P. chlamydospora*. Hydroxyquinoline controlled both *E. lata* and *P. chlamydospora*.

The effects of hot water treatment and anaerobic cold storage on growth of grapevine cuttings. H. Waite, P. May and G. Bossinger. *University of Melbourne, Institute of Land and Food Resources, Water Street, Creswick, Vic. 3363 Australia.*
E-mail: h.waite@pgrad.unimelb.edu.au

Cold storage combined with hot water treatment (HWT; 50°C/30min) has been implicated in the failure, or delayed establishment, of grapevine cuttings in the field. HWT cuttings are commonly stored in sealed plastic bags and it is thought that anoxic conditions and the accumulation of toxic fermentative metabolites injure the tissue. Prolonged anoxia can be lethal, but HWT and anoxia are also reported to break dormancy and promote development of grapevine cuttings. The effects of sealed storage bags and HWT on post storage development of dormant cuttings were evaluated after six weeks (2008) and thirteen weeks (2009) of storage. The effects of 8 hours immersion in water were also examined in 2009. In 2008, HWT had a small stimulatory effect on bud development in the first 3 weeks. Sealed bags had either no effect, or caused slightly delayed bud development in the first 6 weeks. In 2009 sealed storage bags stimulated bud burst, but the effects of HWT and immersion were more equivocal. There was however, a significant positive interaction between HWT, sealed bags and immersion. The results indicate that, depending on headspace, it may take some time at the low temperatures (2–3°C) of cold storage for the atmospheres in sealed bags to become sufficiently anoxic to break dormancy and stimulate growth. However, storage times and head space volumes in storage bags are variable in commercial nurseries, thus storage in perforated bags is recommended to avoid the risks associated with prolonged anoxia.

Impact of ozonation on grapevine scion decontamination. N. Mailhac, J. Pouzoulet, M. Lummerzheim and F. Violleau. *Laboratoire d'Agrophysiologie, Ecole d'Ingénieurs de PURPAN, 75 Voie du TOEC, 31076 Toulouse Cedex 3, France.*
E-mail: nathalie.mailhac@purpan.fr

Fungi responsible of grapevine trunk diseases are able to produce latent infections at the nursery level and cause Petri disease. It has been shown that these pathogens can be transmitted from infected mother vines via contamination on the external bark or internal vascular tissues. Water baths, grafting and callusing boxes are the main propagation steps of the grapevine multiplication process. This contamination is likely to be a contributing factor in poor performance of grapevine planting material. For these reasons, the development of procedures to prevent and / or reduce the infection is crucial. Hot water treatment (HWT) has some beneficial effects, but a complete control of trunk diseases could not be achieved. Ozone is a known and potent oxidative agent able to decontaminate water from fungal pathogens. More recently, this method has proven to be successful for the decontamination of cereals infected by pathogenic fungi. Moreover, ozone was shown to stimulate systemic acquired resistance in plants. In this study, we implement preliminary experiments on ozonation as a tool for grapevine stocks decontamination. A gradient of ozone concentrations combined with various experimental durations have been applied both on aqueous solutions containing fungal spores and on vine shoots (cv. Cabernet Sauvignon). The fugal survival rate has been scored through their capacity to germinate on MEA medium and scion viability was estimated through its bud breaking and rooting ability.

Biofumigation using brassicaceous plant products to control Cylindrocarpon black foot disease in New Zealand soils. C.M. Bleach, E.E. Jones and M.V. Jaspers. *Ecology Department, Faculty of Agriculture and Life Sciences, Lincoln University, P.O. Box 84, Lincoln 7647, New Zealand.*
E-mail: Carolyn.Bleach2@lincolnuni.ac.nz

When plants of *Brassica* species are incorporated into soil, they release volatile isothiocyanates which can suppress pathogenic fungi. In a New Zealand experiment, crops of mustard (*Brassica juncea*), rape (*B. napus*) and oats (*Avena sativa*) were grown for 5 weeks in a vineyard site previously infested with *Cylindrocarpon* spp. The crops were cultivated into the soil at flowering and the area covered with polythene. After 2 weeks, callused cuttings of rootstocks 101-14 Mgt. and Teleki 5C were grown in the soil for 9 months and infection assessed. The mustard treatment was most effective, reducing disease incidence in rootstocks 101-14 Mgt.and Teleki 5C by 11 and 43%, respectively. The following year, the same site was inoculated with *Cylindrocarpon* spp. grown on wheat grains and three mustard treatments were tested; Trt 1: mustard meal cultivated into the soil, Trt 2: mustard grown once to flowering with cultivation and Trt 3: mustard grown twice to flowering with cultivation each time. The site was planted and assessed as in the previous year. Results showed that these treatments reduced disease incidence in rootstock Teleki 5C by 47, 41 and 41%, respectively, but in 101-14 only Trt 1 and Trt 3 reduced disease incidence by 30 and 18%, respectively. *In vitro* bioassays tested the activity of biofumigant treatments for inhibition of radial growth after 7 days. Results showed that the mustard meal (+ and – soil) and ground mustard roots and shoots (+ soil) reduced mycelium growth compared to control plates (P<0.000). These findings show that biofumigation using mustard may be highly effective for reducing soil-borne *Cylindrocarpon* inoculum and so the incidence of black foot disease.

Grapevine rootstock susceptibility to fungi associated with Petri disease and esca under field conditions. D. Gramaje, J. García-Jiménez and J. Armengol. *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.*
E-mail: dagrape@upvnet.upv.es
One-year-old grapevine cuttings of five rootstocks (41 B Millardet-Grasset, 140 Ruggeri, 161–49 Couderc, 1103 Paulsen and 110 Richter) were tested for susceptibility to infection caused by pathogens associated with Petri disease and esca of grapevine. They were vacuum-inoculated with spore suspensions (10^4 or 10^6 conidia mL^{-1}) of *Cadophora luteo-olivacea*, *Phaeomoniella chlamydospora* and five species of *Phaeoacremonium*. Inoculated rootstock cuttings were immediately planted in March 2008 in two fields, where grapevine had never been grown. In July 2008, proportion of vines that sprouted in spring was visually determined. In January 2009, at the end of the growing season, plants were uprooted and taken to the laboratory for disease assessment. Shoot weight was evaluated. Then, the stem of each grapevine cutting was transversally split at 10 cm from the base of the plant to estimate the percentage of vascular tissue discolored on a scale of 0 to 4. All fungal pathogens caused a significant reduction of sprouting and shoot weight, as well as a significant increase of disease severity percentage in all grapevine rootstocks with the exception of 161 49 C rootstock. Both 110 Richter and 140 Ruggeri rootstocks were the most susceptible to inoculation. In general, *Pa. chlamydospora* and *Pm. parasiticum* were able to cause more reduction on cutting sprouting and shoot weight, and a higher increase of disease severity percentage than the other pathogens inoculated. These results suggest that 161–49 Couderc would be a useful component of an integrated management strategy for Petri disease and esca of grapevine.

A survey of current grapevine nursery management practices in Australia: are we clean enough?. H. Waite, P. May and G. Bossinger. *University of Melbourne, Institute of Land and Food Resources, Water Street, Creswick, Vic. 3363 Australia.*
E-mail:h.waite@pgrad.unimelb.edu.au

Nursery management practices are pivotal to the prevention and transmission of trunk disease pathogens in grapevine planting material. Improvements to nursery disease management protocols have been transmitted to the Australian nursery industry via seminars, technical publications and personal communication. To assess the efficacy of extension activities and determine current propagation practices, questionnaires covering the propagation cycle were mailed to all 65 identifiable Australian vine nurseries and followed up with a mailed prompt and/or phone call. Excluding nurseries that had ceased trading (5), the response rate was 41% (25 responses). An improved, but incomplete, understanding of microbial ecology and epidemiology and sanitation was evident in most responses. The use of at least 2 individual sanitary practices (hot water treatment (HWT), cool room sanitation or fungicides) were reported by 23 respondents, but only one nursery used all listed practices. Treated water was used by 21 nurseries, but 13 reported soaking pre-cut buds for grafting, risking cross contamination from microorganisms on the bark. Only six (24%) nurseries exclusively used of cuttings sourced from registered source blocks of known disease status, but another 13 (52%) frequently used registered material, possibly a reflection of supply and demand. HWT was used by 18 (72%) respondents, but reliability was ques-

tioned by 21 respondents. Codification of practical and detailed sanitary protocols for integration into nursery accreditation programs and to reinforce the benefits of registered source material is needed.

Communication and application of grapevine trunk disease research through extension and education. A. Somers, W. Pitt, S. Savocchia, A. Blake and M.A. Whitelaw-Weckert. *National Wine and Grape Industry Centre. Charles Sturt University, Locked Bag 588,Wagga Wagga, NSW 2678, Australia.*
E-mail: anthony.somers@industry.nsw.gov.au

The National Wine and Grape Industry Centre has employees in research, education, training and extension. This team approach has resulted in rapid uptake of new research. The research scientists in the team have studied the distribution, taxonomy, epidemiology and management of the grapevine trunk disease, Botryosphaeria canker. The results have been presented to the wine grape industry using adult education and extension principals as part of existing university courses and at training days for winegrape growers. The incidence and distribution of grapevine trunk disease fungi were surveyed for the major winegrape growing regions of New South Wales and South Australia. The most prevalent species isolated were those belonging to the Botryosphaeriaceae. Other fungi isolated included *Eutypa lata, Phaeomoniella chlamydospora, Phaeoacremonium* spp. and *Phomopsis viticola*. No fungicides are currently registered for the control of Botryosphaeria canker in Australian vineyards however, *in vitro* fungicide screening and field trials have been undertaken and results are pending. A training program using cultural practices, knowledge of fungal species, vine physiology, pruning techniques and awareness of epidemiology and infection risk has been developed. The program is recognised throughout Australia and contributes towards a qualification known as "Certificate III in Food Processing (WINE)". Such quality based training programs are financially supported by the Commonwealth Government and in doing so they financially reward growers for learning about research outcomes that will assist in the management of grapevine trunk diseases.

Disease Management

Evaluation of fungicides for the control of *Phaeomoniella chlamydospora* in soil. M.L. Tello and V. González. *Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Autovía A-2, Km. 38,2. 28800, Alcalá de Henares, Spain.*
E-mail: marisa.tello@madrid.org

Phaeomoniella chlamydospora is one of the main etiological agents of Petri disease of grapevines. Previous studies have demonstrated that *P. chlamydospora* in soil can enter through roots and colonize xylem and pith. Therefore, nursery soils and substrates could potentially become a source of inoculum. In this study, the efficacy of two common soil fungicides, hymexazol (dicarboximide) and thiram (dimethylthiocarbamate), was tested against *P. chlamydospora* infection via soil. Mycelial growth and spore germination inhibition *in vitro* were evaluated for five *P. chlamydospora* isolates and several fungicide concentrations. The two fungicides were also applied at field rates to grafted plants of cv. Tempranillo/140-Ru grown in pots under glasshouse conditions. The soil was inoculated with a mixture of five *P. chlamydospora* isolates at 10^6 and 10^2 conidia mL^{-1}. Results from *in vitro* assays show that both fungicides were more effective in inhibiting conidial germination than mycelial growth. Thiram completely inhibited conidial germination at 100 ppm, while hymexazol was ineffective at doses lower than 250 ppm. In the greenhouse experiments, hymexazol was less effective than thiram which significantly reduced vascular discoloration after two months with only one fungicide application to the soil two days before fungal inoculation. Results suggest that, used as preventive treatments, applications of thiram to soils or substrates could delay *P. chlamydospora* infection of nursery plants through roots.

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Effect of hot-water treatments *in vitro* on conidial germination and mycelial growth of *Cadophora luteo-olivacea* and *Phaeoacremonium* spp. D. Gramaje, J. García-Jiménez and J. Armengol. *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.*
E-mail: dagrape@upvnet.upv.es

This study evaluated the sensitivity of *Cadophora luteo-olivacea* and eight species of the genus *Phaeoacremonium* (*Pm. cinereum*, *Pm. hispanicum*, *Pm. inflatipes*, *Pm. iranianum*, *Pm. mortoniae*, *Pm. scolyti*, *Pm. sicilianum* and *Pm. viticola*) to *in vitro* hot-water treatments (HWTs). Conidial suspensions and plugs of agar with mycelia were placed in Eppendorf vials and incubated for 30, 45 or 60 min in a hot water bath at 49, 50, 51, 52, 53, 54 or 55°C. In general, conidial germination and colony growth rate of all pathogens decreased with increased temperature and time combinations. Conidial germination of *Ca. luteo-olivacea* was inhibited by treatments above 51°C for 30 min, while treatments up to 54°C for 60 min were necessary to inhibit its mycelial growth.

For *Phaeoacremonium* spp., treatments up to 54°C for 60 min were necessary to completely inhibit both conidial germination and mycelial growth. These results suggest that it would be necessary to develop HWTs using higher temperatures rather than the current HWT protocols (50°C for 30 min) to reduce the incidence of *Ca. luteo-olivacea* and *Phaeoacremonium* spp. infections on grapevine planting material.

Evaluation of fungicides as potential grapevine pruning wood protectants against the main grapevine trunk disease pathogens in Chile. J. Auger, I. Pérez and M. Esterio. *Laboratorio de Fitopatología Frutal y Molecular, Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile. Zip Code 8820808, Santiago, Chile. E-mail: jauger@uchile.cl*

Trunk diseases of grapevines cause decline and premature dieback of vineyards across the world. Trunk pathogens, which include *Phaeomoniella chlamydospora* and several *Phaeoacremonium*, *Botryosphaeria*, *Phomopsis* and basidiomycete spp., have been implicated in this complex of diseases. Wounds, especially pruning wounds, are the most important infection portals for these pathogens. Protection of pruning wounds against infection by trunk diseases pathogens is the most efficient and cost-effective method to prevent grapevine trunk diseases. In Chile, pruning wound protection by means of fungicide-amended wound dressings have been recommended as a preventative control measure. However, no studies have been done to determine the effectiveness of chemical pruning wound protectants against the main trunk disease pathogens. Nine fungicides were, tested *in vitro* for their efficacy in mycelial growth inhibition of *P. chlamydospora*, *Phaeoacremonium* spp., *Botryosphaeria* spp. and *Fomitiporella vitis*. Captan, iprodione, tolylfluanid and kresoxim methyl were ineffective for inhibiting the mycelial growth at the highest concentrations tested. Tebuconazole, prochloraz, and prochloraz + carbendazim were the most effective fungicides, with EC_{50} values for the different species ranging from 0,16–0,51, 0,06–4,2 and 0,02–0,9 $g \cdot mL^{-1}$, respectively. Results from bioassays on 8-year-old Thompson Seedless grapevine shoots indicated that prochloraz + carbendazim was the most effective in limiting incidence of *P. chlamydospora* in re-isolations. However, the bioassay results were inconclusive with respect to lesions lengths and re-isolation incidences in pruning wounds inoculated with the other pathogens.

Wood decay diseases: Tests of disinfection methods in French nursery. V. Vigues[1], O. Yobregat[1], B. Barthelemy[1], F. Dias[1], M. Coarer[2], K. Girardon[3], F. Berud[4], M. Muller[5] and P. Larignon[5]. *[1]IFV Pôle Sud-Ouest - V'Innopôle Brames Aigues BP 22 - 81310 Lisle / Tarn, France. [2]IFV Pôle Val de Loire - Château de la Frémoire - 44120 Vertou, France. [3]SPBPVV, 384, route de Caderousse, 84100 Orange, France. [4]Chambre d'agriculture du Vaucluse, 2260, route du Grès, 84100 Orange, France. [5]IFV Pôle Rhône-Méditerranée - Domaine de Donadille - 30230 Rodilhan, France. E-mail: virginie.vigues@vignevin.com*

Fungi associated with wood decay diseases may spread in nurseries. Steps involving risks had been identified so this study aimed to find methods for eliminating fungi associated with these diseases from grapevine plants and to prevent infection of the plants. Different control methods were tested on standard nursery plants, some of which were infected: chemical methods (fungicides and bactericides), biological methods (microorganisms and plant essences) and technological methods [hot water treatment (HWT) and ozonization]. The results were generally disappointing but some trends were interesting. The products discarded because they were ineffective and/or caused high mortality of plants were sodium hypochlorite, Cryptonol® (oxyquinoline) and essences of plants. Ozonization seemed to be inefficient as well. The use of products based on *Trichoderma* spp. brought about the colonization of vine plants by these microorganisms more particularly when the vine plants were soaked in these products (the colonization was less evident when the products were sprayed) but it had no effect on the rate of contamination by the wood pathogens. Two of the fungicides tested provided promising results: Switch® (fludioxonil, cyprodinil), which reduced the incidence of *Diplodia seriata*, *Neofusicoccum parvum* and *Phaeomoniella chlamydospora* in vine plants, and Cabrio Top® (pyraclostrobin, metiram-zinc), which reduced the incidence of *D. seriata* and *P. chlamydospora*. HWT (50°C for 45 min) was the only effective practice that lasted for several years; it reduced incidence of *D. seriata, Botryosphaeria dothidea*, *P. chlamydospora* and *Phomopsis viticola* infections. This treatment was considered most useful when it was performed on vine plants just before selling them. An effective method able to reduce (or prevent infection) by *N. parvum* remains to be found. Use of Switch appeared to be worth further investigation, and if able to reduce infection would complement HWT.

Grapevine rootstock susceptibility to *Cylindrocarpon liriodendri* and C. *macrodidymum*. S. Alaniz[1,2], J. García-Jiménez[2], P. Abad-Campos[2] and J. Armengol[2]. *[1]Departamento de Protección Vegetal, Facultad de Agronomía, Universidad de la República, Garzón 780, CO 12900, Montevideo, Uruguay. [2]Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s / n, 46022-Valencia, Spain. E-mail: salaniz@fagro.edu.uy*

In this work the susceptibility of the grapevine rootstocks most commonly used in Spain to *Cylindrocarpon liriodendri* and *C. macrodidymum* was evaluated. Rooted cuttings of rootstocks 110-R, 1103-P, 140-R, 161-49C, 196-17C, Fercal and SO4 were inoculated by dipping their roots in conidial suspensions (5×10^5 conidia·mL^{-1}) of each pathogen and placed in a greenhouse. One month later, each plant was drench inoculated with 20 mL of the corresponding spore solution to improve root infection. After four months of incubation, a root disease severity index (0 = healthy root, to 5 = dead root) and dry weights of shoots and roots were recorded for each plant. According to the root disease severity index, most of the rootstocks were significantly affected by *C. liriodendri* and *C. macrodidymum*. The root dry weight of 110-R rootstock was significantly reduced by both *Cylindrocarpon* species, however, in the case of shoot dry weight only the rootstock 1103-P was significantly reduced by both *Cylindrocarpon* species. Fercal rootstock showed a significant reduction in both root and shoot dry weights, only when inoculated with *C. liriodendri*. This information should be considered when selecting rootstocks for new plantations.

Effect of Fosfimax 40-20 in the control of *Cylindrocarpon macrodydimum* on table grapes cropped in the III Region of Chile. J.R. Montealegre, S. Sánchez and L. Rivera. *Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile. Casilla 1004, Santiago, Chile.*
E-mail: jmonteal@uchile.cl

The aim of this study was to determine the effectiveness of Fosfimax 40-20 in the control of *Cylindrocarpon macrodydimum* and other phytopathogenic fungi that affect the root systems of table grapes, in the IIIrd Region of Chile. *In vitro* and field tests with Fosfimax 40-20 were conducted in Copiapó, where *C. macrodydimum* and other phytopathogenic fungi of the grapevine root system were causing poor growth and yield of plants in two vineyards that were selected for this experiment. The three treatments included application of Fosfimax 40-20 to soil and foliage, as well as an untreated control. The experimental design was a complete randomized block with three treatments with four replicates. Incidence of the phytopathogenic fungi before and after treatment applications and percentage of yield increase, compared to the control were evaluated. Data were analyzed by ANOVA and Tukey`s test. *In vitro* experiments showed that EC_{50} values of Fosfimax 40-20 to control *C. macrodydimum* strains, varied between 20 and 33 ppm of active ingredient, whereas the field trial results showed that product application caused no statistical differences in the incidences of these fungi. However, Fosfimax 40-20 applied to soil caused statistically significant yield increases compared to the untreated control, in two assays. These results show that Fosfimax 40-20 controlled *C. macrodydimum* in *in vitro* experiments and field soil applications of,it caused increased yields in treated plants, possibly through control of the phytopathogenic fungi that affect the root system of table grapes.

Control of grapevine wood diseases due to Botryosphaeriaceae fungi and *Phomopsis viticola*. C. Rego, T. Nascimento and H. Oliveira. *Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.*
E-mail: crego@isa.utl.pt

The most prevalent fungi associated with excoriose-like symptoms in Portugal are species belonging to the Botryosphaeriaceae and *Phomopsis viticola*. Infections caused by Botryosphaeriaceae fungi are more insidious and harder to control than those caused by *P. viticola*. In this study, the effectiveness of chitosan was compared to that of the conventional fungicides azoxystrobin and pyraclostrobin + metirame to control excoriose-like symptoms under vineyard conditions. Two vineyard trials were conducted for 3 years in the Alentejo region of Portugal to evaluate the effectiveness of these products. Trials were established on two 12-year-old vineyards, one planted with cv. Castelão and one with cv. Aragonez. Each year, during late spring, vines were visually evaluated for the percentage of infected shoots (incidence) and diseased area along each shoot (severity). Data from each field and year were analysed separately. On cv. Castelão, the application of chitosan always resulted in significantly less excoriose-like symptoms incidence and severity, over the three years of experiments, as compared to the untreated control. Moreover, the effectiveness of chitosan was comparable to that achieved by the conventional fungicides. A similar behaviour was observed in the trial conducted on cv. Aragonez, except for disease severity during 2009. Even in this case, the effect of chitosan was not significantly different from that of azoxystrobin. On the whole, these results showed that chitosan could be an excellent alternative to control Botryosphaeriaceae fungi and *P. viticola* under vineyard conditions, as compared to strobilurin fungicides, and so is a valuable option for organic viticulture.

Evaluation of grapevine cultivar susceptibility to *Trichoderma* colonisation of pruning wounds. C. Mutawila[1], F. Halleen[2], P.H. Fourie[1,3] 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. [3]*Citrus Research International, P.O. Box 28, Nelspruit, 1200, South Africa.*
E-mail: lmost@sun.ac.za

Growth and persistence of *Trichoderma* spp. on pruning wounds and the resulting control may depend on intrinsic wound factors, which vary between cultivars. Pruning wound colonization was evaluated on eight wine (Cabernet Sauvignon, Chardonnay, Chenin Blanc, Colombar, Merlot, Pinotage, Sauvignon Blanc and Shiraz) and four table (Prime, Red Globe, Thompson Seedless and Victoria) grape cultivars in the field, during the 2008-2009 season. *Trichoderma atroviride* strains (USPP-T1 and USPP-T2; applied separately or combined) and a registered pruning wound biocontrol agent based on *T. harzianum* were applied to fresh pruning wounds exposed to natural infections. After eight months *Trichoderma* and trunk pathogens, namely *Phaeomoniella chlamydospora*, species of *Phaeoacremonium*, *Phomopsis*, Botryosphaeriaceae and Diatrypaceae were isolated. *Trichoderma* isolations from the pruning wounds showed significant treatment × cultivar interactions ($P<0.01$) in both table and wine grapes. The incidence of *Trichoderma* varied greatly among cultivars, being least variable among treatments within the same cultivar. In the wine grapes, the highest *Trichoderma* incidences were in Chenin Blanc (71.39–82.50%), with 20–50% in the other cultivars. In the table grapes, Thompson Seedless (43.47–76.74%) had the highest *Trichoderma* incidences, with 20-67% incidence in the other cultivars. The effects of the treatments varied amongst the cultivars, with at least one *Trichoderma* treatment significantly reducing pathogen incidence in Cabernet Sauvignon, Chenin Blanc, Merlot, Red Globe, Thomson Seedless and Shiraz. A positive correlation was found between *Trichoderma* incidence and pathogen reduction in all cultivars except in Pinotage, where despite high *Trichoderma* colonization there was no corresponding pathogen reduction. It was concluded that *Trichoderma* wound protection is a result of *Trichoderma*-grapevine-pathogen interactions and not just the suppressive effects of *Trichoderma* on the pathogens.

Microbial metabolites for the biocontrol of the fungi involved in esca of grapevine. A. Raio[1], G. Puopolo[3], A. Cimmino[2], M. Masi[2], L. Mugnai[4], A. Zoina[3], G. Surico[4] and A. Evidente[2]. [1]*Istituto per la Protezione delle Piante, CNR. Via Madonna del Piano, 10, Sesto fiorentino (FI);* [2]*Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Università di Napoli Federico II.* [3]*Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, 80055 Portici, Italy.* [4]*Dipartimento di Biotecnologie-Patologia Vegetale, Università di Firenze, 50144 Firenze, Italy.*
E-mail: raio@ipp.cnr.it

Up to 30 species of fungi have been isolated from the typical wood discolorations in the trunk of grapevines affected by esca. The most important species that cause wood necrosis and vascular streaking are recognized to be *Phaeomoniella chlamydospora* (Pch) and *Phaeoacremonium aleophilum* (Pal). The vascular disease agents Pch and Pal are able to infect the plant both in the nursery and in the field. Control of infections is based on sanitation, especially in the nursery, and on prevention of wound infections in the field. For both of these environments, biological control by antagonistic microorganisms might be a sound alternative to the use of chemicals. In this work, a strain of *Pseudomonas chlororaphis* subsp. *aureofaciens* has been evaluated for its potential in the biological control of the two fungi involved in esca disease, as well as for the utilization of its bioactive metabolites as natural fungicides.

Antifungal activity of saponins against botryosphaeriaceous pathogens of grapevines. F. Mazet[1], S. Farine[1], J. Chong[1], H. Laloue[1], P. Larignon[2] and C. Bertsch[1]. [1]*Laboaratoire Vigne Biotechnologie et Environement, Université de Haute-Alsace, 33, rue de Herrlisheim 68008 Colmar, France.* [2]*Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France.*
E-mail: christophe.bertsch@uha.fr

Grapevine dieback diseases, also called grapevine trunk diseases, are caused by a range of fungi that were described as early as the end of the 20th century. However, disease control has been hampered by the lack of effective fungicides and by favorable environmental conditions during potential infection periods. Sodium arsenite is the only treatment that has so far shown a potential effect against many dieback diseases, but its use has recently been prohibited. Saponins, which occur constitutively in roots and leaves of healthy plants, are plant glycosides that have shown antifungal effects. The growth inhibiting effects of saponins on *Diplodia seriata* and *Neofusicoccum parvum,* two pathogens of grapevine trunks, were investigated by *in vitro* tests, which were performed in Petri dishes containing potato dextrose agar supplemented with a concentration gradient of saponins at pH 4 and pH7. Results showed that an increasing concentration gradient of saponins induced a decreasing dose response of mycelial growth for the two phytopathogenic fungi tested. The efficiency of the treatment was also dependent on the pH of the culture medium. Higher efficiency at acidic pH could imply the absorption capacity of saponin by the different fungi.