Phytopathol. Mediterr. (2009) 48, 159–188

ABSTRACTS

Abstracts of oral and poster presentations given at the 6th International Workshop on Grapevine Trunk Diseases, Florence, Italy, 1–3 September 2008

The 6th International Workshop on Grapevine Trunk Diseases, was held in Florence, Italy, on September 1–3, 2008, and was attended by 120 participants. Thirty-four oral presentations and 59 posters were presented at the meeting, dealing with esca and other trunk diseases of grapevine. Some of these presentations were then completed, submitted and peer-reviewed as original research work, and they are published in full in this issue as full papers or short notes. The abstracts of the remaining presentations are also published here.

Pathogen Identification and Detection

Phenotypic and genetic analysis of *Phaeomoniella chlamydospora* isolates from grapevines in Spain.
M.L. TELLO[1], D. GRAMAJE[2], A. GÓMEZ[1], P. ABAD-CAMPOS[2] and J. ARMENGOL[2]. [1]*Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Autovía A-2, Km. 38,200, 28800 Alcalá de Henares, Spain.* [2]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.*
E-mail: marisa.tello@madrid.org

Phaeomoniella chlamydospora is the main fungal pathogen involved in Petri disease of young grapevines, causing severe losses all over the world. However, up to now, there were no previous studies related to the phenotypic and molecular diversity of this fungus in Spain, which constituted the main objective of this work. For this study, 57 isolates of *Pa. chlamydospora* were selected. They were obtained from symptomatic vines, between 2001 and 2005, and from a wide range of very diverse Spanish viticulture areas and rootstock/scion combinations. Three reference *Pa. chlamydospora* isolates (CBS 117179, CBS 23974 and CBS 101571) and one *Pa. zymoides* (CBS 121168) were included. For their phenotypical characterization, *Phoma*-like synanamorph production, sporulation and mycelial growth rates at 5, 10, 15, 20, 25, 30 and 35°C were studied. Molecular characterization by means of DNA markers was performed with the analysis of 5 ISSR and 24 RAPD markers. This study allowed us to determine the cardinal temperatures of the Spanish *Pa. chlamydospora* isolates and of the *P. zymoides* isolate. Our results showed several differences from previously published work with isolates from other countries. Synanamorph formation was confirmed on all isolates. Both phenotypical and molecular data showed strong homogeneity among all Spanish *Pa. chlamydospora* isolates, regardless of their geographical origin, year of isolation and rootstock/scion combination.

Acknowledgements: This study was supported by the projects RTA2007-00023-C04-03 and RTA2007-0023-C04-04 financed by the Spanish Ministry of Education and Science ("Programa Nacional de Recursos y Tecnologías Agrarias") and the European Union (FEDER program).

Molecular and phenotypic characterization of novel *Phaeoacremonium* species associated with Petri disease and esca of grapevine. S. ESSAKHI[1], L. MUGNAI[1], P.W. CROUS[2], J.Z. GROENEWALD[2] and G. SURICO[1]. [1]*Dipartimento di Biotecnologie Agrarie- sez. Patologia vegetale, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy. 2Centraalbureau voor Schimmelcultures, P.O. Box 85167, 3508 AD Utrecht, The Netherlands.*
E-mail: salwaessakhi@hotmail.com

Petri disease and esca are very destructive grapevine decline diseases that occur in young and old vines in most countries where grapevines (*Vitis* spp.) are cultivated. *Phaeoacremonium* species are among the principal hyphomycetes associated with Petri disease and esca symptoms, producing a range of enzymes and phytotoxic metabolites. The present study compared the phylogeny of a global collection of 118 *Phaeoacremonium* isolates from grapevines, in order to gain a better understanding of their involvement in Petri disease and esca. Phylogenetic analyses of combined DNA sequence data sets of actin and β-tubulin genes revealed the presence of 13 species of *Phaeoacremonium* associated with Petri disease and esca on grapevine. *Phaeoacremonium aleophilum* was the most frequently isolated species with an incidence up to 80% of all isolates investigated. Previously described species, namely *Pm. alvesii*, *Pm. griseorubrum* and *Pm. rubrigenum* are newly reported on grapevine from Turkey, Italy and Croatia, respectively. *Phaeoacremonium viticola* and *Pm. scotyli* represent new records for Italy, as well as *Pm. mortoniae* for Hungary and Croatia. In addition, four new species of *Phaeoacremonium,* namely *Pm. croatiense*, *Pm. hungaricum*, *Pm. sicilianum* and *Pm. tuscanum* are newly described from grapevine based on morphology, cultural characteristics, as well as molecular phylogeny.

Acknowledgements: Research study commissioned from ARSIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forestry) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-Regional Project "Grapevine esca: research and experiment in the nursery and in the field for prevention and cure."

Occurrence of grapevine trunk disease pathogens in rootstock mother plants and nurseries in Spain. A. AROCA[1], D. GRAMAJE[2], P. ABAD-CAMPOS[2], J. GARCÍA-JIMÉNEZ[2], J. ARMENGOL[2] and R. RAPOSO[1]. [1]*Instituto de Investigaciones Agrarias (INIA). CIFOR. Ctra. Coruña Km 7,5, 28040 Madrid, Spain.* [2]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.*
E-mail: dagrape@upvnet.upv.es

Recent surveys of grapevine plants used in new plantings in Spain demonstrated that these plants are infected with trunk disease pathogens. In this work, grapevine mother plants as well as nurseries were surveyed to determine the presence of these pathogens. Seven rootstock mother fields were selected in each 2006 and 2007. Twenty plants were sampled from each field in June and September, collecting two canes of the same plant in each sampling date. One small piece of each cane, taken from the basal end, was peeled out, surface disinfected (in 70% ethanol for 2 minutes), and thin cross sections were incubated in culture medium for fungal isolation and identification. Additionally, five commercial nurseries were sampled at different stages of the Spanish standard propagation process. Samples were taken from scissors (used for bud removing) and grafting tools, by washing with sterile water amended with 0.2% Tween-20. Hydration tanks and peat (used for callusing and rooting) were sampled at different times during the period. Peat was diluted, ground and filtered. Resulting liquid samples were analysed using two different techniques, a) Nested-PCR with specific primers for *Phaeomoniella chlamydospora* and *Phaeoacremonium* species; and b) successive filtration through 5 μm and 0.45 μm of pore size and subsequent incubation in culture medium. Results showed that grapevine mother plants are an important inoculum source of fungal trunk disease pathogens. *Pa. chlamydospora*, *Pm. aleophilum*, *Pm. parasiticum*, several species of Botryosphaeriaceae and *Phomopsis* spp. were isolated from grapevine mother plants. In nurseries, it was shown that potential infection points for plants are: hydration tanks, grafting machines, scissors and peat, where *Phaeoacremonium* spp. and *Pa. chlamydospora* were detected.

Fungi associated with grapevine trunk diseases in established vineyards in New Zealand. M.A. MANNING[1] and D.C. MUNDY[2]. [1]*The Horticulture and Food Institute of New Zealand Limited, Mt Albert Research Centre, Private Bag 92169, Auckland, New Zealand.* [2]*The Horticulture and Food Institute of New Zealand Limited, Marlborough Wine Research Centre, P.O. Box 845, Blenheim, New Zealand.*
E-mail: dmundy@hortresearch.co.nz

Twenty-three-year-old Cabernet Sauvignon grapevines vines removed from a vineyard in the Marlborough region in New Zealand in 2006 were observed to have fungal decay symptoms. A survey was begun in 2007 to find out how widespread these symptoms are in other New Zealand vineyards and to identify the various fungi associated with grapevine trunks. Forty vineyards, most over 10 years old, were selected from the major grape-growing regions of New Zealand for the study. Wood tissue was sampled using a Mattson corer from five vines on each vineyard block. Core samples were observed to be either non-symptomatic or had a range of symptoms including red/brown staining. The

fungi most frequently isolated from grape wood tissue were *Botryosphaeria lutea*, *B. parva*, *B. obtusa*, *Eutypa lata*, *Eutypella vitis*, *Phaeomoniella chlamydospora*, *Cylindrocarpon destructans*, *C. liriodendri* and *Phomopsis viticola*. Fungi isolated less frequently included *Cadophora luteo-olivacea*, *Ca. mellinii*, *Phaeoacremonium rubrigenum*, and *P. aleophilum*. These fungal species included many of the fungi commonly reported from other grape-growing countries. We discuss the incidence of disease and the distribution of fungal species associated with grapevines in the various wine-producing regions of New Zealand.

A multiplex PCR assay detecting several Ascomycetes responsible for grapevine trunk diseases. M. LUMMERZHEIM, L.G. MORELLO and A. MAS. *Laboratoire d'Agrophysiologie, Ecole d'Ingénieur de PURPAN, 75 Voie du TOEC, 31076 Toulouse Cedex 3, France.*
E-mail: marie.lummerzheim@purpan.fr

We have developed a multiplex PCR method allowing the simultaneous identification of *Phaeoacremonium aleophilum, Phaeoamoniella chlamydospora, Botryosphaeria obtusa, Botryosphaeria dothidae* and *Eutypa lata*. These fungal pathogens have been identified, amongst others, to be causal agents of grapevine trunk diseases. Petri disease on young grapevines and esca on older vines are caused by *P. chlamydospora* in association with some other fungi. A number of *Botrysphaeria* species, amongst which *B. dothidae* and *B. obtusa,* 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. These xylem-inhabiting ascomycetes have in common a slow growth and induce extremely complex and variable symptom expression on *Vitis vinifera*, making disease identification using traditional isolation methods problematic. We have tested this multiplex PCR assay on pure fungal DNA, crude mycelium, inoculated vine-stocks and natural infected cordons and wood. It could be a quick and reliable tool for fungal detection and disease diagnosis in suspected vine-stocks in nurseries, natural and greenhouse conditions.

Investigations on grapevine wood colonization using synthetic green fluorescent protein (*sGFP*) tagged *Phaeomoniella chlamydospora*. L. LANDI, S. MUROLO and G. ROMANAZZI. *Department of Environmental and Crop Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131 Ancona, Italy.*
E-mail: g.romanazzi@univpm.it

Phaeomoniella chlamydospora (Pch) is the causal agent of the grapevine Petri disease. It has recently been described as one of the most important trunk diseases of the grapevine as it can induce premature decline and dieback, and reduce the economic life of a vineyard. Recent reports have indicated that Pch infections can occur throughout propagation stages and that the pathogen can spread with propagative material. The aim of this study was to determine the Pch colonization in *Vitis vinifera* cultivars (Montepulciano, Verdicchio, Sangiovese, Biancame, Cabernet Sauvignon) and in some rootstocks (Kober 5BB, SO4, 420A, *V. rupestris*). Cuttings were artificially inoculated with a synthetic green fluorescent protein (*sGFP*) tagged Pch strain CBS 229.95 by dipping them overnight in a conidial suspension (1×10^7 spores ml^{-1}) and incubated them at 4°C and 20°C. The wood colonization was monitored monthly using epifluorescence microscopy. The first results show that the wood colonization is clearly affected by incubation temperature, as it is lower in cuttings stored at 4°C than at 20°C. The green fluorescence, which was localized on xylem vessels at the basal end of the cuttings in the first months after inoculation, has spread along the wood sections over time. The first results show the highest wood colonization in the cuttings of Montepulciano and Verdicchio, with the lowest in the rootstocks SO4 and *V. rupestris*. The sections showing strong fluorescence were infected by Pch, as shown by molecular detection with nested PCR with specific primer pairs. The use of the Pch strain expressing *sGFP* can provide an easy assay procedure for ecological and epidemiological studies of Petri disease.

Acknowledgements: Research study commissioned from ARSIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forestry) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-Regional Project "Grapevine esca: research and experiment in the nursery and in the field for prevention and cure."

The status of *Eutypa lata* in California. F.P. TROUILLAS, W.D. GUBLER, and E.A. WEBER. *Department of Plant Pathology, University of California, Davis, California, 95616 USA.*
E-mail: flotrouillas@ucdavis.edu

Seasonal patterns of ascospore dispersal and the sources of inoculum for *E. lata* in California vineyards were investigated using spore trapping studies and field surveys. Spore trapping studies were conducted through the fall, winter and spring of 2000 and 2001 using Burkard 7-day recording volumetric spore traps. Spore traps were placed in a vineyard in the North Coast region, and around grapevine trunks bearing *E. lata* perithecia at the field research facility of the University of California, Davis. Spore catches were recorded using a compound microscope and spore counts were superimposed with rainfall events and temperature data. Surveys for perithecia were conducted in vineyards, stone fruit orchards and on native trees and ornamental woody plants surrounding vineyards. Pure cultures were obtained for each isolate collected from perithecia on various

host-plants in California as well as for isolates obtained from hyphae in infected wood of grapevine, cherry, apricot and apple trees. The intraspecific diversity of *E. lata* population from California was assessed using morphological studies and phylogenetic analyses of the ITS region of the r-DNA, portions of the β-tubulin and RNA polymerase II (RPB2) genes. The pathogenicity and virulence of *E. lata* isolates from California were assessed using green shoots inoculation of Chardonnay and Sauvignon Blanc grapevines. Finally we evaluated double pruning of grapevines as a cultural management practice for reducing infections by *E. lata* in spur-pruned vineyards.

Identification of *Botryosphaeria* spp. and first report of *Dothiorella viticola* (*'Botryosphaeria' viticola*) associated with bunch rot in Australia. N. WUNDERLICH[1], S. SAVOCCHIA[1], C.C. STEEL[1], G.J. ASH[1,2] and H. RAMAN[2]. *[1]National Wine and Grape Industry Centre, School of Agricultural and Wine Sciences, Charles Sturt University, Wagga Wagga, NSW 2678, Australia. 2EH Graham Centre for Agricultural Innovation, Department of Primary Industries, Wagga Wagga, NSW 2650, Australia.*
E-mail: nwunderlich@csu.edu.au

Botryosphaeria spp. are commonly associated with trunk disease of grapevines but in some situations can cause berry rot. Two hundred vines symptomatic of *Botryosphaeria* canker at two vineyards in the lower Hunter Valley, south eastern Australia, a region with a known history of *Botryosphaeria* canker, were sampled for species of *Botryosphaeria*. Samples were collected from grapevine tissues at different phenological stages: dormant buds, flowers, pea-sized berries and berries at harvest. Fungi isolated from these samples included *Alternaria* spp., *Penicillium* spp., *Epicoccum* spp. *Cladosporium* spp., *Phomopsis* spp. and *Botrytis* sp.. In addition, 25 isolates were suspected to be species of *Botryosphaeria*. These were identified according to spore morphology and sequencing of the rDNA internal transcribed spacer (ITS) region. *Diplodia seriata*, *Neofusicoccum parvum* and *Dothiorella viticola* were isolated from dormant buds and *D. seriata*, *N. parvum*, *N. luteum*/*N. australe* and *B. dothidea* were isolated from berries at harvest. Species of *Botryosphaeria* were not isolated from flowers or pea-sized berries. This suggests that species of *Botryosphaeria* associated with bunch rot infections do not arise from infection of the vegetative tissues earlier in the season. To our knowledge, this is also the first report of D. viticola on grapevines in Australia. Further sequencing of additional gene regions (β-tubulin and EF1-α) will be conducted to confirm the identity of the putative *N. luteum*/*N. australe* isolates. Future work will utilise genetic methods to investigate the homology of isolates originating from the wood and other reproductive tissues. Confirmation of isolate pathogenicity towards different tissues of *Vitis vinifera* is currently in progress.

Detection of Botryosphaeriaceae species occurring on grapevines in Spain by cooperational PCR coupled with dot blot hybridization. S. MARTOS[1], E. TORRES[2], F. GARCIA[2] and J. LUQUE[1]. *[1]Dep. Protecció Vegetal, IRTA Cabrils; Ctra. de Cabrils km 2, E-08348 Cabrils, Spain. [2]Laboratorio de Sanitat Vegetal; Via Circulació Nord, Tram VI, Carrer 3, Zona Franca, E-08040, Barcelona, Spain.*
E-mail: jordi.luque@irta.cat

A sensitive tool for a rapid detection of Botryosphaeriaceae species occurring on grapevines was developed based on the co-operational PCR (Co-PCR) technique coupled with dot-blot hybridization using a probe labeled with digoxigenin at the 3' end. The probe *BotR2D* was designed to target a common region in the ITS segment of the rDNA repeat of all Botryosphaeriaceae species. Forty-nine ITS sequences belonging to 10 Botryosphaeriaceous species (*Botryosphaeria dothidea*, *Diplodia mutila*, *D. seriata*, *Dothiorella viticola*, *Lasiodiplodia theobromae*, *Neofusicoccum australe, N. luteum*, *N. parvum*, *N. viticlavatum* and *N. vitifusiforme*) plus 10 other grapevine-associated fungi were used to design the probe. The ITS sequences obtained in this study or retrieved from GenBank were representative of fungi isolated from different countries and hosts, thus increasing the genetic variability of the study and optimizing probe specificity. DNA extracted from a grapevine plant cv. Red Grenache was included in the validation test as a negative control. The detection technique was tested on eight Botryosphaeriaceae species occurring on grapevines in Spain (12 isolates), 10 non-Botryosphaeriaceous fungi (13 isolates), and grapevine DNA. It recognized unambiguously all the Botryosphaeriaceae species, and showed a negative detection for the non-Botryosphaeriaceous fungi and the plant DNA. All tests were run twice in independent assays with no variation in the results. This technique showed a sensitivity level similar to nested-PCR methods, but Co-PCR only requires one amplification reaction and thus minimizes contamination risks. Additionally, it allows considerable numbers of samples to be processed within a reasonably short time period and at low cost.

Setting up and application of a nested PCR protocol to detect DNA of Botryosphaeriaceous fungi associated with grapevine wood tissues and xylem sap. A. SPAGNOLO, G. MARCHI, F. PEDUTO, S. ESSAKHI and G. SURICO. *Dipartimento di Biotecnologie Agrarie–Sezione Patologia vegetale, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy.*
E-mail: alessandro.spagnolo@unifi.it

Species of Botryosphaeriaceae are frequently isolated from grape-growing areas around the world; so far, thirteen species have been associated with grapevine. Many of these species are considered potentially pathogenic both because

of their association (albeit together with other fungi) with various disease symptoms (grafting failure, stunted growth, leaf chlorosis, delayed bud burst, bud mortality, dieback of canes and shoots, fruit rot and wood deterioration such as xylem brown streaking, cankers and wood necrosis), and also because they cause wood discoloration and necrosis. The aim of this work was to screen Botryosphaeriaceous fungi in the tissues of propagation material and standing vines from Italy. The fungal DNA was analyzed after being extracted from the xylem sap of symptomatic and asymptomatic esca-diseased vines, from samples collected from single vines, from single propagation-material units, and from pooled samples (average weight ± SE) of different types of tissues collected at random. Target fungi were detected by tracing the internal transcribed spacer region (ITS1-5.8S-ITS2) of their nuclear ribosomal DNA. The DNA of the Botryosphaeriaceous fungi occurred in 49% of the samples; amplicon sequencing indicated that the most common Botryosphaeriaceous species were *Diplodia seriata*, *Botryosphaeria dothidea* and *Neofusicoccum parvum*, representing 51.4, 28.6 and 11.4% of sequenced samples respectively. A significant finding was also that DNA of these species and other Botryosphaeriaceae, detected in the discolored wood, was found in the asymptomatic propagation material. Nevertheless no isolate of any target fungi was obtained from any asymptomatic vine wood.

Detection of Botryosphaeriaceae species by real-time PCR. N. LUCHI[1], P. PINZANI[2], M. PAZZAGLI[2] and P. CAPRETTI[1]. *[1]Dipartimento Biotecnologie Agrarie, Sez. Patologia vegetale, Piazzale delle Casine, 28, 50144 Firenze, Italy. [2] Dipartimento Fisiopatologia Clinica, Sez. Biochimica Clinica, Viale Pieraccini 6, 50139 Firenze, Italy.*
E-mail: nicola.luchi@unifi.it

In the present work a real time PCR assay was developed, by using TaqMan chemistry, designing taxon-specific primers and probe able to detect Botryosphaeriaceae species, a fungal genus having its taxonomic position still under revision, that include a number of pathogens often associated to trunk canker and grapevine dieback. Since damage attributed to a single species is not easy to be distinguished, detection of Botryosphaeriaceae at genus level could be useful enough for practical purposes. Study of the homology of the amplicon sequence with other fungal species was performed by the Standard nucleotide-nucleotide BLAST (blast n) of the NCBI. The primers designed with TaqMan chemistry matched with *Diplodia seriata ('B. obtusa')*, *B. stevensii*, *Neofusicoccum luteum* ('*B. lutea*'). The specificity of primers and probe was confirmed on DNA extracted from pure fungal cultures of *D. seriata*, isolated from grape trunks. The amplification plots showed the PCR products with a Ct value that ranged from 18 to 23. Primers and probe designed for Botryosphaeriaceae species are proposed to predict fungal progression, during early stages of infection in asymptomatic plants and particularly in spurs, cordons and trunk. The use of this sensitive and rapid diagnostic tool could also be relevant in checking plant material used for propagation.

A new method for detecting *Phaeomoniella chlamydospora* and *Phaeoacremonium* species in grapevine plants. A. AROCA[1], D. GRAMAJE[2], J. ARMENGOL[2] and R. RAPOSO[1]. *[1]Instituto de Investigaciones Agrarias (INIA). CIFOR. Ctra. Coruña Km 7,5, 28040 Madrid, Spain. [2]Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.*
E-mail: aroca@inia.es

Phaeomoniella chlamydospora and several species of *Phaeoacremonium* are associated to Esca and Petri disease, two of the most destructive diseases of grapevines. These vascular fungi are slow growing in culture media and then, conventional methods of isolating them from a piece of wood incubated in culture media do not work adequately. These pathogens are frequently overgrown by other microorganisms present in the plant increasing the number of false negatives in the sample. In addition, subculturing of fungi growing in the plate may be required, which makes the identification process longer. In this work, an improved method for detecting *Pa. chlamydospora* and *Phaeoacremonium* spp. in grapevine was developed. A fragment taken from the potentially infected shoot was vacuum infiltrated with sterile water, and the vascular content collected. This liquid was then used for detection of *Phaeoacremonium* spp. and *Pa. chlamydospora* by two different methods: a) successive filtration through 5 μm and 0.45 μm of pore size and subsequent incubation in culture media; and b) DNA extraction and later detection by PCR with specific primers. Sensitivity of both methods was compared with the traditional method of fungal isolation in culture media from thin wood disks. Results demonstrated that the use of vacuum-washed vascular juice was better for detecting *Phaeoacremonium* spp. and *Pa. chlamydospora* because they were detected in more samples than using the conventional method. Furthermore, DNA extraction from vascular juice was easier than it was from wood, since it avoided the use of liquid nitrogen for grapevine wood ground and it prevented the risks of PCR inhibitors from wood.

Simultaneous identification of multiple fungal pathogens and endophytes with database t-RFLP. B.S. WEIR[1,2] and A.B. GRAHAM[1]. *[1]Corbans Viticulture, 8 Bristol Road, Whenuapai, Auckland, New Zeaaland. [2]Landcare Research, Private bag 92170, Auckland Mail Centre, Auckland, New Zealand.*
E-mail: bevan.weir@gmail.com

Terminal restriction fragment length polymorphism (t-RFLP) has previously been used to evaluate diversity in

microbial populations in soil and leaf litter. This method was adapted to monitor endophytic and pathogenic fungal populations in the xylem of grapevines during nursery propagation. Tissue samples were taken from xylem of young grapevines for fungal isolation onto MEA and PDA, and DNA was extracted from sub-cultures of isolates using the REDextract ™ kit (Sigma). Isolates were identified by sequencing the ITS region, and morphology. The DNA extracts from up to 3 isolates of each identified genus or species was used to obtain standard t-RFLP profiles. The extracts were PCR amplified with terminally labelled ITS1F and ITS4 primers. The ITS fragments were digested separately with *Hae*III and *Cfo*I and run on an ABI 3100 Genetic analyser, with a 1000-bp standard. The electropherogram was analysed with GeneMapper 3.5. Data including peak size, height, and area, was exported from GeneMapper into an R statistical package TRAMPR, where peaks were analysed and included in the database. This method was able to distinguish to the genus level between all of the fungal pathogens associated with vine decline and dieback diseases, as well as a range of common grapevine endophytes. Most of the common grapevine pathogens including *Cylindrocarpon, Eutypa, Botryosphaeria, Phaeomoniella* and *Phaeoacremonium* were distinguished to species level but *Phomopsis viticola* was distinguished from only three of the four *Phomopsis* and *Diaporthe* species tested. The ability of t-RFLP to simultaneously identify pathogenic and endophytic species in grapevine wood samples is a significant advance.

Identifying the causes of wood cankers and branch dieback in eastern U.S. vineyards. P.E. ROLSHAUSEN[1] and W. WILCOX[2]. *[1]University of Connecticut, Department of Plant Science, 1380 Storrs Road, Unit 4067, Storrs, CT, 06269, USA. 2Cornell University, Department of Plant Pathology, NY State Agricultural Experiment Station, 630 W. North Street Geneva, NY, 14456, USA.*
E-mail: philippe.rolshausen@uconn.edu

Little is known about the occurrence, diversity, and geographic distribution of the causal agents of trunk diseases in eastern U.S. vineyards. Although a few previous reports have documented the presence of these diseases, overall the baseline information is limited and scattered. Eastern US vineyards are quite different from their counterparts in Mediterranean climates (California, Mediterranean basin, Australia, South Africa). The climate is generally humid and the native ecosystem is primarily woodland. Winter temperatures are often extremely low; consequently, cold tolerant native *Vitis* species and inter-specific hybrids predominate although *Vitis vinifera* can also be grown in some of the warmer areas. Viticulture practices are adapted to the local weather patterns and to the type of grapes being grown. The susceptibility of non-*vinifera* grapes to individual trunk pathogens as well as the impact of local cultural practices, native vegetation and climate on disease etiology and epidemiology is unknown. In order to gain a broad perspective on the problem a survey including 12 eastern U.S states and the major grape varieties grown within them is currently underway. Here we present the preliminary results from 6 states. Fungi were identified by sequencing of the ITS1/5.8S/ITS2 region and BLAST search in the GenBank database. These results indicated that all major trunk diseases (Esca, Eutypa dieback and Bot canker) are present in all grape varieties although the individual species of the causal fungal genera remains to be clarified. Our results also showed that the prevalence of the individual diseases varies among geographical region and grape taxa.

Preliminary results of grapevine trunk disease monitoring in two vineyards in Marlborough, New Zealand. D.C. MUNDY[1], V. RAW[1] and M.A. MANNING[2]. *[1]The Horticulture and Food Institute of New Zealand Limited, Marlborough Wine Research Centre, P.O. Box 845, Blenheim, New Zealand. [2]The Horticulture and Food Institute of New Zealand Limited, Mt Albert Research Centre, Private Bag 92169, Auckland, New Zealand.*
E-mail: dmundy@hortresearch.co.nz

The New Zealand wine industry has been expanding since 1993 and has grown to become New Zealand's second largest horticultural export crop by value. With the investment made in planting grapes into new regions and the expansion within established growing areas, vineyard managers want to know how to ensure long-term sustainable production. A Sauvignon blanc and a Riesling vineyard were chosen in the Marlborough district to monitor trunk disease development and to provide information on the potential for control. Our results are from the first three years of a five-year programme. At the Riesling site, 1870 vines were mapped. In summer 2006, 8.3% of these vines were dead. By the summer of 2008, the proportion of dead vines had increased to 9.8%. At the Sauvignon blanc site, where 3772 vines were surveyed, the percentage of dead vines was lower than the Riesling site in 2006, with only 0.9% dead. By 2008, the proportion of dead vines at that site had increased to 2.8%. The two fungi most commonly isolates from those two sites were *Botryosphaeria* spp. and *Eutypa lata*. Regardless of which fungi are killing the vines, the loss of vines is reducing productivity for the grower, and replacement vines take time to restore full production. The rate of vine death will be used to develop an initial model of the potential economic impact of trunk disease fungi.

A comparison of micro-organism populations associated with *Vitis vinifera* cvs. Cabernet Sauvignon and Pinot Noir. H. WAITE, M. COLE and E. POWELL. *Northern Melbourne Institute of TAFE, Cnr, Dalton Rd and Cooper St, PO box 8, Epping, Victoria 3076, Australia.*
E-mail: *helenw-aas@nmit.vic.edu.au*

Under low powered microscopic examination the bark of Pinot Noir (PN) cuttings appeared to have higher populations of microorganisms on the cutting surface than Cabernet Sauvignon (CS). To determine if this was the case, 30 cuttings from each variety were randomly selected from the NMIT vineyard at Eden Park, 50 mm sections were cut from the base of each cutting and soaked in 50 ml of water (1 drop Tween/100 ml) at room temperature for 1 hour. 1 ml of the soaking water was then incubated on PDA for 2 days at 25°C and colonies counted. Following soaking, the 50 mm segments were debarked, flamed and 3 slivers of wood taken from the interior of each and incubated on PDA at 25°C for 5 days. Results of the colony count showed no significant difference ($P<0.05$) in the number of micro-organism colonies cultured from the soaking water from the bark of either variety. However, significantly more ($P<0.05$) colonies were isolated from the wood of PN (6.1 per 3 slivers) than CS (1.8 per 3 slivers). All cultures were grown on to enable identification of fungi and yeasts. Bacteria were not able to be identified. The most common organisms identified were *Cladosporium* sp., *Alternaria* sp., *Epicoccum* sp., *Candida* sp. and *Rhodotorlua* sp. There was no significant difference ($P<0.05$) in the mix of species between bark and wood cultures or between PN and CS. Although not recognised vine pathogens, these results indicate that the wood of PN is more easily colonised by common phylloplane micro-organisms than CS.

Detection of Petri disease fungi by morphological and molecular diagnosis: a preliminary comparison in young grapevine material from Italy. M. BORGO, I. BAZZO, D. BELLOTTO, G. DAL CORTIVO, G. LUCCHETTA, L. MIOTTI, L. STRINGHER, and E. ANGELINI. *CRA-VIT Centro di Ricerca per la Viticoltura, Viale XXVIII aprile 26, 31015 Conegliano Veneto, TV, Italy.*
E-mail: michele.borgo@entecra.it

Conventional detection of *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp., the main fungal species associated with Petri disease, involves fungal isolation and morphological examination *in vitro*. These methods are time-consuming and require well-trained personnel in fungal taxonomy. PCR-based methods, using genus and species-specific primers, are currently preferred over conventional analyses. PCR testing is faster, easy to establish and more sensitive than isolation. However, comparisons of the different methods are still needed for optimizing protocols suitable for routine molecular analyses. In the current study, a 100 young grapevine plants (one to three years old) were analysed by morphological features and PCR using different primer pairs, including universal and specific primers. DNA of infected grapevine wood was extracted by using the Qiagen kit. *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. were isolated *in vitro* respectively in 10% and 40% of the samples. Generally, molecular analyses allowed the detection of both genera in the same sample and in more samples, though the results slightly depended on the primer pairs used. In a few cases, the isolation was positive (1 out of 12 repetitions), but the molecular assay did not detect any fungal infection. The nested PCR gave more reliable results than non-nested PCR.

Isolation and identification of fungi associated with Esca in Vinho Verde Region (RDVV) – northwestern Portugal. M. FELGUEIRAS[1], G. CHICAU[2], A.C.P. DIAS[1]. *[1]Minho University – Biology Department, Campus de Gualtar, 4710-057 Braga–Portugal. [2]Ministry of Agriculture, D.R.A.E.D.M., Rua da Restauração No. 336, 4050-501 Porto–Portugal*
E-mail: acpdias@bio.uminho.pt

In the Portuguese Vinho Verde region (RDVV), during the last years the esca incidence is taking serious proportions. In this context, in the summers of 2004 and 2005 three vineyards were selected (two within the Monção subregion and one outside) in order to isolate associated Esca-fungi. Cordons were taken from 10 infected Alvarinho cultivar grapevines showing foliar esca symptoms. Results confirm the occurrence of the species P. chlamydospora and P. angustius in RDVV. The species, P. viticola and P. inflatipes, was identified for the first time in this wine region and in the Alvarinho grape variety. Results show that there are different strains within one fungus species in the majority of the isolated species, and also that the closer the vineyards, the higher the similarity of the fungal strains.

Acknowledgements: We thank Prof João Cabral and Dr. Silvia Cabral (Universidade do Porto, Dep. Biologia) for assistance in fungal identification.

Esca disease in young and mature vineyards in Marche, central-eastern Italy, and molecular detection of some associated fungi. G. ROMANAZZI[1], S. MUROLO[1], A. TRAMONTANO[1], L. PIZZICHINI[2] and S. NARDI[2]. *[1]Department of Environmental and Crop Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131 Ancona, Italy. [2]Servizio Fitosanitario Regionale - ASSAM, Via Alpi, 21 - 60131 Ancona, Italy.*
E-mail: g.romanazzi@univpm.it

Esca is the most important grapevine trunk disease, and it can induce severe crop declines. In the past this occurred mostly in mature vines, but today this is even a problem in young vineyards. The aim of this study was to investigate the incidence of esca in young (less than 7 years old) and mature (more than 12 years old) vineyards with cvs. Montepulciano, Sangiovese, Verdicchio and Passerina located in the main viticultural areas of the Marche region, central eastern Italy. The average incidence of diseased plants was higher in the mature (35.0%) than the young (5.2%) vine-

yards, and cv. Verdicchio was the most sensitive among the cultivars considered. The analysis of spatial spread of esca carried out in two mature vineyards with cv. Verdicchio and in a young vineyard with cv. Sangiovese showed a fluctation in the numbers of infected plants over the three years of observation. Esca symptoms were associated with the presence of some of the fungi involved in the disease, which were detected by classical and molecular tools. Isolation on agar media yielded colonies of *Phaeomoniella chlamydospora* (Pch), *Botryosphaeria* sp. (Bot), *Fomitiporia mediterranea* (Fomed), and, sporadically *Phaeoacremonium aleophilum* (Pal). In samples from young plants, Bot and Pch were recurrent, while Pch and Fomed were found in mature vines and old rootstocks. DNA extraction from wood scrapings and its amplification with Pch and Pal-specific primers confirmed the results obtained using classical tools, and at times it was more sensitive. This study confirms the role of different causal agents in the expression of esca symptoms, and the importance of molecular tools for the early and sensitive detection of these pathogens on propagative materials.

Diagnosis of fungal diseases implicated in grapevine trunk disease in an Alsatian French vineyard. P. KUNTZMANN[1], S. VUILLAUME[2], P. LARIGNON[3] and C. BERTSCH[4]. *[1]Institut Français de la Vigne et du Vin (ENTAV-ITV France) Station Régionale Alsace, France. 2Comité Interprofessionnel des Vins d'Alsace, Maison des Vins d'Alsace, Colmar, France. 3Institut Français de la Vigne et du Vin (ENTAV-ITV France) Station Régionale Rhônes-Méditerranée, France. 4Laboratoire Vigne Biotechnologie et Environnement, Université de Haute-Alsace, Colmar, France.*
E-mail: christophe.bertsch@uha.fr

The French vineyard, presents three principal wood diseases: eutypiose, esca and black dead arm (BDA). *Phaeomoniella chlamydospora, Phaeocremonium aleophilum, Eutypa lata, Fomitiporia mediterranea, Botryosphaeria obtusa, Botryosphaeria stevensii, Neofusicoccum parvum* are the main fungi isolated in France and associated with grapevines trunk diseases. The aim of this study is to highlight the type of wood lesions and the fungus present in Alsace French vineyard. Therefore, we have studied two vineyard plants with two different grapevine varieties (Auxerrois, Gewurztraminer). The foliar symptoms have shown that the vines planted with Auxerrois and Gewurztraminer varieties had respectively 15% and 18% of grapevine with symptoms. The study of the infected grapevine cartography shows a random distribution. Different cross sections were made on trunks, arms and roots of 66 vines showing foliar symptoms. Visual characterisation of the different lesions was described. The isolation of the different necrosis section allowed the identification of fungal species implied in grapevines trunk diseases as well as other fungi. Microbiological observations show that the majority of these vines are infected with *B. obtusa*, *P. chlamydospora*, *E. lata*, *F. mediterranea* and *P. aleophilum*. In Gewurztraminer vineyards, the fungus the most frequently isolated is *P. chlamydospora*, follow-up by *B. obtusa*. The presence of *B. obtusa* in the wood of the different parts of the grapevine and in young wood should certainly complete action accomplished by *E. lata* and *P. chlamydospora*.

Genetic and virulence diversity of *Cylindrocarpon liriodendri* and *C. macrodidymum* associated with black foot disease of grapevine. S. ALANIZ, J. ARMENGOL, M. LEÓN, J. GARCÍA-JIMÉNEZ, and P. ABAD-CAMPOS. *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022, Valencia, Spain.*
E-mail: alanizferro@gmail.com

Inter-Simple Sequence Repeat (ISSR) analysis was used to investigate genetic diversity of 87 Spanish and five reference isolates of *Cylindrocarpon liriodendri* and *C. macrodidymum*, the causal agents of black foot disease of grapevine. A total of ten ISSR primers were evaluated and four of them, $(GT)_7$, $(CCA)_5$, $(CGA)_5$ and $(TCG)_5$, were able to provide reproducible and polymorphic DNA fingerprint patterns. This analysis detected genetic diversity in both species that was more relevant in *C. macrodidymum*. Estimated Nei´s genetic diversity was $H = 0.06$ in *C. liriodendri* and $H = 0.16$ in *C. macrodidymum*. The cluster analysis of ISSR data showed 21 different genotypes that were grouped in seven groups. Two of these groups corresponded to *C. liriodendri* (G1 and G2) and the other five groups corresponding to *C. macrodidymum* (G3 to G7), with a similarity among the isolates into each group of at least 88%. Nineteen isolates were selected from the seven groups to study the virulence diversity. They were inoculated in grapevine seedlings obtained from cv. Tempranillo by watering the roots with 20 mL of conidial suspension. Controls were inoculated with sterile distilled water. After inoculation, these seedlings were placed in a greenhouse at 25–30°C in a completely randomized design. Plants were observed at five days intervals over two months for the development of foliar symptoms. At the end of experiment, root symptoms and dry weights of shoot and root were recorded. The pathogenicity test detected virulence diversity in *C. macrodidymum*. The isolates belonging to ISSR groups G6 and G7 were significantly more virulent than the other *C. macrodidymum* and *C. liriodendri* isolates.

A double-stranded RNA from *Cylindrocarpon* isolates related to members of *Endornavirus* genus. T. NASCIMENTO[1], F. CARDOSO[2], C. REGO[1] and H. OLIVEIRA[1]. *[1]Instituto Superior de Agronomia, Technical University of Lisbon, 1349-017 Lisboa, Portugal. [2]LNEG-DB-UTPAM, Estrada do Poço do Lumiar, 22, 1649-038 Lisboa, Portugal.*
E-mail: nascimento@isa.utl.pt

The presence of high molecular weight endogenous dsRNA, greater than 10 kbp, is frequently found in various fungi. Actually, these large plasmid-like dsRNA replicons are recognized as members of a new virus genus named *Endornavirus*. dsRNA viruses in the genus *Endornavirus* are distributed in plants, protists and fungi. During our work that comprised the screening of *Cylindrocarpon* isolates, obtained from grapevine, for the occurrence of dsRNAs, we detect the presence of large dsRNA (14–19 Kb). Using degenerate primers corresponding to the RNA-dependent RNA polymerase (RdRp) region of the endornaviruses, we detected by RT-PCR the expected bands. This confirms that the dsRNA molecules present in *Cylindrocarpon* isolates are an endornavirus. The role of this dsRNA endornavirus in virulence of *Cylindrocarpon* isolates is under study.

Multigene sequence analyses reveal novel *Cylindrocarpon* species associated with black foot disease of grapevines (*Vitis* spp.). A. CABRAL[1], C. REGO[1], T. NASCIMENTO[1], H. OLIVEIRA[1], J.Z. GROENEWALD[2] and P.W. CROUS[2]. *[1]Instituto Superior de Agronomia, Universidade Técnica de Lisboa. Tapada da Ajuda, 1349-017 Lisboa, Portugal. [2]Centraalbureau voor Schimmelcultures, P.O. Box 85167, 3508 AD Utrecht, The Netherlands.*
E-mail: anacabral@isa.utl.pt

Black foot is an important disease of grapevines, affecting nurseries and young vineyards. Typical disease symptoms include a darkening at the foot of vines, reduced vigour, retarded sprouting, shortened internodes, sparse and chlorotic foliage, frequently resulting in plant death. In different countries the incidence and severity of this disease are forcing grapevine growers to replant considerable areas. Currently the causal agents belong to *Cylindrocarpon liriodendri*, *Cyl. macrodidymum*, *Campylocarpon fasciculare*, *Campyl. pseudofasciculare,* and possibly, *Cyl. pauciseptatum*. However, the relative importance, frequency, geographic distribution and genetic diversity of populations of these species are still insufficiently understood. The aim of this work is to study a collection of Portuguese grapevine *Cylindrocarpon* isolates using multigene nucleotide sequences (β-tubulin (TUB), histone H3 (HIS), translation elongation factor-1α (EF), and the Internal Transcribed Spacers (ITS) of the nuclear ribosomal DNA operon), with the purpose of elucidating the possible existence of different species. Within the four genes studied, the ITS proved the least informative, whereas HIS was the most informative, showing more nucleotide differences among species. These phylogenetic analyses revealed genetic variability within *Cyl. macrodidymum*, suggesting that this could be a species complex. Some isolates clustered with *Cyl. pauciseptatum*, and *Cyl. liriodendri*, while other *Cylindrocarpon* isolates formed a new lineage close to *Cyl. macrodidymum*, or clustered in the "*C. destructans*" complex. To facilitate morphological identification, isolates of each cluster were described morphologically, and these data correlated with the DNA phylogeny.

Identification of *Cylindrocarpon* species associated with grapevine decline in Castilla y Leon (Spain). M.T. DE FRANCISCO, L. MARTIN, R. COBOS, P. GARCIA-BENAVIDES and M.T. MARTIN. *Dpt. Viticultura, ITACYL, ctra. de Burgos Km.119, E-47071 Valladolid, SPAIN. Servicio de defensa contra plagas e inspección fitopatológica, Centro de Aldearrubia, E-37430 Salamanca, Spain.*
E-mail: marvilte@itacyl.es

Black Foot disease is one of the grapevine decline diseases observed worldwide. Grapevine decline causes economic losses by shortening the productive life of vines. External symptoms could be observed and in some cases symptomless infections was found in mainly young plants. Grapevine decline is present in the 9 "Appellation of origin" areas of Castilla y León (Arlanza, Arribes, Bierzo, Cigales, Ribera de Duero, Rueda, Tierra de León, Tierra del Vino de Zamora and Toro). The incidence reached 10% in 2007. During this study grapevine decline fungi were isolated and identified. The most frequently isolated fungi include *Botryosphaeria* spp., *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp, and *Cylindrocarpon* spp. The *Cylindrocarpon* species was more studied further. Based on molecular techniques two major species: *C. macrodidymum and C. liriodendri* and two minor species: *C. pauciseptatum* and *C. olidum* were identified. As it was expected the four species were mainly isolated from the basal end of the rootstocks and the roots of young grapevines. The analysis of more than one isolates collected from single vines showed that at least two different *Cylindrocarpon* species may coexist on the same plant.

Host-Pathogen Interaction

Physiological effects of esca-associated fungi on *Vitis vinifera* L. cv. Italia. G. BRUNO, M.P. IPPOLITO, F. TOMMASI and L. SPARAPANO. *Department of Biology and Plant Pathology, Università degli Studi di Bari, via G. Amendola 165A, 70126 Bari, Italy.*
E-mail: gbruno@agr.uniba.it

Esca symptoms affect trunk, branches, shoots, leaves and berries, bringing about tiger stripes on leaves, white rot of trunk, black measles on berries, apoplexy, cracking and death of vines. The systematic isolation of *Togninia minima* (*Tmi;* anamorph: *Phaeoacremonium aleophilum)* and *Phaeomoniella chlamydospora* (*Pch*) and the basidiomycete *Fomitiporia mediterranea* (*Fme*) from the discoloured or decayed wood of esca-diseased vines

indicated a close relationship between the individual stages of wood deterioration and particular species of fungi. Very likely, at least a part of the external and internal symptoms of esca are caused by phytotoxic fungal metabolites produced in the discoloured or decayed woody tissue or by the oxidation of some host response substances. A vineyard of currently 15-year-old *Vitis vinifera* cv. Italia (4376 vines) trained by the overhead system ('tendone', located in the countryside of Bari, Apulia, southern Italy), was surveyed for incidence of external and internal symptoms of the esca syndrome. Samples of xylem sap, berries and leaves were collected from healthy vines and from vines showing symptoms of brown wood-streaking caused by *Pch* and *Tmi* or from vines with symptoms of both brown wood-streaking and white rot caused by *Pch*, *Tmi* and *Fme*. The accumulation of biomolecules (α-glucans and pentaketides) produced by the fungi and host defence compounds (resveratrol, benzoic acid derivatives, flavonols) were detected at different stages of grapevine development. Further emphasis was laid on the involvement of antioxidant redox systems, in particular those related to ascorbate and glutathione. The effect of fungi and/or their metabolites on leaf water potential was also monitored.

Alteration of photosynthesis and activation of defense in grapevine before severe esca expression. P. LETOUSEY[1], F. BAILLIEUL[1], M. BOULAY[2], C. CLÉMENT[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 implies to have a better knowledge of the disease, including characterization of changes in grapevine physiology due to esca. Physiological and molecular approaches were used to determinate if the plant physiology is affected before and during the expression of the apoplectic form of esca. This study was investigated in French Moët & Chandon vineyard on Chardonnay cultivar. Different apoplectic samples were analyzed: pre-apoplectic leaves without symptoms or softened and leaves harvested during cane apoplexy (softened or wilting leaves). Control samples corresponded to leaves harvested on vines which did not develop any symptom since 2001. Our results showed an early alteration of photosynthetic mechanisms by severe esca, before the expression of foliar symptoms. Indeed, seven days prior any symptom were visible, net photosynthetic activity, stomatal conductance and activity of photosystem II were dramatically decreased whereas the intercellular CO_2 concentration increased slightly. Thus, gas exchange and chlorophyll fluorescence measurements allowed to early detect grapevine apoplexy. Moreover, analysis by quantitative RT-PCR showed a strong repression of genes encoding photosystem I subunit, Rubisco and enzyme from Calvin cycle in pre-apoplectic leaves and thus explained in part the reduction in net photosynthetic activity. In addition, photosynthesis gene expression was still repressed during wilting of leaves. By contrast, grapevine activated the expression of several stress-related genes encoding for enzymes of phenylpropanoid pathway, chitinases and detoxication enzyme. This indicated that severe esca disease is perceived by foliar cells prior the appearance and during the expression of apoplexy symptoms.

Physiological alterations in esca-diseased vines and the detection of scytalone and isosclerone in the tiger-striped vine leaves. L. CALAMAI[1], G.B. MATTII[2], L. MUGNAI[3] and G. SURICO[3]. *[1]Dipartimento di Scienza del Suolo e Nutrizione della Pianta, Università degli Studi di Firenze, Piazzale delle Cascine 18, 50144 Firenze, Italy. [2]Dipartimento di Ortoflorofrutticoltura, Università degli Studi di Firenze, Viale delle Idee 30, 50019 Sesto F.no (FI), Italy. [3]Dipartimento di Biotecnologie Agrarie–Sezione Patologia vegetale, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy.*
E-mail: gbmattii@unifi.it

Over a period of three years, from 2005 to 2007, gas exchange was measured on entire esca diseased potted 25-year-old grapevines, with or without leaf symptoms and watered with about 10 l of water daily, and on single vine leaves. The leaves on which gas exchange was measured either came from asymptomatic vines, or were completely healthy-looking leaves from symptomatic vines, or healthy portions of symptomatic leaves, or impaired portions of symptomatic leaves. The leaves from the same sample types were also used to measure levels of soluble sugars (fructose, glucose and sucrose), free amino-acids, abscisic acid, starch and the anthocyanins. HPLC combined with ion trap mass spectrometry was used to detect scytalone and isosclerone in the symptomatic leaves of infected vines. The measurements of gas exchange of entire vines and single leaves broadly confirmed what was obviously expected: photosynthesis was strongly affected and decreased going from healthy leaves to the more impaired leaves depending on the degree of their impairment. Leaves growing in the vicinity of diseased leaves had a lower photosynthesis than leaves growing farther away from diseased leaves. Lastly, such alterations in the photosynthesis of leaves appeared about 15 days before the symptoms of esca appeared on these leaves. Symptomatic leaves exhibited an increase in the levels of fructose and glucose and a decrease in the levels of sucrose. The concentration of starch went down and that of abscissic acid went up, as did that of free amino-acids, and especially that of proline. Anthocyanin levels also

increased. On the whole the results gave values typical of leaves suffering from water stress even though there was no water stress as the vines were regularly watered. Despite repeated attempts, it was not possible to detect any scytalone or isosclerone in any of the symptomatic leaves. Specifically, while a peak at retention times corresponding to scytalone was present in the HPLC-UV chromatograms of several leaf tissues extracts, its mass spectrum did not allow the identification as scytalone.

Acknowledgements: Research study commissioned from ARSIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forestry) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-Regional Project "Grapevine esca: research and experiment in the nursery and in the field for prevention and cure."

Histological observations on the petioles of esca symptomatic leaves. L. ANDREINI, R. VITI and G. SCALABRELLI. *Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Coltivazioni Arboree, Università di Pisa.*
E-mail: landreini@sssup.it

This work aimed to study the anatomical characteristics of esca symptomatic leaf petioles sampled from grapevine plants cv. 'Cabernet Sauvignon', a genotype known to be very susceptible to this disease. Petioles were collected from: symptomatic vines (SV); vines that never showed Esca symptoms (NES); vines that in the previous years resulted symptomatic and that could be expected to show again foliar symptoms (PYS). On these vines were selected three shoots from which petioles were collected from basal and apical portions before Esca appearance. After leaf symptoms appeared petioles were collected from the apical leaves that had not yet exhibited Esca symptoms. The tissues were fixed in FAA, embedded in Histoplast and transversally sectioned (7 μm) by a Shandon microtome. The thin sections were stained with Crystal violet and Erythrosin B to identify the lignified cell walls. Morphological observations were carried out by optical microscope. Petiole observations showed different anatomical characteristics in relation to vine health. The main feature of tissues infected by Esca disease was the minor lignification of vascular tissues. This deficiency was detected revealing the presence of lignin by the specific stain. The plants which were symptomatic the previous years showed, at the beginning of fruit set and before appearance of visual Esca symptoms, xylematic tissues of petioles poorly lignified. The same feature was found on petioles of symptomatic leaves that had lower cell wall thickening of xylem vessels, wood fiber, bundle sheath and collenchyma. In addition the cells of pith showed lack of turgidity and appeared almost collapsed. At veraison (end of July) when the Esca disease symptoms were observed only in the basal part of the shoots, the apical petioles of healthy leaves showed tissues poorly lignified and partially damaged. The histological examination of petiole tissues could be a method for the early detection of Esca infections before the symptoms became visible. More work needs to be done to understand the mechanism of such alteration induction.

Acknowledgements: Research study commissioned from ARSIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forestry) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-Regional Project "Grapevine esca: research and experiment in the nursery and in the field for prevention and cure."

Analysis of xylem sap proteins in water-stressed vs. non-stressed *Vitis vinifera* 'Cabernet Sauvignon' and 'Thompson Seedless'. C.B. AGÜERO[1], E.T. THORNE[2], A.M. IBAÑEZ1, A. FABRITIUS[2,3], W.D. GUBLER[2] and A.M. DANDEKAR[1]. *[1]Department of Plant Sciences and [2] Department of Plant Pathology, University of California, Davis, CA 95616, USA. [3]Agri-Analysis LLC, 45133 County Rd 32B, Davis, CA 95618, USA.*
E-mail: wdgubler@ucdavis.edu

In recent years, vineyards have experienced a dramatic increase in esca and Petri disease caused by *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. Various stresses including water deficit, early fruiting, "J" rooting, and salt stress have been suggested as cause of increased virulence of the esca pathogens. Water deficit has been shown to predispose plants to infection from pathogens, including *P. chlamydospora* and *Phaeoacremonium* spp. Preliminary experiments of comparing xylem sap from water-stressed and non-stressed grapevines showed that sap from stressed vines enhances the *in vitro* growth of *Phaeoacremonium* spp. and *P. chlamydospora*. This suggested that xylem sap of non-stressed vines may contain substances that inhibit or slow fungal growth, and are absent under stress conditions. To investigate the xylem sap protein content in water-stressed versus non-stressed vines, young Cabernet Sauvignon and Thompson Seedless vines were exposed to water stress with leaf water potentials between -1.0 and -1.5 MPa. Non-stressed vines were kept at water potentials between -0.2 and -0.6 MPa. Sap was collected under positive pressure from herbaceous shoots. Sap proteins were separated by one-dimensional polyacrylamide gel, protein spots were digested with trypsin, and peptide spectra were searched using GPM software. Several proteins were identified that were present only in non-stressed vines. These included β-xylosidase, SBT1-subtilisin, methionine synthase, α-mannosidase precursor and α-arabinofurosidase. Many of these proteins are involved in cell wall metabolism, and interestingly, in plant defense. These preliminary results suggest that xylem sap may act as a barrier against *P. chlamydospora* and *Phaeoacremonium* spp.

Identification and biochemical characterization of the major proteins secreted by the grapevine pathogen *Eutypa lata*. R. FREITAS[1,3]; C. REGO[2]; H. OLIVEIRA[2] and R. FERREIRA[1,3]. [1]*Departamento de Botânica e Engenharia Biológica, Universidade Técnica de Lisboa, Instituto Superior de Agromonia, 1349-017 Lisboa, Portugal.* [2]*Departamento de Protecção das Plantas e de Fitoecologia, Universidade Técnica de Lisboa, Instituto Superior de Agromonia, 1349-017 Lisboa, Portugal.* [3]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Apartado 127, 2780 Oeiras, Portugal.*
E-mail: regfreitas@isa.utl.pt

The objective of this project is to understand the molecular mechanisms of plant-microbe interactions with particular relevance to grapevine trunk diseases caused by fungi. One of these fungi is *Eutypa lata* (Pers:Fr.) Tul & C. Tul., an ascomycete fungus responsible for severe dieback in numerous woody plants. In grapevine, the pathogen colonises the wood through pruning wounds and slowly kills the vines. In the present study, we confirmed the presence of several enzymes secreted by the grapevine pathogen *E. lata* that are potentially involved in the breakdown of cellulose, xylose and lignin. The fungus also produced polyphenol oxidases that might participate in the breakdown of wall-bound lignin. Nonetheless, more work is needed to characterize the *E. lata* cell wall degrading enzymes. Results from different liquid culture media supplemented with wood powder substrate also support the conclusion that *E. lata* is able to sense the presence of the wood, including the starch component, and produce the enzymes required to utilize these more complex sources of carbohydrates since a high activity rate for amylase was found in the grapevine wood extract media. In addition, a very high cutinase activity was obtained, as well as a very positive test for proteinase activity in the grapevine wood extract media, which clearly indicates a need of this fungus to scavenge for nitrogen in the wood. These extracellular enzymatic activity results necessarily reflect the infection method and needs of this fungus, which will be the aim of further studies.

Further evaluation of the role of fungal secondary metabolites in the expression of Eutypa dieback symptoms. P.E. ROLSHAUSEN[1], N.E. MAHONEY[2], R.J. MOLYNEUX[2] and W.D. GUBLER[3]. [1]*University of Connecticut, Department of Plant Science, 1380 Storrs Road, Unit 4067, Storrs, CT, 06269, USA.* [2]*Western Regional Research Center, Agricultural Research Service, USDA, 800 Buchanan Street, Albany, Berkeley, CA, 94710, USA.* [3]*University of California, Davis, Plant Pathology Department, One Shields Avenue, Davis, CA, 95616, USA.*
E-mail: philippe.rolshausen@uconn.edu

Eutypa lata causes disease on grapevine by producing an array of cell wall degrading enzymes and phytotoxic secondary metabolites. The appearance of foliar symptoms are caused by (a) translocating compound(s) because the fungus resides in the diseased wood and is never isolated from symptomatic green tissues. However, our repeated attempts to find known fungal metabolites (i.e. eutypine, eutypinol, methyleutypinol, methyleutypine, 2-isoprenyl-5-formylbenzofuran, eulatachromene, siccayne, eulatinol) in tissues and sap of affected grapevines have failed repeatedly. Our goal was to; (i) study the time course of apparition of *E. lata* metabolites in liquid medium overtime and clarify the sequence of metabolite production; (ii) grow the fungus in the same medium amended with wood powder to determine if metabolite production was altered; (iii) determine if other metabolites were detected during fungal growth. Our results indicated that eutypinol was the first of the metabolites to be detected and was produced in the highest abundance. Eutypine was produced after eutypinol and in much lower abundance, indicating that eutypine was most likely derived from eutypinol. Methyleutypinol, eulatachromene, methyleutypine, and 2-isoprenyl-5-formylbenzofuran were produced later on and were likely derived from these two main compounds. The pH of the media became increasingly basic going from pH 5 to pH 8. Eutypine and eutypine derivatives production were altered in the presence of wood. Abscissic acid was detected in the media suggesting that this hormone could play a role in the symptoms appearance and could be the, or one of the compounds translocated in the plant.

Pathogenicity of *Cylindrocarpon* propagules on grapevine. C.M. PROBST, E.E. JONES, H.J. RIDGWAY and M.V. JASPERS. *Bio-Protection and Ecology Division, Lincoln University, PO Box 84, Lincoln University, New Zealand 7647.*
E-mail: probstc2@lincoln.ac.nz

Cylindrocarpon liriodendri and *C. destructans* pathogenicity was investigated for three propagule types, in three concentrations, with callused cuttings of rootstock 101-14. Before planting they were soaked in suspensions of chlamydospores or conidia, at 0, 10^3, 3.2×10^4 and 10^6 ml^{-1}, or with 0, 1, 3 or 5 g of infested wheat grains added to planting holes in pots. Assessment after 5 months growth was by isolation from trunk pieces, four taken at 1 cm and one at 5 cm above each base. For the spores, disease severity (percent infected pieces) at 1 cm did not differ between spore types (P=0.17), but differed between species and concentrations (both $P \leq 0.001$). *Cylindrocarpon liriodendri* was the more pathogenic, with severity of 0, 22, 49 and 38%, and for *C. destructans* of 0, 4, 14 and 38%, respectively across the concentrations. Incidence (% infected plants) for *C. liriodendri* was 0, 32, 82 and 54%, and for *C. destructans* was 0, 4, 25 and 54%, respectively across the concentrations. For the mycelium inoculum at 1 cm, severity differed between species (P=0.018) and amounts of inoculum ($P \leq 0.01$); for *C. liriodendri*, severity

and incidence were, 0, 12, 32 and 54% and 0, 14, 50 and 79% respectively while for *C. destructans*, severity and incidence were 0, 5, 21 and 36%, and 0, 7, 36 and 50% respectively, across the concentrations. At 5 cm above the stem base, incidence differed only between species ($P \leq 0.001$), being 29% and 10%, respectively. A field trial currently being assessed will also be discussed.

Accumulation of viniferins and their reactivity to ROS in the reddish-brown wood of esca-diseased grapevine (cv. Sangiovese). C. AMALFITANO[1], D. AGRELLI[1], A. EVIDENTE[1], L. MUGNAI[2] and G. SURICO[2]. *[1]Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Università degli Studi di Napoli Federico II, via Università 100, Portici, Italy. [2]Dipartimento di Biotecnologie Agrarie - Sezione di Patologia Vegetale, Università degli Studi di Firenze, P.le delle Cascine 28,50144 Firenze, Italy.*
E-mail: amalfita@unina.it

Numerous viniferins have been isolated from the wood of esca-diseased *Vitis vinifera* cv. Sangiovese grapevines. They are stilbenic polyphenols with phytoalexinic activity typical of the Vitacee family. We detected higher levels of viniferins in the deteriorated brown-red vine wood that is typical of vines colonized by the esca pathogens, especially *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*. These phytoalexins created *in vitro* condensation products that reached a molecular weight of over 10000 Dalton, in the presence of ROS generated by a Fenton-type reaction. On this basis, the effect of the vine oxydative reaction on the condensation of these polyphenols, which would limit invasion by the fungus is discussed. Essentially, the effect of the rapid and substantial production of these polyphenols should be to create a physical barrier blocking pathogen penetration, delaying their entry into the wood, and probably to a certain extent protecting the wood tissues from the esca fungi. Spectroscopic examination did indeed show that condensed polyphenols became deposited in the brown-red wood and that these polyphenols had features similar to the condensed products derived from the methanolic extract of grapevine wood, which contains viniferins, and which undergoes the Fenton-type reaction.

Acknowledgements: Research study commissioned from ARSIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forestry) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-Regional Project "Grapevine esca: research and experiment in the nursery and in the field for prevention and cure."

An immunological method to detect toxic polysaccarides produced by *Phaeomoniella chlamydospora*. A. ANDOLFI[1], A. CIMMINO[1], A. EVIDENTE[1], M. IANNACCONE[1], R. CAPPARELLI[1], L. MUGNAI[2] and G. SURICO[2]. *[1]Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali; Università di Napoli Federico II, Via Università 100, 80055 Portici, Italy; [2]Dipartimento di Biotecnologie Agrarie – Sezione di Patologia Vegetale, Università di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy.*
E-mail: laura.mugnai@unifi.it

Esca is a very important disease of grapevine and is associated with a number of different fungi, of which *Phaeomoniella chlamydospora* (*Pch*), seems to be the most injurious. The grapevine leaf and berry symptoms of esca are thought to be caused when phytotoxins produced by the esca fungi in the wood are transferred to the apical tissue of the vines and accumulate there. Phytotoxic metabolites are found in the culture filtrates of *P. chlamydospora* and *P. aleophilum,* another fungus with an important role in esca. Among these phytotoxins there are some that are reported to belong to a class of polysaccharides. Thus an immunochemical method based on flow cytometry and antibodies developed against a specific exopolysaccharide could be useful to elucidate the role of these phytotoxins and possibly even to diagnose esca. In this study, native EPSs from culture filtrate of the Pch type strain were used to immunise Fischer rats, and the EPSs-diluted rat antiserum was used to make a sensitive method with a flow cytometer for the specific determination of *Pch* EPS (5 μg *Pch* EPS minimum detectable limit). The test was applied to extracts containing polysaccarides obtained from 1) asymptomatic leaves in apparently healthy vines; 2) asymptomatic leaves in esca-affected, symptomatic vines; and 3) symptomatic leaves at various stages of symptom manifestation. At the sensitivity limit of the method applied, only extracts from symptomatic leaves were positive in the immunological test, supporting the hypothesis that the *Pch* EPSs species had moved from the wood to the leaves of esca diseased vines.

Acknowledgements: Research study commissioned from ARSIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forestry) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-Regional Project "Grapevine esca: research and experiment in the nursery and in the field for prevention and cure."

Trans-resveratrol in leaves and berries of esca proper infected grapevines at different phenological growth stages. F. CALZARANO, V. D'AGOSTINO and M. DEL CARLO. *Università degli Studi di Teramo, Dipartimento di Scienze degli Alimenti, Via C.R. Leric, 1, 64023 Mosciano Stazione (TE), Italy.*
E-mail: fcalzarano@unite.it

Foliar symptoms in two cv. "Trebbiano d'Abruzzo" vineyards affected by esca proper in the Abruzzo region of Italy

have been monitored over a 10-year period. By this means asymptomatic esca-infected vines could be distinguished from possibly healthy vines. Leaves and berries of healthy, asymptomatic, and symptomatic esca-affected vines were sampled over four different phenological growth stages ranging from pre-closure to harvest during a 4-year study period. Trans-resveratrol was measured in all samples using a recently established analytical protocol characterised by rapid pre-treatment in liquid nitrogen. The results indicated a possible defence mechanism of esca-affected vines that varied in intensity in symptomatic and asymptomatic vines and at different growth stages. The sometimes significantly higher concentration of trans-resveratrol found in the leaves and berries of asymptomatic esca-affected vines in comparison to healthy vines suggests the possible triggering of a systemic response, given the absence of any necrosis. Alternatively, it can be hypothesised that toxins are released from the infected wood at levels not sufficient to cause symptoms, but sufficient to elicit trans-resveratrol synthesis in the canopy parts reached. The higher concentrations observed in the leaves and berries of symptomatic vines in comparison to the healthy and asymptomatic sample groups could be a result of the accumulation of trans-resveratrol induced by the plant response in addition to that produced as a consequence of the necrosis of the leaves and berries. Another hypothesis is that trans-resveratrol may play a role in the appearance of symptoms when its concentration reaches such toxic levels that it can generate necrosis of the leaves and berries. Moreover, a possible cross-interaction between trans-resveratrol and toxins produced by fungi could result in the production of toxic compounds.

Acknowledgements: Research study commissioned from ARSIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forestry) by 14 administrative regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-regional project "Grapevine esca: research and experiments in the nursery and in the field for prevention and cure."

Induction of terpenoid biosynthesis in grapevine calluses by *Phaeoacremonium parasiticum*. G. ESCORIAZA[1], M. GIL[2], M. GATICA[1], R. BOTTINI[2] and P. PICCOLI[2]. *[1]Laboratorio de Fitopatología, Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Mendoza, San Martín 3853, Luján de Cuyo, Mendoza, Argentina. [2]Cátedra de Química Orgánica y Biológica, Facultad de Ciencias Agrarias-CONICET, Universidad Nacional de Cuyo, Alte. Brown 500, Chacras de Coria, Mendoza, Argentina.*
E-mail: gescoriaza@mendoza.inta.gov.ar

The grape disease "hoja de malvón" is associated with a xylophages fungi complex that includes *Phaeoacremonium parasiticum*. A normal plant response to pathogenic attack is the production of terpenic phytoalexins throughout the cyclization of pharnesyl di-phosphate (FPP) by terpene-cyclase enzymes (TPS). The aim of this work was to evaluate TPS activity and to assess terpene biosynthesis in grape calluses that had been inoculated with *P. parasiticum* in comparison to non-inoculated plants. TPS activity, evaluated as the amount of radioactive FPP ([^{3}H]-FPP) transformed into hexane soluble products, showed a significant increase with fungi dilutions of 2×10^6 conidia μl^{-1} and 2×10^7 conidia $10\mu l^{-1}$ as compared with controls and calluses inoculated with lower dilutions (2×10^4 and 2×10^5 conidia μl^{-1}). In addition, the metabolic profiles of the hexane fractions from control and inoculated calluses were assessed by capillary gas chromatography coupled with mass spectrometry (GC-MS). In inoculated calluses the biosynthesis of pinene (monoterpene) and *cis*-Nerolidol (sesquiterpene) increased with increasing fungi concentrations and in correlation with TPS activity, whereas the amount of squalene (the triterperne key in sterol synthesis) decreased.

Analysis of expression of defence-related genes in *Vitis vinifera* cv. Vinhão cell cultures elicited by *Phaeomoniella chlamydospora*. M.R.M. LIMA and A.C.P. DIAS. *Universidade do Minho, Departamento de Biologia, Campus de Gualtar, 4710-057 Braga, Portugal.*
E-mail: acpdias@bio.uminho.pt

Esca is a destructive disease that affects *Vitis vinífera* plants around the world, leading to important losses in wine production. *Phaeomoniella chlamydospora* (Pc) is a fungus frequently associated with esca and grapevine decline. To study the defence response – specifically, gene activation – of grapevine to Pc we utilized *in vitro* cultures of *V. vinifera* cv. Vinhão (Vv) elicited with fungus extract. The expression of genes encoding pathogenesis-related proteins (class 6 and class 10 PR proteins, β-1,3-glucanase, and class I and III chitinases) and genes involved in the octadecanoid (lipoxigenase) and phenylpropanoid (phenylalanine ammonia lyase and stilbene synthase) pathways were monitored by semi-quantitative RT-PCR at 3, 12, 24 and 48 hours after elicitation. Pc elicitation resulted in variations in mRNA production in Vv cell cultures, namely an increase in lipoxigenase and stilbene synthase gene expression.

Acknowledgments: This work was supported by an FCT grant (SFRH/BD/17944/2004).

Identification of phytotoxins from *Botryosphaeria obtusa*, a pathogen of black dead arm disease of grapevine. J.D. DJOUKENG[1], P. LARIGNON[2], R. TABACCHI[1] and E. ABOU-MANSOUR[1]. *[1]Institute of Biology, University of Neuchâtel, rue Emile Argand 11, 2009 Neuchâtel, Switzerland. [2]Institut Français de la Vigne et du Vin, Unité Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France.*
E-mail: eliane.abou-mansour@unifr.ch

A bioassay-guided fractionation of culture filtrate of *Botryosphaeria obtusa* resulted in the isolation of four dihydroisocoumarins: mellein 1, 4-hydroxymellein 2,7-hydroxymellein 3 and the new compound 4,7-dihydroxymellein 4. LC-UV-DAD-MS analysis of vine wood infected by *B. obtusa* revealed the presence of mellein (1). *B. obtusa* was also able to oxidise wood δ-resveratrol in the dimer δ-viniferin. The structures of the phytotoxins isolated have been established on the basis of IR, MS, 1D and 2D NMR.

Effect of grapevine hydric status on the development of lesions caused by *Phaeomoniella chlamydospora* in internal wood tissues. C. LAVEAU, N. MAHER, S. BASTIEN and L. GUÉRIN-DUBRANA. *M.R 1065 Santé Végétale - INRA/ENITA de Bordeaux, I.S.V.V., U.F.R. 103, B.P. 81, 33883 Villenave d'Ornon Cedex, France.*
E-mail: l-guerin@enitab.fr

Phaeomoniella chlamydospora is considered to be a pioneering fungus of grapevine esca disease. A survey of this disease in Bordeaux vineyards revealed that the incidence of esca was linked to soil water availability. Therefore, the effect of the vine's hydric status on the invasive capacity of *P. chlamydospora* was investigated by inoculating one-year-old woody shoots of 14-year-old vines in a greenhouse. Potted vine stocks from 3 varieties (Merlot, Cabernet-Sauvignon, Semillon) were maintained under either a non-restrictive or a water deficient (corresponding to about 40% of the normal requirement) hydric status. Physiological parameters such as stem growth and stomatal resistance were measured. Water stress induced a marked reduction in stem growth and a significant increase in stomatal resistance. Inoculation of *P. chlamydospora* resulted in the development of lesions that were significantly shorter in the water-stressed vines compared to the non-stressed vines. No significant effect was observed in relation to the vine variety or to an interaction between the water supply and the cultivar.

Influence of ESCA disease on reduction in grapevine yield. N. LATINOVIC and J. LATINOVIC. *University of Montenegro, Biotechnical Faculty, Department of Plant Protection, Kralja Nikole bb, 81 000 Podgorica, Montenegro.*
E-mail: nlatin@cg.yu

The appearance of wilting of varying intensity on certain grapevine canes has been observed in the vineyards of Podgorica for several years. The fungi *Phaeomoniella chlamydospora*, *Phaeocremonium aleophilum,* and *Fomitiporia mediterranea*, causal agents of ESCA disease, have been isolated from diseased wood tissue. On diseased canes clear symptoms of the disease were noted on the leaves, as well as the drying of a varying number clusters per cane. To quantify the decrease in yield, a sample of infected canes was monitored and the percentage of dried clusters was recorded. Five lots of grapevines with 1000 canes each, of different age and varieties (local varieties Vranac and Kratosija), were studied. The average yield per cane in this vineyard is 3–3.5 kg. In the first lot, consisting of 24-year-old grapevines, 69 canes with expressed symptoms of esca disease were identified and yield reductions of 4.3% were recorded. In the second lot, consisting of 17-year-old grapevines, 86 diseased canes were found that led to a yield reduction of 2.97%. The decrease in yield in the third lot (12-year-old grapevines) with 36 infected canes was 1.57%. In the fourth lot (6-year-old grapevines) only one cane with esca symptoms was observed and there were no dried clusters. There were also no dried clusters in the fifth lot, where 3 diseased canes were noted. Based on the results obtained in this study, it can be concluded that esca leads to yield reductions in the range of 1.57–4.3%, and that the reduction is more significant in older vineyards.

Gas exchange, stem water potential and xylem flux of grapevines affected by Esca disease. R. GUCCI, G. CARUSO, C. BERTOLLA, G. SCALABRELLI, L. ANDREINI and R. VITI. *Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Coltivazioni Arboree, Università di Pisa.*
E-mail: gscalabrelli@agr.unipi.it

A study was carried out on 24-year-old grapevines of four varieties ('Cabernet Sauvignon', 'Sangiovese', 'Trebbiano toscano' and 'Chardonnay') on their own roots or grafted on 1103P and Kober 5BB rootstocks. The study design was a randomized block, with 4 blocks and 10 replicate plants. Gas exchange parameters were measured on three fully expanded leaves of cultivars at fruit set, veraison, and cluster ripening between 10:30 and 12:30 (solar time) using a CIRAS 1 infrared gas analyser at photosynthetic photon flux (PPF) > 900 μmol m^{-2} s^{-1}, a leaf temperature of 32.4 ± 1.1 °C, and an ambient CO_2 partial pressure of 35.3 ± 1.2 μPa. Stem water potential was measured between 09:30 and 11:00 (solar time) after wrapping the fully expanded leaves with aluminium foil for 40 min using a PMS 1000 pressure chamber (Plant Moisture Systems, USA). Gas exchange and stem water potential measurements were performed on healthy vines (that had never shown any Esca symptoms) and symptomatic vines (apparently healthy in the year the measurements were taken, but which had exhibited Esca symptoms in the previous growing season). In addition, we compared the gas exchange and water potential of healthy leaves to that of leaves with Esca symptoms - both taken from Esca-affected vines. Xylem flux was determined by perfusion using an aqueous tracing dye (azosulfamide

1%) on shoot segments: before and after the appearance of Esca symptoms on the leaves of grapevine cv. 'Sangiovese'; in plants that were symptomatic the previous year; and in plants not affected by Esca. Differing trends in xylem flux based on the year and the cultivar were observed during the 2005–2007 period. No differences in dye translocation were found before the appearance of Esca symptoms during the growing season. After the appearance of Esca symptoms, healthy and diseased vines showed differences in xylem flux that were also linked to the cultivar. Esca disease induced a marked reduction in the carbon assimilation rate and stomatal conductance, particularly in 'Cabernet Sauvignon' vines. Higher internal CO_2 concentrations were also detected in symptomatic leaves. Symptoms of Esca disease did not affect the water leaf potential, which reached levels typical of unstressed leaves in both symptomatic and healthy samples. In addition, it may be noted that the gas exchange values observed before symptom appearance in plants affected by Esca were similar to those found in healthy plants. This means that measurements made one month before symptom appearance cannot be used as a screening method to determine whether a vine will develop Esca disease symptoms. It remains to be clarified whether more frequent monitoring could help to identify an early indicator of symptom appearance.

Epidemiology

Potential sources of *Phaeomoniella chlamydospora* inoculum in grapevine nurseries in southern Italy. S. POLLASTRO[1], W. HABIB[1,2], A. PICHIERRI[1], N. MASIELLO[1] and F. FARETRA[1]. [1]*Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy.* [2]*Istituto Agronomico Mediterraneo di Bari, Via Ceglie 9, 70010 Valenzano, Bari, Italy.*
E-mail: faretra@agr.uniba.it

Phaeomoniella chlamydospora is involved in Petri and Esca disease. Potential sources of *P. chlamydospora* inoculum in nurseries were investigated in Southern Italy. Nested-PCR was used to detect the fungus in: i) sapflow of 73 rootstock mother plants; ii) wood of 200 rootstock cuttings, 99 scion cuttings, 99 and 374 graftlings before and after the callusing phase, respectively, and 319 grafted rootstocks; iii) water from 53 pre-grafting and 57 pre-callusing hydration tanks; iv) plant debris from blades and benches of 48 grafting machines; v) 26 soil samples from nurseries; and vi) 57 microbiological tampons randomly collected in buildings where the material was processed. The severity of wood discoloration in plant materials was assessed using an empirical scale with six classes. The frequency of *P. chlamydospora* detection in plant materials increased during the production process. The pathogen, undetected in scion cuttings, was found in rootstock cuttings and graftlings before and after callusing (2–6%), and grafted rootstocks (57%). A similar trend was observed for wood discoloration that reached a McKinney's Index as high as 48% in grafted rootstocks. *P. chlamydospora* was detected in grapevine sapflow (7%), soil (3%), on grafting machines (29%), in water (28% of pre-grafting and 23% of pre-callusing hydration tanks) and tampons (12%). Several potential sources of contamination could be identified. Nurseries should be aware of this and control measures be put in place to prevent infection of plant material and subsequent spread of *P. chlamydospora* in new vineyards.

Acknowledgements: Research study commissioned from ARSIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forestry) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-Regional Project "Grapevine esca: research and experiment in the nursery and in the field for prevention and cure."

***Fomitiporia mediterranea* as a white rotter in esca-diseased grapevine: spores are produced in relation to temperature and humidity and are able to colonize young wood.** M. FISCHER. *Staatliches Weinbauinstitut, Merzhauser Str. 119, D-79100 Freiburg, Germany.*
E-mail: michael.fischer@wbi.bwl.de

Basidiospores of *Fomitiporia mediterranea* (*Fmed*) are found to be released from naturally occurring fruit bodies in relation to average daily temperatures and humidity. Using spore traps affixed to fruit bodies in the field, the majority of spores is detected with average daily temperatures of more than 10°C and a relative humidity higher than 80%. Fruit bodies may switch from non-active to active condition within several days. Rainfall does not influence the amount of produced spores, but results in increased spore deposit in areas free of fruit bodies. With regard to white rot symptoms, *Fmed* is limited to older vines. In young vines the existence of the fungus on the surface of pruning wounds and adjacent wood can be shown by a nested PCR reaction using primer pairs ITS5-4 and Fmed1-2. Multiple infections occur with ongoing years often resulting in different individuals of *Fmed* that occur side by side in the same host plant. A manganese peroxidase, formed both from homo- and heterokaryotic mycelia, contributes to observed decay symptoms. The data show that, via pruning wounds, *Fmed* is able to colonize young plants. In normal years, the active period of fruit bodies lasts from appr. March-April through October-November. Besides temperature, spore release is dependent on humidity. From this point of view, fruit bodies under mediterranean conditions, with a pronounced drought during summertime, might differ in their periods of activity.

Revisiting esca symptoms in the vineyard: results of a four-year survey. P. LECOMTE[1], G. DARRIEUTORT[1], J.-M. LIMINANA[1], G. LOUVET[1], A. MURAMENDIARAZ[2] , F.J. LEGORBURU[2] , E. CHOUEIRI[3], F. JREIJIRI[3] and M. FERMAUD[1]. *[1]INRA, UMR Santé Végétale n° 1065, ISVV, Ave E. Bourlaux, BP 81, 33883, Villenave d'Ornon cedex, France. [2]NEIKER-Tecnalia, Basque Institute for Agriculture Research and Development, Apdo 46, E[2]-01080 Vitoria / Gasteiz, Álava, Spain. [3]Department of Plant Protection, Lebanese Agricultural Research Institute, Tal Amara, P.O. Box 287 Zahlé, Lebanon.*
E-mail: lecomte@bordeaux.inra.fr

In grapevine, Esca is a worldwide trunk disease which develops as a complex syndrome showing a large range of wood and leaf symptoms. Black Dead Arm (BDA) is a more recently described grapevine decline characterized initially by similar foliar symptoms and a brown streaking under the bark. Symptoms of both declines occur frequently on a same mature vine during summer. To better describe the associated symptomatologies, 41 adult vineyards were surveyed between 2004 and 2007 in the Bordeaux area mostly, in other European regions and in Lebanon. Regular leaf observations along summer in 12 vineyards showed a clear pattern of leaf symptom variations in severity and of temporal evolution. First-appearing symptoms corresponded mostly to BDA. The most damaged leaves drop down rapidly (apoplectic form). However, the less damaged leaves (mild form) showed mostly typical esca tiger-striped profiles, after some days or weeks, with different colorations on black cultivars. A multiple correspondence analysis, based on 561 vines, was used to identify relationships between the decline type (BDA, Esca…), symptom occurrence (leaf fall, wood streaking …) and severity on leaves. BDA and Esca were not clearly differentiated, being associated with low and higher leaf symptom severity, respectively. The presence of the orange or brownish streaking in the xylem was associated with vines showing either BDA or esca leaf symptoms (95% out of 647 vines that were peeled off). Thus, our observations lead to define a dominant esca symptomatology, including BDA-like symptoms, as described previously by some other authors.

Spatial pattern analysis of esca in different geographical areas of Italy. G. MARCHI[1], S. BURRUANO[2], S. DI MARCO[3], F. OSTI[3], I. PERTOT[4] and G. SURICO[1]. *[1]Università degli Studi, Dipartimento di Biotecnologie Agrarie, Piazzale delle Cascine 28 , 50144 Firenze, Italy. [2]Università degli Studi, Dipartimento S.En.Fi.Mi.Zo.,Viale delle Scienze, 90128 Palermo, Italy. [3]Ibimet - CNR, Via Gobetti 101, 40129 Bologna, Italy. [4]SafeCrop Centre, Istituto Agrario San Michele all'Adige, Via Mach 1, San Michele-TN, Italy.*
E-mail: guido.marchi@unifi.it

The spatial spread of Esca disease was examined in 6 vineyards chosen as ideally representative of the different cultivars, environmental conditions and agricultural practices that characterize the Italian viticultural system. Surveys were carried out for a minimum of 3 to a maximum of 17 consecutive years. A high level of discontinuity in symptom expression of diseased plants was observed from year to year in all the vineyards. With the purpose of quantifying the spatial pattern of the disease (random vs. aggregated or clustered), for each year of assessment field data on esca incidence were analyzed at three spatial levels (hierarchies) including: 1) adjacent vines within agricultural columns or rows (ordinary runs analysis), 2) within vines grouped into sampling units (distribution analysis) and 3) among groups of plants over some distance to each other (SADIE-Spatial Analysis by Distance Indices). Although in all vineyards in at least one yearly assessment aggregation of disease incidence was indicated, with one exception, random disease patterns, regardless the absolute value of disease incidence, are much more likely to occur. Moreover, the comparison of the maps of clustering indices produced by the SADIE program, showed that in each plot the spatial location of the sub-areas where clustering of symptomatic plants is more intense, fluctuates between years. These results suggest that the predominant factors involved in the discontinuity of foliar symptoms expression, are not peculiar to specific sites within vineyard, but may act on the entire population of host plants although their intensity shifts from year to year.

Acknowledgements: Research study commissioned from ARSIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forestry) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-Regional Project "Grapevine esca: research and experiment in the nursery and in the field for prevention and cure."

Stone fruit trees – an inoculum source of grapevine trunk disease pathogens? U. DAMM[1,2], P.W. CROUS[1,2] and P.H. FOURIE[1,3]. *[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch 7602. [2]Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. [3]Citrus Research International, P.O. Box 2201, Stellenbosch 7602, South Africa.*
E-mail: u.damm@cbs.knaw.nl

Stone fruits trees (*Prunus* spp.) are often grown in close proximity of vineyards, and known to have some wood pathogens in common with grapevine, such as *Diplodia seriata* (syn. *Botryosphaeria obtusa*) and *Eutypa lata*. A study was therefore undertaken to determine whether stone fruit trees in South Africa are inhabited by known grapevine trunk disease pathogens and whether they could act as alternative hosts. Living wood of *Prunus* spp. with dieback, canker or necrotic symptoms as well as pruning debris were sampled in climatically different

grapevine producing areas. Fungi were isolated from the samples, and characterised based on morphology and DNA phylogeny (ITS rDNA, actin, β-tubulin, EF-1α, 18S or 28S rDNA genes). Several species that were reported to be pathogenic on grapevine were identified, for example *D. seriata*, *D. mutila*, *Neofusicoccum ustrale*, *N. vitifusiforme*, *Togninia minima*, *Phaeoacremonium viticola*, *Eutypa lata* and *Cryptovalsa ampelina*. Many other species found on *Prunus* wood are known from grapevine as well. Additionally, a number of taxa constituted new reports on *Prunus* species or new records in South Africa, and many new species have been discovered. A selection of the fungi was tested for their pathogenicity on grapevine. Among the species that caused lesions on grapevine shoots were known grapevine pathogens as well as unknown ones like *Lasiodiplodia plurivora* and *D. pinea*. According to these results, stone fruit orchards should be considered as potential inoculum sources of grapevine wood pathogens.

Pathogenicity and epidemiology of Botryosphaeriaceae from grapevines in California. J.R. URBEZ-TORRES and W.D. GUBLER. *Plant Pathology Department, University of California, Davis 95616, California, USA.*
E-mail: wdgubler@ucdavis.edu

Botryosphaeriaceae species have been identified as the most abundant fungi associated with grapevine cankers in California. Nine different species including *Botryosphaeria dothidea*, *Lasiodiplodia theobromae*, *Diplodia seriata*, *D. mutila*, *Neofusicoccum parvum*, *N. australe*, *N. luteum*, *Dothiorella iberica*, and *D. viticola* were isolated and identified from perennial cankers from dead spurs and cordon and trunk dieback. In order to determine the role that these fungal species play on grapevine health in California, 72 isolates were tested in 5 different pathogenicity tests conducted in both wine and table-grape cultivars. Overall, *in vivo* and *in vitro* experiments showed all 9 Botryosphaeriaceae species to be pathogenic on grapevines. However, virulence varied by species. Based on extent of spread in the wood, *Lasiodiplodia theobromae* and *N. parvum* were the most virulent species, whereas *D. viticola* and *D. iberica* were the least virulent. Epidemiology of Botryosphaeriaceae in California was studied using spore traps in 7 different locations throughout the State. Spore trapping results from the last 2 years showed that Botryosphaeriaceae spores were mainly trapped following rainfall events or overhead sprinkler irrigation from November to March. These findings indicate the importance of Botryosphaeriaceae species as grapevine pathogens as well as contribute to a better understanding of their epidemiology in California.

Factors that affect the infection of grapevine tissues with *Botryosphaeria* species. N.T. AMPONSAH, E.E. JONES, H.J. RIDGWAY and M.V. JASPERS. *Lincoln University, PO Box 84 Lincoln University, Canterbury, New Zealand.*
E-mail: Amponsan@Lincoln.ac.nz

In New Zealand, *B. lutea* (*Neofusicocum luteum*), *B. australis (Neofusicocum australe*), *B. parva* (*Neofusicocum parvum*), *B. stevensii* (*Diplodia mutila*) and *B. obtusa* (*Diplodia seriata*) isolates from symptomatic grapevines were used for pathogenicity experiments. Mycelium was inoculated onto wounds on detached, green shoots of Pinot noir, and after 10 days incubation assessment was by lesion length and isolations. Species differed ($P \leq 0.05$) with lesion lengths of 69–76, 63–66, 49–59, 24–55 and 8–10 mm, respectively. Air-dried lesions developed pycnidia that oozed conidia if incubated under high relative humidity for 1–2 days, numbers per 15 mm of shoot being 1.9×0^6, 1.6×0^6, nil, 1.5×10^6 and 1.5×10^5, respectively. Further experiments with *B. lutea, B. parva and B. stevensii* were on wounded and non-wounded green stems of five grapevine varieties, and by mycelium or conidial inoculation to woody tissue and to different wound ages. Only wounded stems were infected for Chardonnay, Pinot noir, Riesling, Cabernet sauvignon and Sauvignon blanc, but they were equally susceptible. On trunks of 18 month vines, no lesions appeared at the inoculation site after 4 months, but isolations showed that infections reached 81 and 62 mm, respectively, for the inoculum types, with dieback beginning when *B. lutea* reached the pruned tips. Wounds were made in the trunks of 18 month Pinot noir vines at 0-30 days prior to inoculation with conidia or mycelium. For the 0, 1, 2, 7, 14, and 30 day wounds, incidence at 2 months was 100, 100, 100, 80, 40, and 20% for mycelium inoculation, and 100, 100, 40, 0, 0, and 0%, for conidial inoculations, respectively.

First observations on the role of rain in the penetration of *Eutypa lata* into pruning wounds. P. LARIGNON. *Institut Français de la Vigne et du Vin, Unité Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France.*
E-mail: philippe.larignon@vignevin.com

The choice of methods for evaluating fungicides able to protect pruning wounds against *Eutypa lata* depends on a good knowledge of the first stages of infection under natural conditions. The objective of this study was to obtain information on the role of rain in the penetration of ascospores of *E. lata* into the vessels after pruning. In natural conditions, pruning wounds subjected to rain in the presence of wood pieces carrying perithecia of *E. lata* showed that the spores were not distributed homogeneously over the entire length of the wound. They were rather present in woody tissues located between 6 and 15 millimetre below the wound surface (58% of isolations). They were seldom present deeper than 20 millimetres. Experiments with artificial inoculation using a suspension of ascospores showed that the spores were preferentially located in the

first five millimetres when they were inoculated in a dry period whereas under rain, they were distributed deeper in the wound and homogeneously through the first twenty millimetres. This study demonstrated the role of water in migration of the spores into wounds and subjacent tissues. Evaluations of the efficiency of products for protection of pruning wounds against *E. lata* will have to take account of this important factor.

Propagation of pioneer fungi associated with esca disease by vegetative material in French grapevine nurseries. P. LARIGNON[1], M. COARER[2], K. GIRARDON[3], F. BERUD[4] and O. JACQUET[4]. *[1]Institut Français de la Vigne et du Vin, Unité Rhône-Méditerranée, 30230 Rodilhan, France. [2]Institut Français de la Vigne et du Vin, Unité Pays-de-Loire, 44120 Vertou, France. [3]SPBPVV, 384, route de Caderousse, 84100 Orange. [4]Chambre d'agriculture du Vaucluse, 2260, route du Grès, 84100 Orange.*
E-mail: philippe.larignon@vignevin.com

Esca disease of grapevines is caused by a white rot in the wood, which is also correlated with various foliar symptoms (mild form, apoplexy). The white rot results from the action of several microrganisms: pioneer fungi leading to the formation of brown necrosis in a central position (*Phaeomoniella chlamydospora* (*Pch*), *Phaeoacremonium aleophilum* (*Pal*) or in sectorial position (*Eutypa lata*), which is then colonized by *Fomitiporia mediterranea*. The organisms responsible for the foliar symptoms are not known, as Koch's postulates are incomplete. Since no control measures are known in the vineyard, measures must be taken in nurseries to manage this syndrome. To achieve this goal, we studied the life cycle of fungi associated with esca disease in nurseries, then tested some control measures aimed at obtaining healthy plants. Only, *Pch* and *Pal* occurred in the woody tissues of canes (grafts, rootstocks), and at their surface. They were also isolated from plants after planting. Their isolation in higher percentage suggested contaminations during the grapevine propagation process. This possibility was examined during two steps (callusing, planting). *Pch* contaminated the plants during callusing of the wounds located at the base of grafted cuttings. *Pal* would contaminate the aerial part during planting. Among the different treatments tested, only hot water treatment (45 min, 50°C) showed good efficiency against *Pch*. Only by combining different measures associated with HWT will it be possible to achieve our goal.

Occurrence of *Phaeomoniella chlamydospora* in grapevine planting material. S. SERRA, M.A. MANNONI, V. LIGIOS and A. DEMONTIS. *Dipartimento di Protezione delle Piante–Università degli Studi di Sassari, Via De Nicola 9, 07100 Sassari, Italy.*
E-mail: salvase@uniss.it

Petri disease of young grapevines is primarily caused by *Phaeomoniella chlamydospora* (Pch), a fungus also implicated in esca disease of older grapevines. Infected planting material is a means of pathogen dissemination. The occurrence of Pch was investigated during the grapevine propagation process in an Italian nursery. In the three-year period 2005–07, cutting and graft samples (cv. Sangiovese as scion, 140Ru in 2005 and 1103P in 2006–07 as rootstock) were taken during the propagation process at several stages recognized as potential infection ways. In addition, canes from esca-diseased mother plants (140Ru, 1103P and cv. Sauvignon) were sampled. At each stage woody material was collected and grown by avoiding accidental contamination. After one growing season, rooted cuttings and grafts were destructively examined. DNA was extracted from wood collected at different positions and analysed by nested PCR with Pch-specific primers. Despite the extended wood discolouration, Pch occurrence in nursery woody material was scarce in the three-year period. Canes from esca-diseased mother plants were moderately contaminated (about 30% of the samples examined) only in 2005. This result needs further studies to establish whether climatic conditions could have influenced Pch occurrence in canes. No final conclusion could be drawn on which were the factors showing a major role, as the detected planting material contamination may have resulted from infected mother plants or from the propagation process, particularly during stages after grafting.

Fungi associated with wood decay diseases: identification of the steps involving risk in a French nursery. V. VIGUES[1], O. YOBREGAT[1], B. BARTHELEMY[1], F. DIAS[1], M. COARER[2] and P. LARIGNON[3]. *[1]IFV Midi-Pyrénées - V'Innopôle Brames Aigues BP 22 - 81310 Lisle / Tarn, France. [2]IFV Val de Loire - Château de la Frémoire - 44120 Vertou, France. [3]IFV Rhône-Méditerranée - Domaine de Donadille - 30230 Rodilhan, France.*
E-mail: virginie.vigues@itvfrance.com

Wood grapevine diseases (Esca disease, Black dead arm, Eutypa dieback) affect the structure of the trunk and have, in the more or less long term, the death of affected vines as a consequence. Symptoms have been observed in young vineyards, 4 to 5 years old, asking the question of a possible presence of fungi in rootstocks and grafted vines. An investigation led in 2005 in Midi-Pyrénées demonstrated that *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Botryosphaeria* spp. Were the fungi most frequently present in grafted vines once they left the nursery. The following question has been asked too: is the plant material contaminated before the nursery or is it contaminated during the propagation process? We have taken a propagation material sample before and after each step of the propagation process and submitted it to microbiological analysis. This study showed that the propagation material was totally healthy when it entered the nursery

but it was contaminated at the end of the manufacturing process. Fungi associated with wood decay diseases were detected by PCR on the surface of grafted varieties and rootstocks and in hydration and callusing baths. Then, these fungi would contaminate the grafted vines through the wounds (such as callus point or disbudding wounds). Some methods of control (chemical or biological) have been tested to eliminate these fungi from hydration baths and from grafted vines, but none was effective.

Occurrence of *Phaeomoniella chlamydospora* in grapevine rootstocks and grafted rootstocks: results of a three-year monitoring. A. PICHIERRI[1], W. HABIB1,[2], N. MASIELLO[1], S. POLLASTRO[1] and F. FARETRA[1]. *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. [2]Istituto Agronomico Mediterraneo di Bari, Via Ceglie 9, 70010 Valenzano, Bari, Italy.*
E-mail: faretra@agr.uniba.it

Phaeomoniella chlamydospora is a fungus involved in Petri and Esca grapevine diseases. Graft failure, shoot dieback, decline and gradual death of plants are associated with the presence of *P. chlamydospora* in young vines. It is supposed that the fungus can be transmitted to new vineyards through infected grapevine propagation material. The presence of *P. chlamydospora* in rootstocks and grafted rootstocks in grapevine nurseries was investigated in 2004 to 2006 in Southern Italy. On the whole, 1411 samples of 140Ru. and 1103P. rootstocks not grafted or grafted with 'Aglianico', 'Cabernet sauvignon', 'Chardonnay', 'Ciliegiuolo', 'Lambrusco', 'Merlot', 'Montepulciano', 'Moscato Bianco', 'Negroamaro', 'Primitivo', 'Sangiovese', 'Trebbiano toscano', 'Verdeca' (wine grapes) and 'Victoria' (table grape) were collected from several nurseries. Nested-PCR was used to detect *P. chlamydospora*. Wood discoloration reached a McKinney's Index as high as 58% in grafted rootstocks. *P. chlamydospora* was detected in all the nurseries and all the years with a frequency ranging from 2 to 31 of rootstocks and 29 to 61% of grafted rootstocks. The fungus was often detected in discoloured wood near cuts, confirming that wounds are its main penetration way. These finding show that grapevine propagation material can be frequently infected by *P. chlamydospora*. However, further research should be carried out to explain why in Italy the occurrence of Petri disease in young vineyards is much lower than in other countries, such as Australia, South Africa and California.

Acknowledgements: Research study commissioned from ARSIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forestry) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-Regional Project "Grapevine esca: research and experiment in the nursery and in the field for prevention and cure."

Evolution of Esca symptoms on different combinations between grapevine varieties and rootstocks. G. SCALABRELLI, R. VITI and L. ANDREINI. *Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Coltivazioni Arboree, Università di Pisa, Italy.*
E-mail: gscalabrelli@agr.unipi.it

The research was carried out in a vineyard trained to high free cordon, planted in Pisa experimental station twenty-three years ago at spacing m 3×1. Four grapevine varieties, 'Cabernet Sauvignon', 'Sangiovese', 'Trebbiano toscano' and 'Chardonnay' self rooted and grafted on 1103P and Kober 5BB, rootstocks were arranged in 4 randomized blocks disposed in cross-sectional sense, with 10 plants for replication. Periodic observations were done in the period 2004–2007, beginning from bud break to monitor visually the appearance and the evolution of Esca disease symptoms. First of all we observed a fluctuation of the symptoms in the time, only in few cases symptomatic plants in 2004 (approximately 2%), have newly shown the symptoms also after two vegetative cycles. More frequently (14%) the symptomatic plants did not appear affected by Esca the following year and still return to be symptomatic the successive year. The plants that have shown the Esca symptoms only one year during the four-year term of observations, represent approximately 60% of the plants that on the whole have shown the disease, while the symptomatic plants which showed symptoms for more consecutive years were 26%. On 2004 the plants which visibly show symptoms were less than 10%, while on 2005 the incidence increased to 27%, while on 2006 the symptomatic plants lowered to 18%. Around 50% of the symptomatic plants on 2004 newly showed symptoms in following year, while 19% showed leaf symptoms at a distance of two years (2006). On 2006 between the symptomatic plants, 34% had already shown the symptoms the previous year. Finally on 2007 the apparent disease reached 25%, at cluster veraison stage. The percentage of plants that appeared affected by Esca for the first time has not exceeded 10%. Considering the cumulative incidence in the years it passed from 10% approximately on 2004 till to 45% to the end of 2007. The progression of Esca symptoms, classified as chronic, severe and apoplectic, has substantially followed the same trend in the three years of observation (2005–2007). The trend of symptoms observed on plants of Cabernet Sauvignon during 2007 reveals a greater infection in correspondence of veraison with the prevalence of chronic symptoms, followed in the season by increase of severe symptoms and apoplectic strokes. The percentage of cumulative infection from 2004 to 2007 confirmed that 'Cabernet Sauvignon', has a greater sensibility to Esca disease, while Chardonnay, which was in general the less susceptible, showed an unexpected higher incidence in a circumscribed zone of the vineyard, where 'Trebbiano

toscano' and 'Sangiovese' had the higher percentage of Esca incidence too. Self rooted plants of 'Sangiovese', 'Cabernet Sauvignon' and 'Chardonnay' had a lower susceptibility to Esca independently from the position inside of the vineyard, while 'Trebbiano toscano' was less susceptible to Esca when grafted on 1103P. These data suggest the possibility that genetic influence (variety and rootstock) may play a role in Esca susceptibility, moreover soil condition, or to spreading conditions due to unknown contamination may modulate it. Climate conditions varying between years could be also responsible of variability of symptoms appearance, although a plant reaction or the effect of more severe pruning of symptomatic plants could be taken into account.

Susceptibility to esca of grapevine cultivars and clones in Italy. S. SERRA, M.A. MANNONI and V. LIGIOS. *Dipartimento di Protezione delle Piante – Università degli Studi di Sassari, Via De Nicola 9, 07100 Sassari, Italy.*
E-mail: salvase@uniss.it

Esca is one of the most serious grapevine diseases all over the world. Differences in susceptibility among cultivars of *Vitis vinifera* and among rootstocks were reported, but little information is available concerning clone susceptibility. The present study was undertaken to investigate susceptibility to esca of two clones representing cv. Sauvignon (ISV1 and R3, both grafted on SO4), Cabernet Sauvignon (R5 and 338, grafted on 110R) and the local variety Cannonau (70 and 362, grafted on 110R). Field surveys were carried out from 2002 to 2007 in productive vineyards located in the same farm in North Sardinia, pergola - trained and subjected to the same cultural practices. At the beginning of the survey, vineyards of Sauvignon Blanc, C. Sauvignon and Cannonau were 13, 11 and 10 years-old, respectively. Chronic symptoms such as tiger-stripes (specific), edge and limb chlorosis and necrosis (non-specific), often in association with cane defoliation and wilt and/or cluster dehydration, were recorded. Apoplexy and dead arm or plants were also assessed even if chronic symptoms in previous years were lacking. Cumulative esca incidence was higher on cv. Sauvignon with respect to chronic symptoms, apoplexy and death. C. Sauvignon showed intermediate chronic symptom values, but the lowest apoplexy and death incidence. Differences between the kind of chronic symptom were also recorded: many Sauvignon plants showed only non-specific symptoms, while tiger-stripes prevailed on the other varieties, particularly on C. Sauvignon. Clones Sauvignon ISV1 and C. Sauvignon R5 showed a higher incidence of chronic symptoms than clone R3 and 338, respectively, but clone 338 showed higher apoplexy values than R5. No differences were recorded between Cannonau clones, except a higher incidence of dead arm and plants on clone 362. The possible causes of this behaviour are discussed.

Esca infection influenced bud breaking of Cabernet Sauvignon grapevines. L. ANDREINI, R. VITI, S. BARTOLINI and G. SCALABRELLI. *Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Coltivazioni Arboree, Università di Pisa.*
E-mail: gscalabrelli@agr.unipi.it

The aim of this study was to evaluate the influence of Esca infection on 'Cabernet Sauvignon' bud breaking. The phenological observations was made on grapevine plants cv. 'Cabernet Sauvignon' that in previous years (2004, 2005, 2006) had shown Esca disease symptoms (ES) in single (ES04; ES05, ES06) or repeated years (ES04-05; ES05-06; ES 04-05-06) and those that never had showed Esca symptoms (NES). The bud breaking monitoring was carried out in according to the BBCH reference stages of development. Analogous observations had been made on one node cutting obtained from canes sampled at the 23rd February 2007, from every plant type ES and NES, placed in forcing chamber (temperature 23°C, 60% relative humidity, photoperiod 12 hours at 300–400 μE m^{-2} s^{-1}). In vineyard, phenological remarks on half March showed that symptomatic plants which had showed symptoms on all three previous years (ES 04-05-06) had a significant delay in bud break, while the control plants were at the stage 01, correspondent to the appearance of the white tip (value 1,43) the buds of the plants Esca symptomatic in the previous years were still found to the dormant stage 00 (value 1,06). After a month (half of April) still observed a meaningful delay in the development of bud in the symptomatic plants in repeated years (ES 04-05-06), which were in the stage preceding to green button (07), while the control had achieved the stage in which it is possible to appreciate under the brown wool the new leaves (09). In forcing conditions, after 14 days (7200 GDH accumulated), did not show any difference in the phenological stage between the various plant type. After others seven days, the control buds were found in correspondence of the first leaves opening (stage 11), while the buds of symptomatic plants for consecutive years (ES 05-06; ES 04-05-06), showed, as observed in field, a delay in bud break. Buds coming from plants that had shown Esca symptoms in a single season (ES 05, ES 06) exhibited a similar behaviour to those brought from the control plants. These results confirm previous findings that the repeated Esca symptoms provokes a delay of bud break probably due to a reduction of the carbohydrate stock necessary for the first stage of shoot development.

The occurrence and distribution of esca in grapevines of South Africa. C. WHITE[1], F. HALLEEN[2] and L. MOSTERT[1]. *[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.*
E-mail: halleenf@arc.agric.za

Various trunk diseases affect wine and table grape production in South Africa. The occurrence and distribution of esca is not known and has not been studied. It has only been reported from one production area and was believed to be of no economic importance. Consequently, since 2001, grapevines suspected of being affected by esca, were collected from various regions in the Western and Northern Cape of South Africa. These regions were a representation of all the main production areas. Grapevines were selected according to the presence of foliar and/or internal symptoms, where isolations were made from the various symptom types. Internal wood symptoms included brown to black wood streaking, a cream/ white or yellow soft/spongy rot surrounded by necrotic tissue which was mostly brown, black or red-brown. These symptom types were similar to those associated with esca-affected grapevines in Europe. Foliar symptoms in South Africa do not always show the typical 'tiger stripes' and foliar symptoms sometimes do not even appear at all. This phenomenon is believed to be linked to the specific white rot Basidiomycete species found in South African grapevines. The other genera involved including *Phaeomoniella chlamydospora, Phaeoacremonium* spp. (mostly *aleophilum*), Botryosphaeriaceae, *Eutypa lata* and *Phomopsis* spp., are similar to those associated with esca elsewhere. It has been established that esca occurs in all 33 locations sampled from and on all of the cultivars investigated (wine and table grapes). Research is currently being conducted to characterise the different Basidiomycete species involved in the esca-complex in South Africa.

The *Botryosphaeria* species from vineyards of Apulia. A. CARLUCCI, F. LOPS, M.L. RAIMONDO, V. GENTILE, M. MUCCI and S. FRISULLO. *Dipartimento di Scienze Agro-ambientali, Chimica e Difesa Vegetale, Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy.*
E-mail: s.frisullo@unifg.it

In recent years, surveys carried out in Puglia on vineyards showing symptoms commonly associated with Esca disease, made it possible to frequently isolate several Botryosphaeriaceae species from browning wood . The plants affected by Botryosphaeriaceae show the first symptoms during the vegetative resumption, as some shoots do not open or as the young shoots die after reaching a length of 10-20 cm. In other cases, the shoots become branches with yellowish leaves, in which the yellow area evolve later in necrosis of interveinal tissues until the leaves drop. The branches have shorter internodes, not well lignified, and have whitish areas on the surface nearby the nodes. In the wooden stem the disease is characterized by sub-cortical brown streaking with variable length (10 to 150 cm and, sometimes, extending up to graft area) and width (up to 75% of the cross-section of the stem). Fungal isolates belonging to the Botryosphaeriaceae were analysed using morphometric and molecular characterization through the amplification of the ITS ribosomal DNA gene region. The results obtained revealed the presence of anamorphs of *B. btuse* (*Diplodia seriata*), *B. rhodina* (*Lasiodiplodia theobromae*), *B. stevensii* (*D. mutila*), *B. parva* (*Neofusicoccum parvum*), *B. lutea* (*N. luteum*), *B. sarmentorum* (*Dothiorella sarmentorum*), *B. iberica* (*D. iberica*), *B. dothidea* (*Fusicoccum aesculi*) and *B. quercum* (*D. corticola*). This is the first report of *B. quercum* occurring on wine grapes.

Grapevine decline syndrome associated with *Botryosphaeria* spp.: a risk for Umbrian vineyards? NATALINI[1] and L.TOSI[2]. [1]*Agenzia Regionale Umbra per lo Sviluppo e l'Innovazione in Agricoltura (A.R.U.S.I.A.), Via Fontivegge, 51, 06124 Perugia, Italy.* [2]*Department of Agricultural and Environmental Sciences, University of Perugia, Borgo XX Giugno, 74, 06121 Perugia, Italy.*
E-mail: patveg@unipg.it

Surveys in four Umbrian vineyards suffering for decline syndrome were carried out between 1995 and 2003. Field symptoms varied from mild chlorosis, leaf and fruit wilting, cankers on canes, sometimes in association with brown wood streaking of branches. Diseased vines showed a gradual decline in vigour and yield. In 1995, decline syndrome was observed on 1% of the plants localized in one row whereas in 1999, a vineyard (3000 m^2) of white wine cv Trebbiano Toscano was seriously damaged (100%). In 2002 and 2003, in two vineyards, disease incidence ranged from 60 to 70%. Isolations were made from cankers onto potato-dextrose agar (PDA) plates. Dark grey colonies with dense aerial mycelium developed and produced black pycnidia within a month, under near-UV light. The isolates were identified as the anamorph of *Botryosphaeria* spp. on the basis of conidial shape (length/width ratio), cultural and morphological characters. Pathogenicity tests were performed in 1999 by inoculating ten 1-year-old rooted grape Trebbiano Toscano cuttings. Mycelium plugs were placed on wounds drilled into canes and covered with parafilm whereas control vines were inoculated with sterile PDA. Artificial inoculations, using the same technique and three isolates, were carried out in 2003 on red wine cv Cabernet Sauvignon rooted cuttings. No symptoms developed on the inoculated vines. In 1995 and 1999 three spray applications of thiophanate-metyl and the removal of pruning debris resulted to be good sanitation practices as no disease symptoms were observed on vines in the following years. Further studies and surveys are in progress.

Increasing evidence that *Eutypa dieback* of grapevines is widespread in New South Wales. W.M. PITT, R. HUANG, S. SAVOCCHIA and C.C. STEEL. *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

Eutypa dieback, caused by the fungus *Eutypa lata* is a serious disease of grapevines that affects vineyard productivity and longevity. Between November 2006 and April 2008, grapevines displaying foliar symptoms typical of Eutypa dieback or evidence of *Botryosphaeria* canker such as dead spurs, cankers, and bleached or discolored tissue, were surveyed from 75 vineyards throughout New South Wales, Australia. Wood samples were taken from 1835 vines, surface sterilised and transferred to potato dextrose agar (PDA). Samples were incubated at 25°C and monitored for the appearance of fungi. *Eutypa lata* was tentatively identified based on cultural and conidial morphology. Molecular identification confirmed the identity of *Eutypa lata* and *Cryptovalsa ampelina*, a diatrypaceous ascomycete from the same family, based on amplification of a species-specific marker and sequencing and comparison of ribosomal DNA internal transcribed spacer (ITS) sequences. These surveys have shown that Eutypa dieback is more widespread in New South Wales than first thought, and may be increasing in prominence in the cooler climate regions where lower temperatures and higher rainfall favor its growth. Vigilant monitoring, protection of pruning wounds from infection, and removal of dead infected wood from the vineyard remain the best methods of managing these diseases.

Diseases incited by Botryosphaeriaceae fungi in Portuguese vineyards. C. REGO, A. VAZ, T. NASCIMENTO, A. CABRAL and H. OLIVEIRA. *Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal.*
E-mail: crego@isa.utl.pt

Species of *Botryosphaeriaceae* are important grapevine pathogens commonly associated with wood symptoms, such as cankers, dieback, wood streaking, shoot dieback, cane bleaching, bud necrosis or graft failure. A three year field survey was conducted in six grape-growing regions of Portugal (Vinhos Verdes, Douro, Dão, Estremadura, Alentejo and Algarve) in order to establish the identity of *Botryosphaeria* species involved in grapevine trunk diseases, to record associated symptoms as well as to determine their pathogenicity. Decline symptoms, black streaks and hard necroses were the dominant symptoms observed in young vines; esca-like symptoms prevailed in mature vines, while black dead arm was recorded in both young and mature vines. Regional differences between *Botryosphaeria* species were not evident. Morphological and cultural characterisation of isolates along with ISSR fingerprinting allowed the identification of six "*Botryosphaeria*" species: *B. dothidea* (*Fusicoccum aesculi*), *"B". obtusa* (*Diplodia seriata*), *"B". stevensii* (*Diplodia mutila*), *Dothidotthia viticola* (*Dothiorella viticola*), *"B". lutea* (*Neofusicoccum luteum*) and *"B". parva* (*Neofusicoccum parvum*). Pathogenicity studies carried out on grapevine potted plants, cultivar "Castelão", provided evidence on the pathogenic variability among isolates assessed by the wood lesion extension. Foliar symptoms associated with "*Botryosphaeria*" species were observed one year after inoculation.

Monitoring *Diplodia seriata* and *Eutypa lata* spore dispersal in vineyards of Rioja Alavesa. A. MURUA-MENDIARAZ[1], J. LUQUE[2] and F.J. LEGORBURU[1]. *[1]NEIKER-Tecnalia, Basque Institute for Agriculture 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: amuruamendiaraz@neiker.net

The aerobiological dispersal pattern of *Diplodia seriata* conidia and *Eutypa lata* ascospores was monitored from December 2006 to July 2007 in two vineyards of Rioja Alavesa, in order to investigate the epidemiology of these pathogens and to define the highest infective risk periods. Each spore trap consisted of four vertical microscope slides, set in a cross pattern and coated with petroleum jelly. The traps were located on a vertical stand, at a random point among the vines. In vineyard "A" a second trap was hanged from an almond tree on a bank. In vineyard "B" two additional, individual slide-traps were set up, facing putative *E. lata* stromatic tissue on died grapevine wood, 5 cm away from the canker. The slides were replaced weekly to be examined under the microscope. Temperature and rainfall were locally recorded. No conclusive results were obtained from individual slides facing stromatic tissue. Additionally, *E. lata*-like diatrypaceous ascospores were only erratically caught in randomly placed traps. For *D. seriata*, the highest catches were recorded in midwinter and decreased steadily until late spring. During the spring, peaks of spore dispersal deviating from this general trend were coincident with warm periods following rainy spells. The trap located under the almond tree showed a very different temporal pattern, with spore catches increasing over late winter and peaking in spring. Although, the total number of spores caught was quite low and these results are only preliminary, we recommend delaying the pruning period, avoiding warm periods following high precipitation.

Pome fruit trees as alternative hosts of grapevine trunk pathogens. M. CLOETE[1], U. DAMM[2], P.W. CROUS[2], P.H. FOURIE[3] and L. MOSTERT[1]. *[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa. [2]Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, Netherlands. [3]Citrus Research International, P.O. Box 2201, Stellenbosch 7602, South Africa.*
E-mail: mia@sun.ac.za

Grapevine trunk diseases impede the sustainability and competitiveness of the South African wine and table grape industry. Effective disease management is based on disease prevention through wound protection and sanitation. Several pathogens that cause grapevine trunk diseases were shown not to be host specific, and were also described from other hosts such as pome fruit (Family Rosaceae). Grapevines are frequently cultivated in close proximity to pome fruit trees in South Africa. Furthermore, grapevines are often established in soils previously cultivated with pome fruit trees. To study the extend in which pome fruit trees are inhabited by known grapevine trunk disease pathogens, fungi were isolated from dieback, canker and wood necrosis symptoms in commercial apple and pear orchards in the Western Cape in South Africa. Identification was based on morphological and cultural characteristics and DNA sequence data (5.8S rDNA, ITS-1, ITS-2 and β-tubulin). Species belonging to Botryosphaeriaceae , *Phaeoacremonium* and *Phomopsis* were isolated from wood symptoms of pome fruit trees. Species of Botryosphaeriaceae were dominating and included *Diplodia seriata* and *Neofusicoccum australe* that are known grapevine trunk disease pathogens. Further examinations will include pathogenicity trials on grapevine and pome fruit material.

Wood rot agents of fruit trees as potential pathogens of vineyards. A. CARLUCCI, F. LOPS, M.L. RAIMONDO, V. GENTILE, M. MUCCI and S. FRISULLO. *Dipartimento di Scienze Agro-ambientali, Chimica e Difesa Vegetale, Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy.*
E-mail: s.frisullo@unifg.it

The occurrence of fruiting bodies of Basidiomycetes on plants grown close or inside the vineyard, may be a significant source of infection to promote wood rotting in grapevine. In order to ascertain the ability of certain fungal species belonging to Basidiomycetes to infect the grapevine, several fruiting bodies collected from plants such as olive, peach, cherry, almond, apricot, orange and plum were subjected to morphological characterisation. Furthermore, fungal isolates were subjected to investigations by molecular amplification of the ITS ribosomal DNA region. Subsequently, the amplicons were sequenced and analysed by phylogenetic methods. Therefore, two *Phellinus pomaceus* strains from peach and cherry, two *Trametes pubescens* strains from peach and apricot, two *Fomitiporia mediterranea* strains from wine grapes, two *Fomitiporia* sp. from orange, two *Fomitiporia* sp. strains from olive trees, one strain of *P. punctatus* from willow, one strain of *Stereum hirsutum* from eucalyptus and a strain of *Chondrostereum purpureum* from peach were artificially inoculated on five-year-old grapevine plants (cv. Italia and Lambrusco). After two years, the results obtained indicated that the *Fomitiporia* isolates from olive and orange were able of producing incipient white rot, while *Phellinus punctatus*, *Stereum hirsutum* produced large browning areas around the inoculation site.

Olive trees as a potential source of inoculum of esca-associated fungi of grapevine in southern Italy. A. CARLUCCI, F. LOPS, V. GENTILE, M.L. RAIMONDO and S. FRISULLO. *Dipartimento di Scienze Agro-ambientali, Chimica e Difesa Vegetale, Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy.*
E-mail: s.frisullo@unifg.it

During the last two decades, a number of esca-associated fungi were isolated from the discoloured or decayed woody tissue of both grapevine (*Vitis vinifera*) and olive trees (*Olea europaea*) in several areas of Apulia (southern Italy). These included *Fomitiporia mediterranea, Phaeomoniella chlamydospora, Lecythophora lignicola, Phaeoacremonium aleophilum, P. rubrigenum* and *P. inflatipes*. Selected isolates of the above fungi from both host plants were cross-inoculated on 3-year-old potted plants of grapevine cv. Italia and Lambrusco and 4-year-old potted plants of olive cv. Leccino, Coratina and Frantoio. The results of the trial indicated that within six years after inoculation, isolates of *Fomitiporia mediterranea* from grapevine and of *Fomitiporia* sp. from olive caused brown wood streaking and esca symptoms on the inoculated grapevine cultivars, as well as similar wood and leaf symptoms on the inoculated olive cultivars. Isolates of *Phaeomoniella chlamydospora* from grapevine induced brown streaking both on inoculated grapevine and olive plants, although the fungus was not reisolated from olive cv. Leccino. *Lecythophora lignicola* from olive produced disease symptoms on all inoculated grapevine and olive plants. *Phaeoacremonium aleophilum, P. rubrigenum* and *P. inflatipes* isolates from olive and grapevine produced brown wood streaking when inoculated on grapevine and olive plants. These findings suggest that the olive trees represent a potential source of inoculum for esca disease of grapevine in southern Italy, were the two crops are widely grown on the same areas.

Disease Management

Effect of hot-water treatment on the fungal community of grapevine nursery-plants. L. CASIERI, V. HOFSTETTER, K. GINDRO and O. VIRET. *Agroscope Changins-Wädenswil ACW Research station, CP1012, 1260 Nyon, Switzerland*
E-mail: Leonardo.Casieri@acw.admin.ch

Young vine decline is a serious problem in most of the grapevine-producing countries of the world (Whiting et al., 2001). Within 5 years after planting, this disease compromises water and nutrient transport in the plants by forming black gum and tyloses in vascular tissues.

Understanding of the etiology and epidemiology of some of the fungi responsible for the disease has progressed considerably, but an adequate sanitary test for the propagation material and an effective treatment to control the disease is still lacking. The use of hot water treatment (HWT) to decontaminate nursery stock from fungal pathogens has been proposed by different authors, although the efficiency of such treatment remains controversial. In this study we examined the fungal community in nursery plants before and after hot-water treatment (45 minutes at 50°C). Five different cultivars were studied: Gamay, Arvine, Humagne, Gamaret and Chasselas. Pith and wood from de-barked and surface-sterilized plants were taken from the foot, pruning wound areas and grafting point, plated on PDA medium and regularly checked over a period of 10 days for the emergence of fungi. After isolation in pure culture, each isolate was identified by a morphological and/or molecular procedure (sequencing the ITS and tef-1 regions). Preliminary results show that HWT causes changes in the fungal community depending on the plant cultivar, and results in a general isolation and frequency reduction of several plant pathogen species. The potential of the tef-1 gene as molecular marker for specific and supra-specific ranks was explored.

Hot water treatment for elimination of *Cylindrocarpon* species from infected grapevines. C.M. BLEACH, E.E. JONES AND M.V. JASPERS. *Bio-Protection and Ecology Division, Lincoln University, PO Box 84, Lincoln University, Canterbury7647, New Zealand.*
E-mail: bleachc2@lincoln.ac.nz

The black foot pathogens *Cylindrocarpon liriodendri*, *C. macrodidymum* and *C. destructans* are common in New Zealand grape-growing regions. Hot water treatment (HWT) of young grapevine plants does reduce incidence, but in New Zealand the standard HWT protocols sometimes damage young plants, possibly due to poor heat acclimatisation in a cool climate. This research examined the effectiveness of different heat treatments in field and laboratory experiments. Callused rootstock cuttings were grown for 8 months in nursery soils previously inoculated with the three *Cylindrocarpon* spp. above, and then the dormant plants were given standard HWT. Isolations from trunks showed that HWT was similarly effective in plants assessed immediately or grown in pots for 6 months, having 6–13% severity compared to 38–72% in control plants ($P<0.001$). The efficacy of different HWT temperatures (40–70°C) and times (5–30 min), in killing the pathogens (three isolates per species) was tested with mycelium discs and conidia, which were assessed by their ability to grow or germinate. Trunk lengths of 1-year vines were also deeply inoculated using mature cultures of these isolates. After 7 days they received HWT at 47–50°C for 30 min, with isolation at 1–2 cm from the inoculation site. Results showed that the different treatments affected isolates and species differently, with mycelium generally being less susceptible to HWT than conidia, which had no germination after 15 or 30 min at 40–50°C. A recent experiment, with dormant rootstock plants from an infected field site being given HWT at 47, 48.5 and 50°C for 15 and 30 min, will also be discussed.

Activity of electrolyzed acid water for the control of *Phaeomoniella chlamydospora* in the nursery. S. DI MARCO and F. OSTI. *Istituto di Biometeorologia, CNR, Via Gobetti 101, 40139 Bologna, Italy.*
E-mail: s.dimarco@ibimet.cnr.it

Phaeomoniella chlamydospora (Pch) and *Phaeoacremonium* species are associated with infections in grapevine cutting at the nursery level and with the development of Petri disease. Nursery protocols are being developed to reduce the incidence and severity of plant infections. Electrolyzed acid water (EAW) is a novel disinfectant obtained by water electrolysis with the addition of KCl, that has a pH 2.5, and an oxidation reduction potential (ORP) of 1100 mV. Washing and sanitizing treatments with EAW against fungal and bacterial diseases have been studied in fruit and vegetables. Laboratory, greenhouse and field trials are being carried out to evaluate the potential use of EAW in cutting hydration after the cold-stored period, for the control of Pch and *Phaeoacremonium aleophilum* (Pal). Laboratory assays have demonstrated that after 36 hours EAW was effective in reducing conidial germination of Pch and Pal by 70% and 50%, respectively, compared to water. No differences between the absorption of EAW and water were observed in the cuttings; more than 50% of EAW had been absorbed after 5 hours and the cuttings were completely saturated after 24 hours. Similar results were obtained at low temperatures (4–11°C). No morpho-histological differences were ever noticed on the treated cuttings, apart from a certain discoloration of the surface that can probably be attributed to chlorine. At the end of cutting hydration, the total water content was extracted from the cuttings and analyzed. The EAW hydrated cuttings showed a pH of 3.4 and an ORP of 600 mV. EAW treatment did not seem to affect plant vegetation in the nursery field, except for a reduction in vegetation for vines grafted on K5BB. Further investigations are underway.

Inhibitory interactions between *Trichoderma* isolates and grape wood pathogens. M.V. JASPERS[1] and L. MOSTERT[2]. *[1]Bio-Protection and Ecology Division, Lincoln University, PO Box 84, Lincoln University, Canterbury, New Zealand 764. [2]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa.*
E-mail: Jaspersm@Lincoln.ac.nz

This research investigated the modes of action of three *Trichoderma* isolates against 11 isolates of the grapevine wood pathogens *Phaeomoniella chlamydospora*, *Eutypa lata*, *Phomopsis viticola*, *Botryosphaeria parva*, *B. rhodina* and *B. obtusa*. Dual cultures made with all possible combinations of the *Trichoderma* isolates and pathogens demonstrated a range of antagonistic behaviours by the *Trichoderma* isolates including coiling and lysis. The production of volatile and non-volatile products by the *Trichoderma* isolates was assessed by their inhibitory effects on development of the pathogenic fungi. For volatile products, a culture of each *Trichoderma* isolate was taped to another plate on which a pathogen was growing either as mycelium or conidium germination. All *Trichoderma* isolates were able to reduce the rate of mycelium extension of all pathogen isolates. Similarly, the conidium germination rate was reduced for all pathogens, being delayed by at least 24 h beyond the control. For non-volatile products the autoclaved broth in which each *Trichoderma* isolate had grown was used as a base for making the broth media in which pathogen mycelium or conidia were introduced for growth or germination. Mycelium growth in liquid medium was only satisfactory for the *Botryosphaeria* species, these having 30–40% of the mycelium dry weight recorded for the control plates. Conidium germination of pathogen isolates was greatly inhibited in the broth extract. By 96 h, it was nil for the *P. chlamydospora* isolates and only 6–28% that of the controls for the *P. viticola* conidia. For *B. rhodina* and *B. obtusa* conidia, germination was 3–26% and 0–3%, respectively, in comparison with the controls.

Identifying potential biocontrol agents for grapevine pruning wound protection against trunk pathogen infection. C. KOTZE[1,2], J.M. VAN NIEKERK[1,3], F. HALLEEN[4] and P.H. FOURIE[1,2]. *[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602. 2Citrus Research International, Private Bag X1, Matieland, 7602. 3Westfalia Technological Services, P.O. Box 1103, Tzaneen, 0850. [4]Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa.*
E-mail: halleenf@arc.agric.za

Trunk diseases lead to premature decline and dieback of grapevine and are caused by a complex of pathogens, including *Eutypa lata*, *Phaeomoniella chlamydospora*, species of *Botryosphaeriaceae*, *Phomopsis* and *Phaeoacremonium*. The aim of this study was to evaluate promising biocontrol agents in *in vivo* trials for the protection of pruning wounds against infection by all of these pathogens. Grapevine cultivars Merlot and Chenin Blanc were spur-pruned in August 2006. Fresh pruning wounds were treated by spray inoculation with USPP-T1, USPP-T2 (*Trichoderma atroviride*), ECO 77® (*Trichoderma harzianum*), Biotricho (*T. harzianum*), Vinevax® (*T. harzianum* and *T. atroviride*), and *Bacillus subtilis*. Control treatments were with benomyl or sterile water. Seven days after pruning, treated pruning wounds were spray-inoculated with a spore suspension of either *E. lata, Pa. chlamydospora* and species of *Botryosphaeriaceae* (*Neofusicoccum australe*, *N. parvum*, *Diplodia seriata* and *Lasiodiplodia theobromae*), *Phomopsis viticola*, or sterile water as the control inoculation. The treated pruning wounds were removed after 8 months. The incidence of the inoculated pathogens and biocontrol agents was determined by means of isolations onto potato dextrose agar. The results obtained clearly indicate that the biological control agents gave protection of pruning wounds against infection by a range of trunk disease pathogens that were similar, and in some cases even better, than benomyl treatment. Furthermore, the successful re-isolation of *Trichoderma* spp. indicates that pruning wounds were colonised by these fungi and that they can provide long-term protection of pruning wounds, which remain susceptible to infection for 2–16 weeks after pruning.

Evaluation of wound treatments to control Eutypa dieback in grapevines. M.R. SOSNOWSKI, A.P. LOSCHIAVO, T.J. WICKS and E.S. SCOTT. *South Australian Research and Development Institute, GPO Box 397, Adelaide 5001 Australia.*
E-mail: sosnowski.mark@saugov.sa.gov.au

Eutypa dieback, caused by the fungus *Eutypa lata*, infects grapevines through pruning wounds and colonises the wood of cordons and trunks. Wound treatments can reduce infection by *E. lata* although no fungicides are registered for this purpose in Australia. Due to the withdrawal of products such as benomyl from the market, and in order to delay the occurrence of fungicide resistance, it is important to continue to examine new options for the protection of wounds on vines. Twelve fungicides and 5 non-fungicide alternatives were evaluated *in vitro*. A spore suspension of *E. lata* was applied to potato dextrose agar (PDA) plates amended with treatments. After 48 h incubation at 23°C under continuous fluorescent light, spore germination was measured. Mycelium plugs of *E. lata* were also placed in the centre of amended PDA plates and incubated for 7 days at 23°C under 12-h cycles of fluorescent light/dark before the colony diameter was measured. Germination and mycelial growth were inhibited most by tebuconazole, fenarimol, myclobutanil, tetraconazole, garlic juice and lactoferrin. Carbendazim, cyprodinil + fludioxonil and tea tree oil restricted the growth of mycelium only. Field evaluation is underway on established grapevines in the Barossa Valley, South Australia. In winter 2008, treatments were applied to fresh pruning wounds on 1-year-old canes using a paintbrush, and on the following day the wounds were inoculated with 500 ascospores of *E. lata*. Canes will

be removed in the following winter and the presence or absence of *E. lata* determined by isolation on PDA with streptomycin.

Double pruning, a potential method to control Bot canker disease of grapes, and susceptibility of grapevine pruning wounds to infection by Botryosphaeriaceae. J.R. ÚRBEZ-TORRES and W.D. GUBLER. *Plant Pathology Department, University of California, Davis, California 95616, USA.*
E-mail: wdgubler@ucdavis.edu

Grapevine canker diseases are one of the main factors limiting vineyard longevity and productivity. Botryosphaeriaceae species are wood pathogens that infect mainly through pruning wounds. Consequently, knowledge of low-risk infection periods and pruning wound susceptibility are critical in deciding the appropriate timing for pruning and wound treatment. Double pruning of grapevines, which allows for final pruning in late winter, has been shown to reduce infections caused by *Eutypa lata*, because infections on pre-pruning wounds do not develop further than the final pruning point. In this study we evaluated the efficacy of double pruning to reduce infections caused by *Lasiodiplodia theobromae* and *Neofusicoccum parvum*, two Botryosphaeriaceae species capable of colonizing wood tissue much more rapidly than *E. lata*. Chardonnay and Cabernet Sauvignon grapevines were pre-pruned and separately inoculated with a spore suspension from mid-October to February, with a final pruning in March. Canes were examined and the length of vascular discoloration was measured from the point of infection to determine whether fungal infection developed beyond the point of final pruning. The duration of susceptibility of pruning wounds to infection by these members of the Botryosphaeriaceae was studied in the same site. Vines were pruned from mid-November to February and inoculated with the same fungal species at 12-day intervals after pruning for a period of 50 days. Data collection was still ongoing at the time this abstract was being written. Therefore, results from both double pruning and grapevine pruning wound susceptibility to infection will be presented and discussed at the meeting.

Sensitivity of pathogens associated with Petri disease and Black foot to hot-water treatments *in vitro*. D. GRAMAJE, S. ALANIZ, P. ABAD-CAMPOS, 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

In this study, the sensitivity of *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Pm. parasiticum* (causal organisms of Petri disease) and *Cylindrocarpon liriodendri,* and *C. macrodidymum* (causal organisms of black foot disease) to hot-water treatments (HWTs) was evaluated *in vitro*. HWT has been reported as an effective method to control grapevine trunk diseases. However, little is known about the effects of different temperature and times of HWT on these pathogens. Conidial suspensions and plugs of agar with mycelia were placed in Eppendorf vials and incubated in hot water baths at 41, 42, 43, 44, 45, 46, 47 and 48°C for 30, 45 or 60 min for *Cylindrocarpon* spp. and at 49, 50, 51, 52, 53 or 54°C for 30, 45 or 60 min for *Pa. chlamydospora* and *Phaeoacremonium* spp. In general, conidial germination and colony growth rate of all pathogens decreased with increased temperature and time combinations. Conidial germination of *Cylindrocarpon* spp. was inhibited at temperatures above 45°C, while temperatures above 48°C were necessary to inhibit the mycelial growth. For *Pa. chlamydospora*, conidial germination was inhibited at temperatures above 52°C, while treatments above 53°C for 45 min were necessary to inhibit the mycelial growth. Regarding *Phaeoacremonium* spp., temperatures above 53–54°C were necessary to completely inhibit both conidial germination and mycelial growth. These results demonstrate that current HWT protocols at 50°C for 30 min are useful to control *Cylindrocarpon* spp. However, it would be necessary to develop HWT using higher temperatures to reduce the incidence of *Pa. chlamydospora* and *Phaeoacremonium* spp. infections.

Effect of combined hot water and cyproconazole treatments on the eradication of *Phaeomoniella chlamydospora* from grapevine planting material. S. SERRA, M.A. MANNONI, V. LIGIOS and A. DEMONTIS. *Dipartimento di Protezione delle Piante – Università, Via De Nicola 9, 07100 Sassari, Italy.*
E-mail: salvase@uniss.it

Phaeomoniella chlamydospora (*Pch*) is the cause of important grapevine decline diseases and it can spread through planting material. Combined chemical and hot water treatments (HWT) were carried out to limit this spread. Cuttings and callused graftlings were treated by dipping in a hot water bath at 50°C for 30 min (HWT) or in a cyproconazole suspension (0.1 g a.i. l^{-1}) for at least 12 h, in different combinations. In 2005–06 canes from esca-diseased grapevines (rootstock cvs 140Ru and 1103P, grafted to cv Sauvignon) were treated at different stages of the propagation process. In 2005 and 2006, natural contamination on nursery material was insufficient to determine the effectiveness of treatments. In 2007, 1103P cuttings were treated after inoculation with *Pch* (10^7 conidia ml^{-1}). Cuttings and graftlings were grown in a field nursery or in pots for one season and then destructively examined. DNA was extracted from wood collected at different points and analysed by nested PCR with Pch-specific primers. One-mm thick woody slices from artificially inoculated cuttings were plated onto

malt extract agar containing antibiotics and fungicides. HWT performed on cuttings before or after cold storage influenced vegetative growth depending on both the cultivar and the growth conditions, but it was deleterious to callused graftlings. HWT or chemical treatments alone were not effective in reducing the percentage of infected cuttings. Only a fungicide treatment immediately followed by HWT significantly reduced the incidence of infecton in cuttings, but it was not sufficient to eradicate the pathogen from all cuttings.

Acknowledgements: Research study commissioned from AR-SIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forestry) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-Regional Project "Grapevine esca: research and experiment in the nursery and in the field for prevention and cure."

Application of hot water treatment to reduce *Phaeomoniella chlamydospora* incidence in grapevine propagation materials. W. HABIB[1,2], A. PICHIERRI[1], N. MASIELLO[1], S. POLLASTRO[1] and F. FARETRA[1]. *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. [2]Istituto Agronomico Mediterraneo di Bari, Via Ceglie 9, 70010 Valenzano, Bari, Italy.*
E-mail: faretra@agr.uniba.it

Hot water treatment (HWT) is commonly used in different countries for the elimination of pests and pathogens from grapevine propagation materials. *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & Mugnai) Crous & W. Gams is frequently associated with wood discoloration and it is believed to be one of the main causes of Petri and Esca diseases of grapevine all over the world. Naturally infected rootstock cuttings, scion cuttings, rootstocks and grafted rootstocks (140Ru. and 1103P. grafted with 'Negroamaro') were treated at 50°C for 45 min. Wood discoloration and *P. chlamydospora* occurrence were evaluated just after the treatment and after one growing season in soil outside; shoot development and growth of the propagation materials were also measured. HWT did not affect the intensity of wood discoloration. *P. chlamydospora* was never detected in scion cuttings. The fungus was isolated from 11% of untreated rootstock cuttings and never detected in the treated ones. The frequency of pathogen detection was reduced up to 99% in HWT rootstocks and grafted rootstocks as compared to the untreated materials. At the end of one growing season the fungus was isolated from 23 to 63% of the HWT grafted rootstocks and young vines and from 44 to 100% of the untreated ones. Shoot development and growth of graftlings, rootstocks and grafted rootstocks were always significantly ($P \leq 0.05$) reduced by the treatment. These results suggest that HWT is effective against *P. chlamydospora*; however, further research should be carried out to clarify the reasons of the increasing frequency of pathogen detection after one-growing season and to reduce negative side-effects on vine development.

Acknowledgements: Research study commissioned from AR-SIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forestry) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-Regional Project "Grapevine esca: research and experiment in the nursery and in the field for prevention and cure."
Hot water treatment was carried on by Dr. Mannini, National Research Council, Institute of Plant Virology – Turin, Italy

Antagonism of the endophytic *Bacillus subtilis* strain AG1 to fungal pathogens that cause tracheomycotic deterioration of vine wood. A. ALFONZO, G. CONIGLIARO, L. TORTA, S. BURRUANO and G. MOSCHETTI. *Dipartimento S.En.Fi.Mi.Zo., Sez. Patologia vegetale e Microbiologia agraria, Viale delle Scienze, 90128, Università degli Studi, Palermo, Italia.*
E-mail: santella@unipa.it

Antagonistic substances produced by the *Bacillus subtilis* strain AG1, previously isolated from grapevine (cv. Catarratto) with symptoms of "esca", were investigated in an artificial medium. After a growth period, these metabolites were separated from the cell-free medium by precipitation and acidification to less than pH 2.5. The active fraction was extracted from the precipitate with 96% ethanol. The extract was tested against *Phaeoacremonium aleophilum* (PAL) and *Phaeomoniella chlamydospora* (PCH) (both isolated from grapevines with esca symptoms), *Verticillium dahliae* (obtained from a decaying vine) and *Botryosphaeria rhodina* (isolated from vine wood with cortical cankers). Antagonistic activity was assayed by the agar-well diffusion and critical dilution assay. One active unit AU ml^{-1} was defined as the reciprocal of the highest dilution that gave growth inhibition of the indicator species. The results showed a high inhibitory activity of *B. subtilis* metabolites against all assayed pathogenic fungi, particularly *B. rhodina* and *V. dahliae*, whose growth was inhibited respectively by 4480 AU ml^{-1} and 960 AU ml^{-1}, while growth of PAL and PCH was inhibited by 440 and 480 AU ml^{-1}. The antifungal activity of the metabolites was stable at high temperature (121°C) and resistant to enzymatic degradation (trypsin, pepsin, pronase, α-chymotrypsin, papain, α-amylase and proteinase K). Further studies are needed to determine the chemical nature of such metabolites and their potential use in biological control programmes.

Acknowledgements: Research study commissioned from AR-SIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forest) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agricultural and Forestry Policy) to implement the inter-

Regional Project "Grapevine esca: research and experiment in the nursery and in the field for prevention and cure."

Penetration and protection of grapevine pruning wounds with *Trichoderma* sp. from Vinevax® pruning wound dressing. I.C. HARVEY[1], J. MCDERMIT[2], P.D TURPIN[1] and D. GALE[2]. *[1]PLANTwise Services Ltd, PO Box 181, Lincoln, New Zealand. [2]Agrimm Technologies Ltd, PO Box 35, Lincoln, New Zealand.*
E-mail: harveyi@plantwise.co.nz

Pathogens in grapevine trunk wood are a cause of plant debilitation, lowered vine production and death. One portal of entry of these pathogens is pruning wounds. The application of a wound dressing to protect against the ingress of these fungi is being encouraged in the wine growing industry. However, the scale of vineyard operation often dictates that these treatments are not feasible. Products containing a *Trichoderma* sp. (viz. Vinevax®) and methods of large-scale application are under development and evaluation. Vinevax is the only registered product in New Zealand for such wound protection in grapevines. This work outlines the trials set up in Canterbury and results show that the bioprotectant establishes well in the wood when spur or cane pruned vines are treated with Vinevax at various concentrations. Optimal timing of applicaiotion is shown to be at the end of the day. Several common wood inhabiting fungi were found to be either controlled or uppressed by the treatment.

Effect of Bellis (boscalid & pyraclostrobin) on the physiological stress and grape quality of Red Globe grapevine plants with decline symptoms. J. AUGER[1], C. CARRERAS[1], I. PÉREZ[1], R. MUNITIZ[2], J. NITSCHE[2] and M. ESTERIO[1]. *[1]Depto. de Sanidad Vegetal, Fac. de Cs. Agronómicas, Universidad de Chile. Código Postal 8820808, Santiago – Chile. [2] BASF Chile S.A., Carrascal 3851, Santiago-Chile.*
E-mail: jauger@uchile.cl

This study aimed to determine the physiological effect of Bellis (boscalid & pyraclostrobin) applications on the development and maturity of berries in grapevines cv. Red Globe, which were affected by the decline syndrome (caused by *Botryosphaeria obtusa* and *Acremonium alternatum*) in comparison with berries from healthy vines. The comparative efficacy of Bellis applications was evaluated in terms of xylem formation and functionality, as well as berry size, soluble solids percentage, fresh and dry weight, berry colour development and rachis condition. The Bellis applications were made at flowering, formed fruit, pre-veraison and veraison, using 1,200 to 1,500 l ha^{-1} and at a rate of 0.8 Kg ha^{-1}. Controls were the healthy and untreated diseased vines. The treated diseased vines had a xylem functionality that was lower than the non-treated vines. The berries of treated diseased vines had increased accumulation of berry soluble solids and developed a vessel size that was similar to those from healthy vines. The grapes post-harvest rachis condition and colour in Bellis-treated plants was better than the ones found in non-treated, diseased plants ($P \leq 0.05$). In addition, there was less loss of colour in berries of Bellis-treated diseased plants after 60 and 90 days of cold storage than in fruits from non-treated diseased plants.

(U. de Chile / Basf Chile S.A. Research Agreement, 2005-06).

Effect of fungicides on germination and mycelial growth of *Cylindrocarpon liriodendri* and *C. macrodidymum*. S. ALANIZ, P. ABAD-CAMPOS, J. GARCÍA-JIMÉNEZ and J. ARMENGOL. *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.*
E-mail: alanizferro@gmail.com

In this study, 14 fungicides (azoxystrobin, captan, carbendazim, copper oxychloride, cubiet, didecyl dimethyl ammonium chloride, flusilazole, hydroxyquinoline sulphate, imazalil, iprodione, prochloraz, tebuconazole, thiophanate-methyl and thiram) were evaluated for their *in vitro* effects on conidial germination and mycelial growth of two isolates of *Cylindrocarpon liriodendri* and two of *C. macrodidymum*, the causal agents of black foot disease. For each fungicide, four concentrations (0.1, 1, 10 and 100 mg a.i. l^{-1}) were assessed. Conidial suspensions (5.0×10^5 conidia ml^{-1}) of each isolate were exposed to each concentration of the different fungicides. After 24 hours, the percent germination relative to the control treatment was assessed. Additionally, the different fungicides were added to potato dextrose agar in order to achieve the experimental concentrations. Mycelial plugs of each isolate were transferred to the centre of the fungicide amended plates. After 10 days, the daily growth rate relative to the unamended control was estimated. The data of each isolate were plotted against $\log_{10}$ of the fungicide concentrations. Probit regression analysis was used to estimate the 50% effective concentration values (EC_{50}). The fungicides, captan, didecyl dimethyl ammonium chloride, copper oxychloride, and thiram, effectively reduced conidial germination in both species; while carbendazim, prochloraz, imazalil and hydroxyquinoline sulphate showed good performance in reducing the mycelial growth. The potential of these fungicides to control *C. liriodendri* and *C. macrodidymum* in 110 R rootstock cuttings in callusing boxes was evaluated.

Managing eutypa dieback in grapevines by spraying pruned vines with fungicide. M.R. SOSNOWSKI, A.P. LOSCHIAVO and T.J. WICKS. *South Australian Research and Development Institute, GPO Box 397, Adelaide 5001 Australia.*
E-mail: sosnowski.mark@saugov.sa.gov.au

Eutypa dieback is caused by the fungal ascomycete

Eutypa lata, and is a serious disease of grapevines worldwide. It contributes to vineyard decline by reducing growth and yield and eventually kills vines. Vines are infected by airborne ascospores, which enter the vascular system through pruning wounds, germinating in the xylem vessels and colonising woody tissue. Fungicides such as carbendazim, biocontrol agents and physical barriers such as paints and pastes applied to wounds with a paint brush can control eutypa dieback. However, in large-scale viticulture this is not economically viable due to the labour costs. This study compared the efficacy of carbendazim, applied at 2.5 g active ingredient l^{-1} to pruning wounds using two commercial spray machines, a SARDI fan sprayer (output 596 l ha^{-1}) and a Hardi air-assisted sprayer (366 l ha^{-1}), with manual paint brush application for the control of *E. lata* infection. Over two consecutive seasons the carbendazim applied by paint brush provided 96–100% control of infection by *E. lata*. Application with the SARDI fan sprayer provided 92–100% control and the air-assisted sprayer provided 50–77% control. Trials are currently underway to optimise water output rates and spray deposition with a range of different spray machines. Spray application of pruning wound protectants has potential to improve control of eutypa dieback on large large-scale vineyard plantings and could also be used for other fungicides, biocontrol agents and alternative products effective against *E. lata*.

***In vitro* fungicide sensitivity of Petri disease pathogens and their potential use in the grapevine propagation process.** D. GRAMAJE[1], A. AROCA[2], R. RAPOSO[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]Instituto de Investigaciones Agrarias (INIA). CIFOR. Ctra. Coruña Km 7,5, 28040 Madrid, Spain. E-mail: dagrape@upvnet.upv.es

Fourteen fungicides were evaluated for their effect on mycelial growth and conidial germination of two isolates of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* at 0.1, 1, 10 and 100 mg a.i. l^{-1}. Comparison of the effective concentrations that inhibited mycelial growth and conidial germination by 50% (EC_{50}) showed that carbendazim, flusilazole and tebuconazole were most effective in reducing mycelial growth for both species. Azoxystrobin, captan, carbendazim, copper oxychloride, cubiet, didecyldimethylammonium chloride and thiram were most effective in reducing conidial germination for both species. Additionally, the effects of cubiet and hydroxyquinoline sulphate (0.5 mg a.i. l^{-1}) and didecyldimethylammonium chloride (0.18 mg a.i. l^{-1}) during the hydration stage in the grapevine propagation process were determined. Cuttings of 110 R rootstock (15 cm long) previously hot-water treated at 54°C for 30 min, were vacuum-inoculated with a conidial suspension of *Pa. chlamydospora* or *Pm. aleophilum* at two different concentrations (10^4 and 10^8 conidia ml^{-1}). One inoculated and three non-inoculated cuttings were placed in a glass (300 cc.) filled with each fungicide solution. For each inoculum and fungicide treatment combination, there were three replications; the experiment was laid out in a completely randomized design and repeated twice. These cuttings were soaked at 25°C for 4 days. Detection of pathogens was done for all cuttings by isolation onto growth media. Results showed that non-inoculated cuttings were colonized by *Pa. chlamydospora* or *Pm. aleophilum* in the untreated control treatment. All fungicides reduced infection by both pathogens when cuttings were vacuum-inoculated with 10^4 conidia ml^{-1}. The most effective fungicide was didecyldimethylammonium chloride, which reduced *Pm. aleophilum* infection and prevented *Pa. chlamydospora* infection at 10^8 conidia ml^{-1}.