

ABSTRACTS

Abstracts of oral and poster presentation given at the first COST Action FA 1303 workshop on Grapevine Trunk Diseases, Cognac, France, 23-24 June 2015

The International COST Action FA1303 Workshop on “Sustainable control of Grapevine Trunk Diseases: current state and future prospects” was held in Cognac, France, on June 23-24 2015. The meeting was attended by 90 participants. Forty-eight oral and posterpapers were presented, in four sessions: Pathogen characterization, detection and epidemiology; Microbial ecology; Host-pathogen and fungus-fungus competitive interactions; and Disease management. A field trip to the nursery Mercier at Vix was undertaken on June the 22th and 25th, including a demonstration of the MYCORRAY detection tool in the nursery research lab. A second field trip was to the Hennessy cellar, on the afternoon of June 23th.

The workshop was organized by members of the COST Action FA1303 (www.managtd.eu), in collaboration with Hennessy.

Session 1: Pathogen characterization, detection and epidemiology

The disease cycles of fungi associated with grapevine trunk diseases. P. LARIGNON¹ and D. GRAMAJE². ¹Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, 7 avenue Cazeaux, 30230 Rodilhan, France. ²Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas – Universidad de La Rioja – Gobierno de La Rioja, Ctra. de Burgos, km. 6, Finca La Grajera, 26007 Logroño, Spain. E-mail : philippe.larignon@vignevin.com

Disease cycles for the pathogens associated with Botryosphaeria dieback, esca, black-foot and Petri diseases have been defined from an analysis of the relevant literature from several sources. Botryosphaeriaceae spp. overwinter in diseased woody material or in pruning debris. From pycnidia, conidia are discharged during rainfall or overhead sprinkler irrigations. The seasonal spore dispersion patterns varied among different locations, most likely dependent on the different climatic conditions present. The pathogen may invade host tissue to form cankers through injuries caused by pruning, vineyard operations and adverse weather events. The susceptibility of fresh pruning wounds is greater than that of the oldest wounds, and less when vines are pruned in late season compared to early pruning. Inoculum can also be spread by arthropods but this is likely to be of secondary importance. Fungi associated

with esca or Petri diseases, *Phaeoconiella chlamydospora* (Pch) and *Phaeoacremonium aleophilum* (Pal), are considered to be airborne fungi. The sources of inoculum, pycnidia (Pch) or perithecia (Pal), are located on pruning wounds, on exposed vascular tissues on grapevine cordons and spurs and on protected wood surfaces inside deep cracks. They can also produce conidiophores inside deep cracks and crevices of the wood, which provide a protected humid environment, favourable for sporulation. Spore liberation occurs during winter (Pch) and the vegetative host period (Pch, Pal), and is closely linked to rainfall or to irrigation practices (Pal). Arthropods and pruning shears may also contribute to pathogen dispersal. These fungi can invade tissues through pruning wounds during winter (Pch) or after the sap bleeding stage, except in very mild winters (Pal). The susceptibility of pruning wounds occurs for a longer time when grapevines are subjected to early pruning. Contrarily to Pch, Pal can infect grapevines through the root systems, but the importance of root infection is still unknown. *Eutypa lata* is responsible for Eutypa dieback. The pathogen overwinters in diseased woody parts. From perithecia, spores are released during and after rainfall or snowmelt and are disseminated by wind. They penetrate into plants through pruning wounds during winter dormancy. Wound susceptibility is greatest when vines are pruned early in the dormant season and less when vines are pruned later in the dormant season. Within the host vessels, spores germinate and the mycelia proliferate slowly, at first within

the vessels and later through associated elements of the functional wood leading to the formation of cankers. The disease cycles of the black-foot pathogens (*Cylindrocarpus* spp., *Cylindrocladiella* spp., *Cylindrodendrum* spp., *Dactylonectria* spp., *Ilyonectria* spp. and *Neonectria* spp.) are relatively poorly understood. These fungi infect hosts through natural openings or wounds on the roots or through rootstock crowns, to cause black necroses. The production of chlamydospores allows the pathogens to survive in the soil for extended periods.

This research was supported by COST Action FA1303.

Development of molecular detection and identification tools for young grapevine decline and esca related pathogens. M. CHRISTOPOULOU, S.E. TJAMOS and E.J. PAPLOMATAS. *Department of Plant Pathology, Agricultural University of Athens, Athens 11855, Greece. E-mail: epaplom@aua.gr*

The etiology of esca is complex. Although white heart rot is caused by closely related species of *Fomitiporia*, dark brown discoloration can be attributed to many different pathogens which are also responsible for young grapevine decline. The main objectives of this study were (a) to develop a sensitive, molecular method for detection of the two main pathogens causing young grapevine decline in Greece, *Phaemoniella chlamydospora* and the *Ilyonectria destructans* complex, directly in young tissues, and (b) to discriminate *Fomitiporia mediterranea* and *F. punctata*, using molecular technology, and also to determine the geographic distribution and host preference of these two species in Greece. *P. chlamydospora* and *I. destructans* complex were detected in vine woody tissues (80 samples at various developmental stages) after DNA extraction and nested PCR. At the first stage of the nested PCR, universal ribosomal primers were used to amplify microbial DNA present in vine tissues. Species-specific primers were then applied to detect the presence of *P. chlamydospora* and *I. destructans* complex in healthy and artificially inoculated rootstocks. Both fungi were successfully detected in artificial and naturally infected rootstocks. High percentages of infection were observed at very early stages of rootstock production, and both pathogens were detected by PCR even in asymptomatic cuttings. The discrimination between *F. mediterranea* and *F. punctata* was achieved using species-specific primers, designed from ITS sequences of eight characterized isolates from sequence databases. Four characterized isolates (two *F. mediterranea* and two *F. punctata*) and 42 *Fomitiporia* isolates collected from four different hosts and nine regions of Greece, were used to test the species-specific primers. Furthermore, 11 isolates of closely related genera and species were used to test primer specificity. The results of this PCR based screening demon-

strated the efficacy of the method to discriminate the two *Fomitiporia* species, and that the 42 Greek isolates were grouped into *F. mediterranea*.

One fungus, one name: a case for retaining the genus name *Phaeoacremonium* above *Togninia*. D. GRAMAJE¹, L. MOSTERT², J.Z. GROENEWALD³ and P.W. CROUS^{3,4,5}. ¹*Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. de Burgos, Km. 6, Finca La Grajera, 26007 Logroño, Spain.* ²*Department of Plant Pathology, University of Stellenbosch, P/ Bag X1, Matieland, 7602, South Africa.* ³*CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.* ⁴*Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa.* ⁵*Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands. E-mail: david.gramaje@icvv.es*

The amendment of Article 59 and the abolishment of dual nomenclature for fungi in the International Code of Nomenclature for algae, fungi and plants (ICN), means that *Togninia* (1900) has priority over *Phaeoacremonium* (1996). *Togninia* presently has 26 epithets, 13 of which are insufficiently known (most have not been reported since they were originally described at the turn of the 20th Century), and nine already have names in *Phaeoacremonium*. *Phaeoacremonium* presently has 46 species, all of which are known from culture, and their DNA sequences are available. The genus name is also favoured by plant pathologists, viticulturists and medical mycologists, and is the more common name used in published literature. Choosing *Phaeoacremonium* over *Togninia* will also result in the least name changes, as only eight new combinations are required and proposed. *Phaeoacremonium* species have been associated with decline and dieback diseases of numerous woody plants as well as phaeohyphomycotic infections in humans. *Phaeoacremonium* strains from grapevines have been studied intensively because of the involvement of these taxa in two complex fungal diseases, namely Petri disease in young vines and esca in adult vines. New advances in the epidemiology, genetic variation and detection of *Phaeoacremonium*, as well as the merits for retaining of *Phaeoacremonium* over the sexual name *Togninia* are presented.

Taxonomy, pathogenicity and phylogeny of fungi associated with *Botryosphaeria dieback* in Sicily. V. MONDELLO¹, S. GIAMBRA¹, G. CONIGLIARO¹, L. SANTOS², A. ALVES² and S. BURRUANO¹. ¹*Agricultural and Forestry Sciences Department, University of Palermo, viale delle Scienze, 4, 90128 Palermo, Italy.* ²*Departamento*

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Since the first report of “Botryosphaeria dieback” caused by *Lasiodiplodia theobromae* in 2008, other Botryosphaeriaceae were found associated with declining vines in grape-growing areas of Western and Central Sicily. In a recent study, *Diplodia seriata*, *Lasiodiplodia* sp., *Neofusicoccum parvum* and *Neofusicoccum vitifusiforme* were isolated from declining grapevines. In order to fulfill Koch’s postulates and verify any genetic variability among isolates, pathogenicity, morphological, molecular and phylogenetic analyses were performed. The pathogenicity of eighteen isolates was tested by inoculating 2-year-old rooted grapevine cuttings (cv. Inzolia) and evaluating vascular discoloration length after 6 months. Morphological identification was based on microscopic (conidial morphology and dimensions) and macroscopic (growth rate) parameters. ITS and EF1- α regions were sequenced and compared to those deposited in sequence databases through Blastn searches, followed by phylogenetic analyses. All the tested isolates caused vascular discoloration *in planta*, sometimes with significant lesion length differences among strains of the same species, even if genetically identical, confirming the difficulty to consider several Botryosphaeriaceae species as primary or secondary pathogens on grapevine. The phylogenetic analyses confirmed our previous identifications, but showed that *Lasiodiplodia* isolates, first identified as *L. theobromae*, where the recently described species *Lasiodiplodia mediterranea*. The studies conducted on grapevine trunk disease pathogens in Sicily confirm the presence of different botryosphaeriaceous fungi as pathogens in several grape-growing areas of Central Western Sicily, and highlight their potential economic impacts on Sicilian viticulture.

First assessment of the distribution of trunk diseases on young and adult grapevines in Europe. L. GUERIN-DUBRANA¹ and L. MUGNAI². ¹ Université de Bordeaux, ISVV, UMR1065 Santé et Agroécologie du Vignoble, Bordeaux Sciences Agro, F-33175 Gradignan, France. ² Department of the Agro-food Production and Environmental Sciences (DISPAA), Section of Plant pathology and Entomology, University of Florence, Piazzale delle Cascine 28, 50144 Florence, Italy. E-mail: lguer@bordeaux.inra.fr

A large survey based on a reporting questionnaire of qualitative information was carried out in European and Mediterranean viticulture regions. The purpose was to: (1) give an overview of the presence and frequency of the main fungal grapevine trunk diseases (GTDs), which are the esca complex [apoplexy, Grapevine Leaf Stripe Disease (GLSD)], *Eutypa* dieback, dead cordon, *Botryosphaeria* dieback and cankers, excoriose,

black foot, Petri disease, or general young vine decline. A bacterial disease (crown gall) was included in the reporting; (2) to collect general information on the characteristics of the viticultural areas at a regional scale that can be linked to GTDs; and (3) to establish a European network for further multisite GTD surveys. Respondents from 18 countries and more than 50 viticultural regions contributed to the survey. Vintage wine, table wine and nurseries represent, respectively, the first, second and third types of production in each country. A broad diversity of cultivars and rootstocks is grown with 13 cultivars representing 50% of the vines. The vines are mainly trained according to the cordon or Guyot methods. Grapevines are mostly hand-pruned, but the use of pneumatic pruning shears and mechanical pruning is becoming increasingly more frequent in some countries. A wide range of disease profiles was reported according to the country or the region surveyed. Apoplexy and GLSD symptoms corresponding to the esca syndrome were widespread in numerous countries and related together. The frequency of both symptoms seems to be increasing and they are becoming worrying phytosanitary problems in the main grapevine producing countries of Europe. Dead cordon was also widespread, from known or unknown origins, varying according to the country surveyed. Excoriose remains a frequent disease in approx. half of the countries surveyed. In contrast to the large presence of trunk diseases on adult vines, GTDs on young vines is not so frequent in most European countries, but remains a subject of concern in some. All the collected information and the developed network represent an important starting point for the development of further in-depth epidemiological studies, and for further surveys based on recording GTD data. This survey depended on the information provided by numerous researchers throughout Europe and the Mediterranean region, and we thank them for their valuable contributions.

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Grapevine trunk disease research in South Africa. F. HALLEEN^{1,2} and L. MOSTERT². ¹ Plant Protection Division, ARC Infruitech-Nietvoorbij. Private Bag X5026, Stellenbosch, 7599, South Africa. ² Department of Plant Pathology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa. E-mail: halleenf@arc.agric.za

The first significant volume of research with regard to grapevine trunk diseases (GTD) in the modern era was carried out by J.H.S. Ferreira in the 1980’s, when he identified *Eutypa lata* as the causal organism of *Eutypa* dieback. In the 1990’s Ferreira also played an instrumental role in the identification of Petri disease fungi (then known as *Phialophora parasitica*) and its association with “*Phialophora* dieback”, “Black Goo”, “young

vine decline” and ultimately Petri disease. Ferreira was also one of the founding members of the International Council on Grapevine Trunk Diseases. This is also the period when P.W. Crous established the genus *Phaeoacremonium*, and later also *Phaeomoniella*, to accommodate the ever-increasing number of related species associated with this disease. Several in-depth studies followed to resolve GTD complexes, including the *Cylindrocarpon/Campylocarpon/Ilyonectria* complex associated with Black foot disease, Botryosphaeriaceae species associated with Botryosphaeria dieback, Diatrypaceae species associated with dieback, and *Phomopsis* spp. associated with Phomopsis cane and leaf spot, as well as *Phomopsis* dieback. Collaboration between ARC Infruitec-Nietvoorbij, Stellenbosch and the University of Stellenbosch is well established. Identification and characterization of fungi associated with GTD is ongoing and one of the cornerstones of our research. Several molecular detection techniques have been developed to aid species identification and the detection of trunk disease pathogens in plants, the nursery processes and other inoculum sources such as the air, soil and insects. Our epidemiology studies revolve around the identification of inoculum sources within vineyards and rootstock mother blocks, the study of spore release patterns and dispersal of pathogens within and between vineyards in order to provide a knowledge base for management strategies. Research regarding management strategies concentrates on prevention of infections in grapevine nurseries in order to improve the phytosanitary status of grafted vines. Management in vineyards includes successful establishment of vines, complemented with knowledge and tools to effectively prevent infections (i.e. pruning wound protection). Biological control forms an integral part of our approach, including the identification of possible control agents, development of these agents into commercial products and developing understanding of pathogen-host-biological control agent interactions.

Incidence of esca symptoms in different cultivars and cultivar/rootstock combinations. S. MUROLO and G. ROMANAZZI. *Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131 Ancona, Italy. E-mail: g.romanazzi@univpm.it*

Esca is one of the main causes of losses of production and plant death in vineyards of most viticultural areas of the World. We determined the foliar esca symptoms (grapevine leaf stripe disease symptoms, GLSD) on a range of grapevine cultivars and cultivar/rootstock combinations in three vineyards located in Marche region, central-eastern Italy during summer 2008, when conditions were conducive for the disease. In vineyard AP1 (established in 1989, Kober 5BB rootstock,

vines trained as cordon with 3 m between rows and 2 m between vines in the rows) the incidence of symptomatic plants was recorded on 67 *Vitis vinifera* cultivars. Among white-berried cultivars, Passerina, Sauvignon blanc, Manzoni bianco, and Riesling had the greatest incidence of symptomatic plants (28–35%), while cvs. Chiapparù, Grechetto, Perdea, Verdicchio, Fiano and Pinot blanc had the least (0–5%). Among red-berried cultivars, Rebo had greatest incidence of symptomatic plants (>35%), with the least for cvs. Lacrima, Brugenile, Sirah, and Limberger (0%), followed by Gallioppo, Merlot, and Nebbiolo (<5%). Mean values of symptomatic plants among red and white cultivars were similar. The six cultivars monitored in vineyard AP2 (with the same properties of AP1) confirmed this cultivar susceptibility ranking. Furthermore, in vineyard AN1 (established in 1995, different rootstocks, vines trained as cordon with 3 m between rows and 1.2 m between plant in rows), the incidence of esca symptoms was recorded on seven cultivars grafted onto different rootstock. Cvs. Fiano and Sauvignon grafted onto SO4 had greater disease incidence than combinations with 1103P. This can be ascribed to the greater drought resistance of 1103P compared with SO4, supporting the hypothesis that GLSD symptoms occur mostly in stressed plants. Significant differences in the symptom expression were recorded just among one clone of Sauvignon, while they were not found in Chardonnay, Riesling or Pinot blanc. Our data provide useful information for growers in planning cultivar/rootstock combination choices for new vineyards, and indicate possible temporal evolution of GLSD symptoms in established vineyards.

Grapevine trunk diseases in British Columbia: challenges in controlling a complex disease under difficult environmental conditions. J. R. ÚRBEZ-TORRES. *Agriculture and Agri-Food Canada, Science & Technology Branch, Pacific Agri-Food Research Centre, Summerland, British Columbia V0H 1Z0, Canada. E-mail: joseramon.urbeztorres@agr.gc.ca*

Grapevines represent a farm gate value of over \$35 million CAD in British Columbia (BC) and though the overall cultivated area (4,000 ha) is considerably smaller compared with most grape-growing regions worldwide, grapes significantly influence BC's economy contributing with over \$2.01 billion CAD. Negative impacts on fruit yield and quality are of particular concern to the BC wine industry, especially with the added difficulty of producing high quality wines under BC's short-season growing conditions. Moreover, winter temperatures can occasionally drop below -20°C, causing serious injury to *Vitis vinifera*, which exacerbates plant stress conditions. Therefore, special interest is shown by BC grape-growers in understanding the effect that diseases and their management practices have on vine

physiology, particularly on winter hardiness, an important aspect to minimize the risk of winter injury. Since the mid-2000s, significant young plant mortality and reduction in productivity has been observed in BC vineyards. Research started in the late 2000s showed GTD to occur in BC, and over 40 different fungi have been identified as possible causes. Additionally, field survey data have highlighted the high canker infection rates in mature vineyards, which have, in most cases, the greatest incidence of winter injury. One added difficulty is the absence of registered pesticides for control of GTD, which make grape-growers totally vulnerable against the problems they cause. Accordingly, development and implementation of effective control strategies are the focus of our research. Successful control strategies require knowledge of disease epidemiology; in particular the role that environment plays on inoculum availability and dispersal, which is of special importance under BC's unique climatic conditions. Epidemiological studies are underway in BC, using the latest technology. BC is considered an emerging grape-growing region with a significant number of new vineyards established during the last decade, which could explain why the young vine decline complex has been shown to be the primary biotic cause of mortality in young vineyards. It has long been known that grapevine propagated material can be infected with GTD pathogens. The lack of a domestic clean plant propagation programme makes the Canadian grape industry dependent on importation of planting material from different sources. Accordingly, a significant effort has been put on the development of more sensitive and high-throughput pathogen detection and diagnostic molecular tools, which are currently being implemented to determine the health status of nursery propagated material coming to BC.

Diversity of Diatrypaceae species in three Spanish grapevine producing regions. S. GIAMBRA¹, J. ARMENGOL², S. BURRUANO¹ and M. BERBEGAL². ¹Dipartimento SAF, Università degli Studi di Palermo, Viale delle Scienze, 4, 90128 Palermo, Italy. ²Istituto Agroforestal Mediterraneo, Universidad Politécnica de Valencia. Camino de Vera s/n 46022, Valencia, Spain. E-mail: mobermar@etsia.upv.es

The objective of this study was to identify species of Diatrypaceae associated with trunk diseases in three different Spanish grapevine producing regions: Albacete, Cádiz and Valencia provinces, located, respectively, in geographically distant areas of Central, South and Eastern Spain. Species identification of 71 isolates was performed using a combination of morphological characters and phylogenetic analyses based on the internal transcribed spacer regions of the rDNA (ITS) and the β -tubulin gene. Four species of Diatrypaceae were identified: *Cryptovalsa ampelina* was the predominant

species (63.4%) followed by *Eutypa lata* (19.7%), *Eutypella microtheca* (4.2%), and *Eutypella citricola* (2.8%). Additionally, three taxa could not be identified at the species level; one (2.8%) was closely related to species such as *Diatrype stigma* or *Diatrypella iranensis* and two (1.4 and 5.6%) were closely related to species such as *Eutypa tetragona* or *Eutypa leptoplaca*, based on the phylogenetic analyses. The distribution of Diatrypaceae species differed between the three regions. *Cryptovalsa ampelina* was the prevalent species in Albacete and Valencia provinces, while *Eutypa lata* was the most frequently isolated species in Cádiz. This province showed the greatest species diversity, since all the species were isolated there except the putative new species closely related to *Diatrype stigma* or *Diatrypella iranensis*. This was found only in Valencia province. Possible relationships between the different species distributions and climatic conditions in the three regions are discussed.

Molecular diagnostics for grapevine trunk disease pathogens in one SO4 rootstock mother plant in the Czech Republic. A. EICHMEIER¹, E. PEŇÁZOVÁ², J. PEČENKA¹, K. BARÁNKOVÁ¹ and Z. MYNARZOVÁ¹. ¹Mendeleum Institute of Genetics and Plant Breeding, Faculty of Horticulture, Mendel University in Brno, Valtická 334, 69144 Lednice, Czech Republic. ²Department of Vegetable Science and Floriculture, Faculty of Horticulture, Mendel University in Brno, Valtická 337, 69144 Lednice, Czech Republic. E-mail: ales.eichmeier@mendelu.cz

A study was conducted to determine the fungal pathogens associated with grapevine trunk diseases (GTDs) in the Czech Republic, particularly in the wine-producing region of Moravia. Wood material from a rootstock mother plant was chosen as the point of focus due to the likely key role in disseminating the causal pathogen. One 10-year-old mother plant of Selection Oppenheim 4 (SO4) was chosen for analysis, as it exhibited symptoms such as stunted growth, short internodes and interveinal chlorosis transitioning from yellow to red and brown (tiger-stripe leaf). Isolates were obtained by plating symptomatic material (canker-necrotic wedges, circular necrosis, exudate from cut wood and soft wood) onto potato glucose agar. The isolates were identified through DNA sequencing of the internal transcribed spacer 2 (ITS2) region using the primers ITS3 and ITS4 (White *et al.*, 1990) and comparing the resulting sequence with those in sequence databases. PCR with specific primers was also carried out to identify isolates of *Phaeoacremonium* species, *Phaeoconiella chlamydospora*, *Eutypa lata*, *Fomitiporia mediterranea* and Botryosphaeriaceae species. Up to seven different species were found in this single vine. Symptoms such as vascular secretion, canker and necrotic wedges were associated with *Fomitiporia mediterranea*, circular necrosis was associated with *Bjerkandera* species, soft wood

was associated with *Talaromyces trachyspermus*, *Phomopsis* species and an as yet undetermined Basidiomycete species. This study presents the first overview of the diversity of fungi associated with GTDs in the Czech Republic in one mother rootstock plant.

Reference: White T.J., T. Bruns, S. Lee and J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *Pcr Protocols, A Guide to Methods and Applications*. (Innis M.A., D.H. Gelfand, J.J. Sninsky, T.J. White, ed.). Academic Press, San Diego, CA, USA, 315–322.

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A new molecular diagnostic tool for detection of Botryosphaeriaceae species. L. MARTÍN¹, F.J. CASTAÑO¹, G. DURANTE², C. CHATILLON³ and J. LUQUE⁴. ¹Fundació Parc Tecnològic del Vi (VITEC), Ctra. de Porrera km 1, 43730 Falset, Spain. ²International Plant Analysis and Diagnostics srl Via Einstein - Loc. Cascina Codazza -26900 Lodi, Italy. ³Qualiplante, Cap Alpha - Avenue de l'Europe - 34830 Clapiers (France). ⁴Institut de Recerca i Tecnologia Agroalimentàries (IRTA) Patologia Vegetal, Ctra. de Cabrils km 2, 08348 Cabrils, Spain. E-mail: laura.martin@vitec.cat

Botryosphaeriaceae spp. have been reported as major pathogens causing grapevine trunk diseases (GTDs) worldwide. On grapevines, infection with these fungi causes internal wood necrosis, cankers, dieback, cane bleaching and graft union failure. Isolation of these pathogens is time consuming and morphological identification to species level can be difficult. Development of culture independent PCR based methods could help to promote accurate disease control in grapevine nurseries as well as new epidemiology research. This study demonstrated a reliable and optimized molecular tool that allowed rapid and simple detection of multiple Botryosphaeriaceae species in a few hours. A collection of Botryosphaeriaceae isolates was used to verify multiple species detection of the *Botryosphaeria* spp. Qualiplante SAS End-Point Nested PCR kit (PCR. Botr-25/100Liq). DNA from 12 different tested Botryosphaeriaceae species, including all of the main species pathogenic to grapevines, showed a single positive amplicon band of 365 bp. A specificity *in vitro* test of the molecular kit was performed using DNA from pure culture of other GTD fungal pathogens that may occur in grapevines (i.e. *Phaeoconiella chlamydozpora*, *Phaeoacremonium* spp., *Eutypa lata*, *Cryptovalsa ampelina* and *Phomopsis viticola*). Results confirmed that the kit was specific for the Botryosphaeriaceae. The sensitivity threshold of the Qualiplante nested PCR kit was evaluated using purified genomic DNA from mycelia

of a *Diplodia seriata* isolate. The assay could detect as little as 5 pg DNA of *D. seriata*. The effectiveness of the assay was validated with environmental samples from naturally infected wood and rainwater traps collected in a mature vineyard where GTDs are known to be present. The viability and virulence of Botryosphaeriaceae pathogens was confirmed through isolation from water and wood material. The specificity of the assay was demonstrated even in the presence of competing DNA templates since other microorganisms were isolated from environmental samples. Detection of Botryosphaeriaceae DNA in rainwater revealed dispersion of these pathogens throughout the rain and the applicability of the assay for epidemiological studies. The results of this study showed that the specificity and sensitivity of this new molecular tool could be used to establish sanitary controls over the nursery propagation processes, in order to possibly prevent Botryosphaeriaceae infection.

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Session 2: Microbial ecology

Comparison of wood-colonizing microbial communities of Cabernet-Sauvignon grapevines planted in Catalonia (Spain) and Bordeaux (France). E. BRUEZ^{1,2}, S. COMPANT³, J. LUQUE⁴, G. E. JIMENEZ⁴, S. BASTIEN^{1,2} and P. REY^{1,2}. ¹Université de Bordeaux, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), Bordeaux Sciences Agro, F-33140, Villenave d'Ornon, France. ²INRA, ISVV, UMR1065 SAVE, F-33140, Villenave d'Ornon, France. ³Austrian Institute of Technology GmbH, Bioresources Unit, Health & Environment Department, Konrad Lorenz Strasse 24, 3430 Tulln, Austria. ⁴IRTA, Recerca I Tecnologia Agroalimentàries, Centra de Cabrils, 08348 Cabrils, Barcelona, Espanya. E-mail : emilie.bruez@bordeaux.inra.fr

This compared the fungal and bacterial communities that colonize grapevines of cv. Cabernet-Sauvignon, grown in Catalonia in Spain and Bordeaux in France. Sampled plants were either foliar esca-symptomatic or asymptomatic. Our sampling design aimed to determine the diversity of microbial communities, the various pathogens possibly involved in esca and the potentially protective microflora colonizing grapevine wood tissues. Methods used included DNA fingerprinting (Single Strand Conformation Polymorphism), to compare the general microflora of plants, and high throughput sequencing to identify microbial species. The most interesting cultivable fungal strains have also been sequenced. Imaging confocal microscopy (Double labeling of Oligonucleotide Probes-Fluorescence *In Situ* Hybridization, DOPE-FISH) was also used to study the locations of microbial communities within plants and

the plant-microbe interactions. The high diversity of fungi and bacteria colonizing the wood tissues has been confirmed. These microorganisms were particularly abundant in the healthy wood tissues in comparison with the necrotic tissues. DOPE-FISH observations revealed that alpha-proteobacteria were very frequently detected in the non necrotic tissues in comparison with those in the necroses. Other bacteria communities colonized these damaged wood tissues. Our presentation focused on the various microbial communities that colonize various wood tissues of the inner parts of grapevines.

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Fungal community of *Eutypa dieback* symptomatic plants and diversity of Diatrypaceae associated with grapevine plants in a single vineyard. V. HOFSTETTER¹, B. BUYCK² and K. GINDRO¹. ¹Agroscope Changins-Wadenswil Research Station, Department of plant protection, Mycology and Biotechnology, Rte de Duiller, 1260, Nyon, Switzerland. ²Muséum National d'Histoire Naturelle, Département Systématique et Evolution CP39, UMR7205, 12 Rue Buffon, F-75005 Paris, France. E-mail: valerie.hofstetter@agroscope.admin.ch

Fungi in wood of Chasselas plants suffering from *Eutypa dieback* in a single plot (Perroy, Vaud, Switzerland) were isolated and characterized, based on their ITS1-5.8S-ITS2 sequences. When comparing the *Eutypa dieback* fungal community with that associated with the wood of asymptomatic plants, as well as with that associated with the wood of esca-diseased plants, isolated from the same vineyard plot, we found no significant differences. Even the isolation frequency of Diatrypaceae isolates, the causal agents of *Eutypa dieback*, was quite similar for the three fungal communities. A phylogenetic analysis was performed on a representative sampling of Diatrypaceae sequences downloaded from GenBank, and 83 *Eutypa*-like sequences were obtained from all three fungal communities. Our *Eutypa*-like sequences represented 13 different ITS genotypes which clustered within four clades, one corresponding to *E. lata* for the high majority of the isolates, a second to *E. armenicae* and the two remaining clusters represent first sequences for two unidentified *Eutypa* species. This phylogeny suggests that disease expression does not result from particular strains, as the most often isolated ITS genotypes were associated with all plant categories. A selection of our isolates was then further characterized by sequencing three more loci (*RPB2*, β -tubulin and calmodulin) and single locus phylogenetic analyses were conducted together with a representative sampling of the genetic variability of *E. lata* in California.

We found no incongruence when comparing these *E. lata* phylogenies, except between β -tubulin and calmodulin, suggesting that recombination occurs. When combining the four loci, most of our isolates of *E. lata* are unresolved from Californian *E. lata* strains, with the exception of four genotypes from our Chasselas vineyard that are highly supported as distinct.

Influence of leaf tiger stripe disease, caused by grapevine trunk fungi, on the composition of leaf bacterial microbiota. L. VIGUIER¹, M. PAPER¹, H. BERGER¹, A. SESSITSCH¹ and S. COMPANT¹. ¹AIT, Austrian Institute of Technology, Health & Environment Department, Bioresources Unit, Konrad-Lorenz-Strasse 24, 3430 Tulln, Austria. E-mail: stephane.compant@ait.ac.at

The impact of leaf tiger stripe disease on the whole leaf bacterial microbiota was assessed by cultivation approaches. Data showed that the leaf disease caused by fungi inside the trunks of grapevine plants *V. vinifera* L. cv. Zweigelt can influence the composition and structure of the leaf microbiota. Results particularly showed that on the phyllosphere and inner tissues of the leaves, the composition and structure of various genera belonging to Actinobacteria, Alpha-, Beta-, Gamma-proteobacteria and Firmicutes can be influenced by the disease, and that a switch occurs due to the leaf physiology. This was not surprising, however, as leaves with symptoms have different physiology from the leaves without visual symptoms. These data suggest, however, that although leaf tiger stripe disease is caused by fungi inside grapevine trunks, the disease not only impacts systemically the leaf physiology but also the microbiota leaving on the phyllosphere or inside the leaf endosphere.

Session 3: Host-pathogen and fungus-fungus competitive interactions

Phytotoxic secondary metabolites from Botryosphaeriaceae of Grapevine. E. ABOU-MANSOUR and J-L. DEBIEUX. Département de Biologie Végétale, Université de Fribourg, Rue du Musée 10, 1700 Fribourg, Suisse. E-mail: eliane.abou-mansour@unifr.ch

Plant pathogenic fungi are prolific producers of bioactive small molecules, known as secondary metabolites (SMs). Phytoalexins are SMs that play an important role in infection and lead to the damage or killing of plants. Some of these phytotoxins are host specific while others have activities against a broader range of plant hosts. Fungi and their hosts are not isolated in the environment, but interacting with other organisms, such as microbes, endophytes and fungivores. Therefore, many SMs from plant pathogenic fungi also pos-

sess antibacterial, antifungal and cytotoxic activities. *Neofusicoccum parvum* and *Diplodia seriata*, associated with grapevine, both produce the well-known dihydroisocoumarins compounds mellein and its hydroxy derivatives, while *N. parvum* produces also a family of epoxyquinols compounds. Dihydroisocoumarins and epoxyquinols were reported to show a wide spectrum of biological activities. Here, we present the structure of the isolated metabolites and a review of their biological activities.

The phytotoxic exopolysaccharides produced by fungi involved in grapevine trunk diseases. A. CIMMINO¹, T. CINELLI², L. MUGNAF², G. SURICO² and A. EVIDENTE¹. ¹Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte S. Angelo, Via Cintia 4, 80126 Napoli, Italy. ²Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA), Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: alessio.cimmino@unina.it

The phytotoxic metabolites involved in the grapevine diseases induced by different pathogens including *Phaeoacremonium*, *Phaeomoniella* and species of *Botryosphaeriaceae* belong to different classes of natural compounds. However they could be grouped in two distinct groups: lipophilic low molecular weight metabolites and hydrophilic high molecular weight compounds. Within the first group some new and previously known naphthalenones, melleins and polyphenol were isolated together with jasmonic acid and its ester with different butanolides. While the isolation and the chemical and biological characterization of these phytotoxins has been extensively studied, there is less knowledge on the exopolysaccharides (EPSs) produced by the different grapevine pathogens. The first preliminary results were reported for the exopolysaccharide produced by *P. aleophilum* and *P. chlamydospora*, which – from chromatographic (HPLC), IR spectroscopic and elemental analysis – were identified as pululans. These EPSs caused clear phytotoxic symptoms when assayed on different grapevine tissues including, leaves, cuttings and stems. The ESPs were also used as antigen to immunize rats to produce specific polyclonal antibodies. These were used to develop an ELISA cytofluorimetric method to recognize the EPSs in crude extracts obtained from grapevine leaves showing initial and fully developed grapevine leaf stripe symptoms (esca complex). Further investigations based on chemical, GC-MS and NMR spectroscopy, disagree, however, with these structure determinations. The chemical and biological characterization of the EPSs produced by *P. aleophilum*, *P. chlamydospora*, *N. parvum* and different botryosphaeriaceous species are reported, including a first evaluation of their phytotoxic activity on host and non-host plants.

Differing transcriptomic alterations of two esca-associated fungi, *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora* on lto co-cultured *Vitis vinifera* calli. J. FISCHER¹, S. COMPANT², R.J.G. PIERRON^{3,4}, M. GORFER^{2,5}, A. JACQUES³, E. THINES¹ and H. BERGER². ¹IBWF, Institute of Biotechnology and Drug Research, Erwin-Schrödinger-Str. 56, 67663 Kaiserslautern, Germany. ²AIT, Austrian Institute of Technology, Health & Environment Department, Bioresources, Konrad-Lorenz-Strasse 24, 3430 Tulln, Austria. ³Université de Toulouse, Institut National Polytechnique de Toulouse – Ecole d'Ingénieurs de Purpan, Département des Sciences Agronomiques et Agroalimentaires, Equipe Agrophysiologie et Agromolécules, 75 voie du TOEC, BP 57611, F-31076 Toulouse Cedex 03, France. ⁴Université de Toulouse, LGC UMR 5503 (CNRS/UPS/INPT), Dept BIOSYM, INP-ENSAT, 1 avenue de l'Agrobiopole, 31326 Castanet-Tolosan, France. ⁵BOKU, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz Strasse 24, A-3430 Tulln/Donau, Austria. E-mail: harald.berger@ait.ac.at

Phaeoacremonium aleophilum (*P.al*) and *Phaeomoniella chlamydospora* (*P.ch*) are filamentous fungi that have been frequently isolated from wood of grapevine trunks, and are suspected as playing roles in trunk disease development. Grapevine trunk/dieback diseases are on the rise in vineyards all over the world and no effective remedies against these diseases are available. Typical symptoms of esca disease are discoloured trunks and white rot, brown spots on fruits and “tiger stripes” on leaves, but these symptoms can vary greatly, depending on the age of the vines, the cultivar and external factors such as climate and terroir. *P. al* and *P.ch* have also been isolated from apparently healthy plants, suggesting that the biological activity of these fungi may be more important than their presence in host plants. Knowledge of the transcriptome can provide insight into this biological activity. In order to exclude as many factors as possible impacting on the fungi we used *Vitis vinifera* callus culture to detect changes in the transcriptome upon exposure to active plant cells. We present the specific transcriptional responses of these two fungi to callus cell exposure and the different strategies they develop to cope with the same environment (endophytic growth). This work will give new insights into the complexities of host/pathogen interactions, and may lead to effective disease management strategies.

Confocal scanning microscopy and imaging of *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora* colonisation inside cuttings of grapevine plants. R.J.G. PIERRON¹, H. BERGER², M. GORFER^{2,3}, A. JACQUES¹, A. SESSITSCH¹, J. STRAUSS^{2,3} and S. COMPANT². ¹Université de Toulouse, Institut National Polytechnique de Toulouse – Ecole d'Ingénieurs de Purpan, Département des Sciences Agronomiques et Agroalimentaires, Equipe

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Phaeoacremonium aleophilum and *Phaeoconiella chlamydospora* are considered pioneers of the esca complex pathosystem, but their colonisation behaviour inside plants has not been investigated in detail. In this study, *P. aleophilum* and *P. chlamydospora* colonisations were assessed 12 weeks post-inoculation in internodes and nodes of 1-year-old rooted Cabernet Sauvignon cuttings, using *gfp*-transformed and wild-type strains, and examination with confocal scanning microscopy, 3D reconstruction, image J analyses and Imaris® viewer. At internodal and nodal levels, bark, pith and xylem fibres were strongly colonised by *P. aleophilum*. The fungus can progress up to 8 mm from the point of inoculation, using pith, bark and fibres. *P. aleophilum* was additionally detected in the lumen of xylem vessels in which tyloses blocked progression. In the case of *P. chlamydospora*, at nodal or internodal levels, the pathogen was mainly restricted to xylem vessels. Tyloses were also visualised and few hyphae were recorded in other tissues. Tyloses were also observed in mock-treated plants but different plant responses in specific tissues at nodal or internodal levels were additionally visualised for *P. aleophilum* and *P. chlamydospora* in comparison to mock-treated plants. 3D modelling also showed the fungi inside xylem vessels and that they were attached to the xylem cell walls during the process of colonisation. These results demonstrate that *P. aleophilum* and *P. chlamydospora* colonisation can vary according to the type of tissues, that *P. aleophilum* can spread short distances in stem pith, bark and fibres but is restricted inside the xylem vessels, while *P. chlamydospora* is restricted to the xylem vessels and only slightly colonises other tissues. Woody tissues can also respond to the injury and to the presence of the fungi when the fungi colonise xylem vessels or other tissues.

Metabolites produced by agents of dieback of grapevine in Sicily: *Lasiodiplodia mediterranea*, *Neofusicoccum parvum* and *N. vitifusiforme*. A. ANDOLFI¹, S. BASSO¹, M. DELLAGRECA¹, S. GIAMBRA², V. MONDELLO² and S. BURRUANO². ¹Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte Sant'Angelo, Via Cintia, 4, IT-80126 Napoli, Italy. ²Dipartimento di Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze 4, IT-90128 Palermo, Italy. E-mail: andolfi@unina.it

Botryosphaeria dieback was recently reported in West Sicilian vineyards. The disease complex was only asso-

ciated with isolates of genus *Lasiodiplodia* in 2007 and together with *Diplodia seriata*, *Neofusicoccum parvum* and *Neofusicoccum vitifusiforme*, this latest reported for the first time on *Vitis vinifera* in Italy. Moreover, a representative isolate of each species was inoculated on green shoots and fulfilled Koch's postulates, although the lengths of vascular discolourations were variable depending on the species. As well as the other GTD causal agents, the expression of foliar symptoms (pale colour along the margins and between veins) was also occasional in Sicily, it was hypothesized that leaf symptoms could be due to fungal toxins, being produced in the woody tissues and translocated to the leaves through the transpiration streams. In order to improve the knowledge on host-pathogen interactions of the fungi associated with Botryosphaeria dieback, a study was undertaken of the pathogenicity of Sicilian botryosphaeriaceous fungi and their ability to produce phytotoxins. Artificial inoculations *in planta* confirmed the pathogenicity of all tested isolates. In addition, a strain of *Lasiodiplodia mediterranea* produced several metabolites in liquid medium. Among these, two new dimeric γ -lactols, lasiolactols A and B were characterized by 1D- and 2D-NMR and HR-ESI-MS, and another four metabolites were identified as botryosphaerioidiplodin, (5R)-5-hydroxylasioidiplodin, (-)-(1R,2R)-jasmonic acid, (-)-(3S,4R,5R)-4-hydroxymethyl-3,5-dimethyl-dihydro-2-furanone. The tested compounds at different concentrations (0.125, 0.25, 0.5 and 1 mg mL⁻¹) on grapevine cv. Inzolia leaves were phytotoxic: among these, jasmonic acid showed the greatest toxicity. All metabolites did not show *in vitro* antifungal activity against four phytopathogens. Moreover, some lipophilic low molecular weight metabolites were isolated from *N. parvum* and *N. vitifusiforme* culture filtrates. Preliminary spectroscopic investigation showed that two strains produced metabolites belonging to different classes of natural compounds. In particular, some naphthalenone polyketides were isolated from organic extracts of *N. parvum*. The chemical and biological characterization of all metabolites is now in progress.

Secondary metabolites by *Lasiodiplodia theobromae* strains and influence of growth conditions on their production. C. FELIX¹, A. ANDOLFI², S. BASSO², M. M. SALVATORE², A. CORREIA¹, A. ALVES¹ and A. C. ESTEVES¹. ¹Departamento de Biologia and CESAM, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal. ²Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte Sant'Angelo, Via Cintia 4, IT-80126 Napoli, Italy. E-mail: andolfi@unina.it

Climate changes are taking place throughout the world, with major consequences for the agricultural and forestry systems. Abiotic factors, such as temperature,

can influence host/pathogen interactions, that could result in changes in disease impact. These changes are important for fungal pathogens, because they are very sensitive to small alterations in weather and climate. The Mediterranean countries are particularly prone to climate changes and an increase in temperature is predicted. Several species from the family Botryosphaeriaceae have been recognized in many areas as relevant pathogens of grapevine, with *Lasiodiplodia theobromae* commonly found in vineyards. This is a phytopathogenic fungus that is found in a diverse group of hosts. Although the fungus is typically found in tropical and subtropical regions, it has a great capacity of adaptation to different environments, and synthesizes a variety of lipophilic and hydrophilic metabolites that exhibit interesting biological activities. We focus on the physical environmental effects on the biosynthetic activities of two strains of *L. theobromae*, since the production of secondary metabolites by filamentous fungi, although very common, performs functions that are not always known. Frequently, these compounds are related to development of spores, but they can also play important roles in the pathogenicity of several fungi. Recent studies have shown that *Lasiodiplodia* spp. produced phytotoxic metabolites that may be involved in host/pathogen interactions. The aim of this study was to characterize the secondary metabolites produced *in vitro* by two different isolates of *L. theobromae* – CAA019 and CBS339.90 – grown at 25°C and 37°C. By employing mainly GC/MS and ¹H NMR techniques, we have shown that the two strains grown at 25°C produced different lipophilic low molecular weight metabolites. The organic extract of strain CAA019 contained 3-indolcarboxylic acid (ICA) as the main metabolite, while the organic extract of CBS339.90 grown at same temperature contained mainly a number of common dicarboxylic acids, with ICA and jasmonic acid as minor compounds. Chemical and biological characterization of secondary metabolites produced by the above *L. theobromae* strains grown at two different temperatures is now in progress.

Complementary research of molecular and physiological markers of grapevine sensitivity to *Eutypa lata*. C. CARDOT^{1,2}, G. FERRARI¹ and P. COUTOS THEVENOT².¹ BNIC - Station Viticole, 69 rue de Bellefonds - BP 90018 - 16101 Cognac Cedex – France. ²Université de Poitiers, UMR CNRS 7267 EBI Ecologie et Biologie des Interactions, Equipe “SEVE Sucres & Echanges Végétaux-Environnement”, Batiment Botanique B31, 3 rue Jacques Fort TSA 51106 86073 POITIERS CEDEX 9, France. E-mail: chloe.cardot@univ-poitiers.fr

Grapevine wood decay diseases are a threat to the sustainability of wine industries. Esca, BDA and Eutypiosis are the most common of these diseases. These are caused by vascular fungi, are characterized by wood

necroses and death of host plants, and result in significant economic losses for the most grapevine sensitive varieties. There is no technically effective and non-destructive diagnostic for use in vineyards, and no available effective chemical treatments. This research focuses on the molecular markers in different grapevine varieties for sensitivity to *Eutypa lata*. This fungus is a vascular ascomycete responsible for Eutypiosis. Healthy plants or those experimentally infected with *E. lata* mycelium are produced in greenhouses. The study focuses on five varieties; two hybrids (RV4 and RV5 (muscadine and *Vitis vinifera* origins)) of unknown sensitivity to the pathogen and three varieties with known levels of sensitivity, including Merlot (tolerant), Ugni Blanc (sensitive) and Cabernet Sauvignon (moderately sensitive). Wood and leaves of six inoculated plants and six control plants are sampled at 3, 15, 30 and 120 d post infection. Total RNAs are extracted. Following a retro-transcription, six plants are combined into three-pools to be analysed by real time qPCR. This analysis is used to study the expression of five genes in leaves and seven genes in the wood. These genes have been selected during a previous study (CASDAR V908). The aim is to validate some of these genes as molecular markers of the variety sensitivity. This study is based on 3 years of infection with three campaigns. After the first year, three genes appear to be good potential markers of sensitivity: VvHT5, which encodes a hexose transporter, one that encodes a nitrate transporter and one that code for a cell wall invertase: Cwlnv1. After validation, these genes could be used by breeders to accelerate the selection of less sensitive new varieties or selecting clones. Subsequently, a functional study will be made on plants to correlate gene expression with the physiological mechanisms of the tolerance against *E. lata*.

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Development of a simplified model to characterize pathogenicity and evaluate foliar symptom expression for *Botryosphaeria dieback*. P. REIS¹, T. NASCIMENTO¹, C. REGO¹ and F. FONTAINE². ¹Departamento de Ciências e Engenharia de Biosistemas, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. ²Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire Stress Défenses et Reproduction des Plantes, Moulin de la Housse, BP 1039, 51687 Reims Cedex 2. E-mail: pedroreis@isa.ulisboa.pt

Botryosphaeria dieback is a worldwide grapevine trunk disease, associated with several Botryosphaeriaceae species. Among the most common species isolated from grapevine growing regions around the world are *Diplodia seriata* and *Neofusicoccum parvum*. These are

xylem-inhabiting fungi that cause leaf and berry symptoms, and eventually the death of infected plants. These symptoms include leaf spots, fruit rots, shoot dieback, bud necrosis, vascular discoloration of the wood and perennial cankers. Research has been developed to gain a better understanding of the mechanisms that are involved in symptom expression by artificial symptom reproduction. The aim of our work was to develop a simplified model with *D. seriata* and *N. parvum* in order to better characterize their pathogenicity and evaluate the percentage of vines developing foliar symptoms. During a 4 year period, green stems of one month's growth of grafted grapevine cuttings of cv. Aragonez (= Tempranillo) were infected with *N. parvum* (Np 19; Np 67) and *D. seriata* (Ds 98-1, Ds 99-7 and Ds AR) from Portugal and Spain, and with different degrees of virulence. *Phyllosticta ampellicida* (Gb 32, Gb 17), *Cladosporium* sp. and *Penicillium* sp. isolates were selected for positive controls. After incubation, necrosis width, length and surface area on green stems were evaluated. In 2011, 2012, 2013 and 2014, mean lesion widths associated with infections by isolates Ds 98-1, Ds 99-7 and Ds AR were slightly less than those associated with Np 19 and Np 67. Mean lesion lengths associated with Np 67 and Ds AR were greater than those of Ds 98-1, Ds 99-7 and Np 19. Mean lesion surface areas associated with Np 67 and Ds AR were greater resulting from Ds 98-1, Ds 99-7 and Np 19. Seven to 8 months after the infection, the percentage of infected plants showing foliar symptoms was greater for Np 67 and Np 19 than Ds 98-1, Ds 99-7 and Ds AR, while none of the control plants showed any symptoms. Results obtained for positive control assays revealed that both Gb 17 and Gb 32 isolates produced necrosis on green stems but no foliar symptoms were observed. In opposite, *Cladosporium* sp. and *Penicillium* sp. caused necroses similar to those recorded for the negative controls, and again no foliar symptoms were observed. The developed simplified model allows accurate characterization of isolate pathogenicity, and connection with foliar symptoms of Botryosphaeria dieback.

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Session 4: Disease management

Evaluation of grapevine propagation protocols against trunk diseases, through a European nursery survey. D. GRAMAJE¹, and S. DI MARCO². ¹Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. de Burgos, Km. 6, Finca La Grajera, 26007 Logroño, Spain. ²CNR, IBIMET, Via Gobetti 101, 40129 Bologna, Italy. E-mail: david.gramaje@icvv.es

A survey covering all aspects of grapevine propagation, including cultural and sanitation practices in mother blocks, nursery operations and practices in nursery fields, was mailed to all Management Committee members of the European COST Action FA1303 "Sustainable Control of Grapevine Trunk Diseases", for distribution through identifiable nurseries in European countries. The main objective was to develop understanding of the current propagation practices and identify those likely to have the greatest impacts on the quality of planting material, especially with regards to the control measures used against fungal trunk pathogen infections. A questionnaire was sent to 546 vine nurseries, and 130 replies were received (response rate, 23.8%). Our study identified several risks of fungal trunk pathogens infection during propagation processes, as well as a clear need for further research into the effects of treatments on grapevine viability. These include hot-water treatments, and the potential for biological agents to control grapevine trunk diseases in nurseries.

REMEDIER® (*Trichoderma asperellum* and *Trichoderma gamsii*): a new opportunity to control the esca disease complex. Five years of results of field trials in Italy. C. ALOI¹, F. REGGIORI¹, G. BIGOT², A. MONTERMINI³, P. BORTOLOTTI³, R. NANNINI³, F. OSTI⁵, L. MUGNAI⁴, S. DI MARCO⁵. ¹ISAGRO S.p.A. via Caldera 21, 20153 Milano, Italy. ²PERLEUVE s.r.l., via Isonzo 25, 34071 Cormons (GO), Italy. ³CONSORZIO FITOSANITARIO PROVINCIALE MODENA-REGGIO EMILIA, via Santi 14, 41123 Modena, Italy, ⁴Department of the Agro-food Production and Environmental Sciences (DISPAA), Section of Plant pathology and Entomology, University of Florence, P.le Cascine, 28, 50144 Firenze (Italy), ⁵IBIMET-CNR, Via Gobetti 101, 40129 Bologna, Italy. E-mail: s.dimarco@ibimet.cnr.it

A microbiological product for pruning wound protection was applied for 5 years in field trials carried out in six viticultural regions in Italy, to evaluate efficacy for control of the "esca disease complex" of grapevine. The product (Remedier®) is an organic fungicide containing two selected strains, of *Trichoderma asperellum* and *T. gamsii*. The trials were performed on susceptible cultivars including Cabernet Sauvignon, Sauvignon and Sangiovese, in young vineyards to test for preventative effects, and in mature vineyards (≥ 10 years old) with esca complex foliar symptoms to evaluate effectiveness of the treatments for reducing the increase in the numbers of symptomatic vines. The effective and rapid pruning wound colonization by the *Trichoderma* isolates allowed preventative activity by protecting pruning wounds from infection from artificial *Phaeoconiella chlamydospora* inoculations. In field applications with a commercial sprayer, reductions of symptomatic vines and foliar symptom severity were

recorded, from the second year after the first application. This activity was mainly related with reduction in the spread of new infections; moreover, after the fourth year, a decrease in the number of dead plants was noticed, also on particularly susceptible varieties. Remedier®, applied especially after spring pruning at the sap bleeding stage, proved to be effective and of practical use. The best condition for *Trichoderma* colonization was achieved with applications at the sap bleeding stage. The product was fully compatible with all the main strategies for disease control in vineyards, and is the only microbiological agrochemical authorized in Italy to prevent one of the most serious diseases of grapevine.

Phytoprotectors of grapevine: exploiting their potential to protect grapevine. C. PINTO^{1,2}, V. CUSTÓDIO¹, A. SPAGNOLO², C. RABENOELINA¹, C. CLÉMENT², A. GOMES¹ and F. FONTAINE². ¹*Biocant, Centro de Inovação em Biotecnologia, Biocant-Park, Parque Tecnológico de Cantanhede, Núcleo 04, lote8, 3060-197 Cantanhede, Portugal.* ²*Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire Stress, Défenses et Reproduction des Plantes, BP 1039, 51687 Reims Cedex 2, France. E-mail: catia.pinto@biocant.pt*

Vitis vinifera hosts a complex microbiome composed by neutral, beneficial or pathogenic microorganisms that are in close associations with this host. This microbiome or ‘second plant genome’ is essential for the plant health status, to promote plant growth, stress resistance or nutrient mobilization. Furthermore, these organisms play important roles in plant productivity and product quality. Unveiling the phytoprotector microorganisms from grapevine represents a challenge, to develop new sustainable strategies for control of grapevine diseases. The aims of this research were to isolate, identify and characterize potential phytoprotectors naturally present in vineyards and to characterize their interactions with grapevine. A total of 254 isolates (bacteria and yeasts) from Bairrada Appellation (Portugal) were isolated during 2010 and 2011 vine campaigns, and tested for their antagonistic potential against different trunk disease phytopathogens, including strains responsible for the *Botryosphaeria dieback*. Three promising phytoprotectors were selected and a significant decrease ($P < 0.05$) of the normal pathogen growth was observed *in vitro*. Two of the potential phytoprotectors produced siderophores and solubilized phosphate. Their ability to colonize and to live within grapevine was also analyzed using *in vitro* plants. Two of the phytoprotectors were endophytic. In order to better understand the interactions between phytoprotectors and grapevine, a gene expression analysis of host defense responses is undergoing. These have identified potential grapevine

phytoprotectors. As these strains are naturally present at vineyards, they are likely to be well adapted and stable to the vineyard environment, which constitutes an advantage for future grapevine management.

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New approaches for the reduction of foliar symptom expression in the esca complex of grapevine: nutrients and defense induction. S. DI MARCO¹ and L. MUGNAI². ¹*Institute of Biometeorology (IBIMET), National Research Council (CNR), Via P. Gobetti, 101, 40129 Bologna, Italy.* ²*Department of the Agro-food Production and Environmental Sciences (DÍSPAA), Section of Plant pathology and Entomology, University of Florence, Piazzale delle Cascine 28, 50144 Florence, Italy. E-mail: s.dimarco@ibimet.cnr.it*

The esca complex is the most important, widespread and destructive trunk disease in the grape growing areas of Europe, and is important to viticulture worldwide. The appearance of the typical foliar symptoms was, in the last decade, described as the Grapevine Leaf Stripe Disease (GLSD). Several studies agreed on the involvement of toxins produced by tracheomycotic fungi such as *Phaeoconiella chlamydospora* and *Phaeacremonium* spp. in determining the appearance of the typical leaf necrosis symptoms. Nevertheless, the occurrence and development of foliar symptoms is a complex process associated with different factors resulting in a decrease in chlorophyll, activation of defense responses and changes in metabolic patterns in leaves. No correlation was found between the severity of wood deterioration and the severity of leaf symptoms; leaf symptoms were found on vines that had only wood discoloration without any decay. Other studies stated that white rot in the cordons was the best predictor for the so called “chronic form” of esca. However, foliar symptom expression is strictly correlated with grape yield and quality reductions. Strategies aimed at reducing the incidence and/or severity of foliar symptoms would also limit losses in quality. The use of fungicides failed to provide satisfactory results, except for fosetyl-Al formulations (applied against downy mildew), which reduced the incidence of symptomatic plants and cumulative vine mortality. Recent studies suggest that host physiology and defense mechanisms, which in turn are correlated with climate, nutrition and agronomic conditions, play important roles in symptom development. Three-year

trials have demonstrated that foliar applications of a mixture of calcium chloride, magnesium nitrate and Fucal seaweed extract led to a significant reduction of foliar symptoms. Treated vines showed increased content of trans-resveratrol and flavonoids, and accumulation of calcium oxalate in crystal druses in the leaf mesophyll tissues. Ongoing trials with products based on copper, zinc and formulated with effective and/or innovative substances, such as hydracid of citric acid or hydroxyapatite crystals, also reduced foliar symptom expression and open new perspectives and opportunities for disease control. The potential of an approach that stimulates the host reaction in reducing interveinal leaf yellowing and necrosis is discussed.

Synthesis and systemic activity of profungicides for controlling vascular diseases. H. WU¹, S. MARHADOUR¹, J.-L. BONNEMAIN² and J.-F. CHOLLET¹. ¹IC2MP (Institut de Chimie des Milieux et des Matériaux de Poitiers), UMR CNRS 7285, Université de Poitiers, 4 rue Michel Brunet, TSA 51106, F-86073 Poitiers cedex 9, France. ²Laboratoire EBI (Écologie et Biologie des Interactions), UMR CNRS 7267, Équipe SEVE (Sucres, Échanges Végétaux, Environnement), Université de Poitiers, 3 rue Jacques Fort, TSA 51106, F-86073 Poitiers cedex 9, France. E-mail : jfcholle@univ-poitiers.fr

With the banning of sodium arsenite, it is necessary to find alternative fungicides with good activity against grapevine trunk disease pathogens. The uptake and distribution of fungicides in plants influence their activity on pathogens localized in vascular tissues. This study aimed to develop foliar-applied fungicides, which can be translocated to vascular tissues. We proposed two strategies to develop systemic profungicides, which involve modification of the non-systemic fungicide fenpiclonil by adding: 1) a group with a carboxylic acid function or 2) an amino acid or a sugar. The first strategy was based on the ion-trap mechanism. Three acidic derivatives and two ester derivatives of fenpiclonil were synthesized. The stability test in water and a systemicity test on castor bean (*Ricinus communis* L.) seedlings were carried out. The esters SM 26 and HW 34 were metabolized to the corresponding carboxylic acid compounds which move in plant sieve tube elements. The aim of the second strategy was to develop active transport of fungicide via nutrient carriers. The resulting molecules were amino acid and glucose conjugates. F 30-Lysine conjugate was detected in phloem sap of *Ricinus* seedlings. Our study suggested that modifying the fungicide fenpiclonil based on ion-trap and the active transport mechanism is a feasible approach to develop systemic fungicides.

Grapevine trunk diseases: establishing a simplified model study for the evaluation of new control strat-

egies. A. SPAGNOLO¹, H. WU², J.-F. CHOLLET², C. CLÉMENT¹ and F. FONTAINE¹. ¹Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire Stress, Défenses et Reproduction des Plantes, BP 1039, 51687 Reims Cedex 2, France. ²IC2MP (Institut de Chimie des Milieux et des Matériaux de Poitiers), UMR CNRS 7285, Université de Poitiers, 4 rue Michel Brunet, TSA 51106, F-86073 Poitiers cedex 9, France. E-mail: alessandro.spagnolo@univ-reims.fr; florence.fontaine@univ-reims.fr

Considering the increasing economic impact of trunk diseases on viticulture worldwide, the identification of efficient and viable control strategies is urgently needed. However, understanding plant-pathogen interactions and plant physiological changes related to these diseases is essential for such accomplishment. A simplified study model which involved the use of greenhouse-grown 'Chardonnay' and 'Sauvignon' cuttings was set-up to: i) study the effect of artificial infection with the *Botryosphaeria* dieback agents *Neofusicoccum parvum* and *Diplodia seriata* on the plant physiology, and ii) evaluate the efficacy and feasibility of a control strategy involving dual of a potential biological control agent and a systemic profungicide. The plant growth-promoting rhizobacterium *Burkholderia phytofirmans* (strain PsjN: *gfp2x*) was tested as a biological control agent and applied to the soil 4 (T-28) and 3 weeks (T-21) before fungal inoculation. The ester derivative of the fungicide fenpiclonil "SM 26" was then applied as a foliar profungicide 2 d (T0-2) before fungal inoculation. The plants were each inoculated (at T0) on green stems under one of the SM 26-treated leaves, by applying a mycelial plug into a wound made by scratching the surface with a cork borer. One apical leaf was sampled 48h after the last profungicide application in order to study fungicide systemicity and metabolization in the plant, and leaf sampling for gene expression analysis was performed 3 (T0+3) and 7 d (T0+7) after inoculation. The possible effect of bacterial application, profungicide treatment or artificial fungal infection on photosynthetic activity was estimated by measuring the PSII activity once a week beginning from T0+4. Lesions associated to artificial infections were measured at T0+72. First results indicated that: i) no effect on photosynthetic activity was detected for any of the treatments and ii) no statistically significant effects of the PsjN or SM 26 treatments were recorded in relation to the lesion sizes associated to artificial infections. However, a synergistic effect of the two treatments was detected, especially for cuttings inoculated with *N. parvum*. This may have been due to the direct antifungal activity and/or to the eliciting effect of one or both treatments when applied together. Confirmation or disproof to this indication is expected from further ongoing experiments.

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Impacts of vine training systems on the occurrence of grapevine trunk diseases. Z. BIHARI, GY. ZSIGRAI, S. ELÉS, P. BALLING and R. FISCHINGER. *Research Institute for Viticulture and Oenology, Tokaj, Könyves K. u. 54. 3915 Tarcal, Hungary. E-mail: biharitokaj@gmail.com*

Grapevine trunk diseases (GTDs) are increasingly significant throughout the world, and these can lead to the death of vines (*Vitis vinifera*). GTDs have significant effects on the profitability of the grape production in the Tokaj Wine Region of Hungary. The vines, which were destroyed and not replaced, cause losses of 3-4 million Euros per year. The vine training systems in vineyards can have very important effects on the frequency of the symptoms of the diseases, as highlighted by several research projects. Two high-trained and two middle-trained systems were studied to identify any differences in the frequency of GTD symptoms. The experimental vineyard was planted in 1984, made up of four training systems with 126 vines each rows. type 104T was used as rootstock used for all the vines. Tillage, spraying and canopy management were the same over the past 20 years. No vines had been replaced since the vineyard was planted. The four training systems studied were Moser's high culture, single curtain, double curtain (GDC) and umbrella. No significant differences in frequency of symptoms were detected for the four systems. Different training systems impact on the severity of GTD as large pruning wounds result in more infections, as shown by previous research. The four training systems examined are very similar from the pruning wound point of view. Our research has confirmed that the height of training systems alone does not cause differences in the occurrence of GTD.

Timely trunk renewal to manage grapevine trunk diseases. R.E. SMART. *Smart Viticulture, 31 North Corner, Newlyn, Cornwall, TR185JG, United Kingdom. E-Mail: vinedoctor@smartvit.com.au*

"In Nature, grapevines are multi-trunked and unpruned." The progenitors of Vitaceae have existed on earth since the early Jurassic period, some 180 M years ago; the forest climbing American and Eurasian forms of the early *Vitis* spp. were present in the late Eocene, some 40 M years ago. Until their cultivation by man, 5-10,000 years ago, these plants survived "happily" as multi-trunked and un-pruned perennials. Dormant (and summer) pruning are man's invention, as is single-trunking. Both are associated with the modern problem of Grapevine Trunk Diseases (GTDs) which occur world-wide. Farmers, viticulturists and scientists have long been aware of trunk renewal to overcome defective trunks, and this is commonly used in Eastern Europe, and Eastern and Western North America, to replace trunks injured by freezing winters and/or *Agrobacterium*. It has also

been traditionally used to overcome trunk disease in Europe, and has been researched for Eutypa control in Australia. Despite this awareness, trunk and/or cordon replacement has not received the widespread attention it might, as a possible "cure" and management option for GTDs. This contribution will offer a systematic approach to GTD control, which aims to minimise crop loss, cost and management input. It is based on early intervention, and classifying "risk" and "disease incidence" on a vineyard block by block basis as a guide for appropriate intervention. It is possible to train new trunks and cordons before surgery, and so avoid any crop losses. A draft proposal to this paper received helpful comments from many GTD researchers.

Bridging the gaps between scientific literature and grower perceptions of trunk disease management. K. BAUMGARTNER¹, R. TRAVADON¹, M. COOPER², A.V. HILLIS³, M. LUBELL³ and J. KAPLAN⁴. ¹United States Department of Agriculture-Agricultural Research Service, Davis, California, USA. ²University of California, Cooperative Extension, Napa, California, USA. ³Department of Environmental Science and Policy, University of California, Davis USA. ⁴Department of Economics, California State University, Sacramento, USA. E-mail: kendra.baumgartner@ars.usda.gov

Trunk diseases significantly limit the productivity of California vineyards. Field trials have shown three practices to minimize infection of pruning wounds: delayed pruning, double pruning, and the fungicide thiophanate-methyl (Topsin M). For optimal efficacy, such preventative practices must be adopted in young vineyards with little to no disease incidence, but it is difficult to convince growers to manage trunk diseases in vineyards that appear healthy. More often, trunk disease management is not considered until the vineyard is mature and disease incidence is high. At this point, the most effective options are remedial vine surgery and replanting the vineyard. Such post-infection practices are labour-intensive and costly. Our goal is to develop an outreach programme that minimizes the need to resort to the most costly practices. To understand the usage and perceptions of these practices, we surveyed pest control advisors (PCAs) and growers. Our online survey of PCAs revealed their awareness of trunk diseases as a widespread problem in California vineyards. Respondents perceived post-infection practices as more costly. Preventative practices, in spite of being perceived as relatively cost effective, nonetheless were not recommended more often by the PCAs. High disease incidence was correlated with greater frequency of recommendation for all practices; there was no preference for preventative practices in cases of low disease incidence (i.e., in young, healthy vineyards). Instead, advisors recommended more frequent-

ly the practices (both preventative and post-infection) perceived as more effective. Our interactive survey of growers, which focused on preventative practices, revealed delayed pruning as the most common practice, followed by Topsin applications to pruning wounds. Growers ranked all preventative practices as “effective”, but practiced usage corresponded to grower perceptions of cost effectiveness; the most costly practice, double pruning, was used the least. Just as with PCAs, our survey of growers revealed no strong preference for timing preventative practices with low levels of disease incidence. The majority of growers said they adopted preventative practices in mature vineyards, after trunk diseases became apparent. Our findings underscore the importance of an outreach programme that effectively communicates the utility of different practices for different levels of disease incidence. To provide convincing evidence in support of this approach, we initiated the following projects: spore trapping experiments to demonstrate the risk of infection for young vineyards, economic analyses to evaluate the long-term benefits of adopting preventative practices, and field trials in young vineyards to demonstrate the efficacy of preventative practices.

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Identification of biocontrol agents for Esca, a grapevine trunk disease. J. FISCHER¹, A. BERNAL-MARTÍNEZ¹ and E. THINES^{1,2}. ¹Institut für Biotechnologie und Wirkstoff-Forschung gGmbH, Erwin-Schrödinger-Straße 56, D-67663 Kaiserslautern. ²Institut für Biotechnologie und Wirkstoff-Forschung, Deuserbergweg 10-14, Johannes-Gutenberg Universität Mainz, D-55128 Mainz. E-mail: thines@ibwf.de

Effective treatment of Esca-affected plants is impossible, once the pathogens have entered the host plants and spread into woody plant tissues. Therefore the only durable alternative is to protect grapevine cutting wounds after pruning. In the last decade researchers have described various methods for wound protection, and two promising approaches have been defined. The first is to seal the crafting wounds with physical barriers, for example wax layers supplemented with fungicides. The second is to inoculate the wounds with spores of antagonistic fungi or cells of bacteria. A search for potential wound-protecting biocontrol agents was outlined by Pellegrini *et al.* (2012). Since most of the previously tested organisms failed to protect pruning wounds as effectively as chemical formulations, we

tried to optimize the first step of the selection process for new antagonistic fungal strains by developing more reliable test systems. These were based on the interactions of all three organisms involved: grapevine plants, pathogenic and antagonistic fungi. We established several test systems useful to interrogate the interactions of two fungal species involved as well as the interdependence of these fungi with the plant host. We used these test systems, based on *in vitro* tests in 96-well plates or on agar plates, to conduct experiments at a rapid laboratory scale. Eleven fungi were identified as suitable to protect pruning wounds from the infection by Esca-associated fungi. We also analyzed the secondary metabolites and enzymes produced by the antagonistic fungi with biological activity. These organisms will be further tested in greenhouse experiments and field trials to determine their ability as plant protection agents in the future. In addition these trials will assist determination of the period for which the fungi are able to protect plants after the incubation of the cuts with inoculated with beneficial fungi.

References: Pellegrini A., V. Leoni and I. Pertot, 2012. Survival of *Trichoderma atroviride* SC1 on grapevine pruning wounds and efficacy against esca disease agents. *IOBC/WPRS bulletin*, 78, 315–318.

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Effects of hot water treatment and fungicides on *Phaeoconiella chlamydospora* and *Phaeoacremonium aleophilum* in grapevine propagation material. M. RIEDLE-BAUER and M. MADERČIĆ. *Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau Klosterneuburg, Wienerstraße 74, Klosterneuburg, Austria.* E-mail: Monika.Riedle-Bauer@weinobst.at

In a first step, four fungicides registered in viticulture in Austria (penconazole, spiroxamine, 8-chinolin sulfate, sulfur) were tested for their effects on mycelial growth of *Phaeoconiella chlamydospora* (Pch) and *Phaeoacremonium aleophilum* (Pal) in Petri dishes. Penconazole, spiroxamine and 8-chinolin sulfate completely inhibited the growth of Pch, while sulfur had a reducing effect. Growth of Pal was completely suppressed by spiroxamine and 8-chinolin sulfate, whereas penconazole had only a slight effect and sulfur was ineffective. Healthy cuttings (cv. “Grüner Veltliner”) were then vacuum infiltrated with conidial suspensions (5×10^2 conidia mL⁻¹ and 10^5 conidia mL⁻¹) of the two fungi. The cuttings were then given three different treatments including: hot water treatment (HWT; 50°C, 45 min); HWT followed by soaking/infiltration with the fungicides penconazole,

spiroxamine or 8-chinolin sulfate; or only soaking/infiltration with penconazole, spiroxamine or 8-chinolin sulfate. One part of each cutting was analyzed directly after the treatments. Two cross sections were taken from different parts of the cutting, disinfected and incubated on malt agar for 2–3 weeks. Mycelial growth was assessed using a stereo microscope, and emerging colonies were identified. For Pch no infections were detected after HWT or HWT in combination with fungicides. Soaking of canes in penconazole or spiroxamine for 24 h had some effect but infections were not fully eliminated. Pal infections were significantly reduced by HWT, but in few cases infections were present even after HWT. Infiltration of spiroxamine and 8-chinolin sulfate reduced infections of canes inoculated with 5×10^2 conidia mL⁻¹ but not of canes inoculated with 10^5 conidia mL⁻¹. part of each cutting has been rooted in order to study the effects of HWT and fungicide treatments on growth and vigour during the coming vegetation period.

Towards a cultural approach to reduce the prevalence of esca of grapevine. P. LECOMTE¹, B. DIARRA¹, C. CHEVRIER² and A. CARBONNEAU³. ¹INRA, UMR SAVE, 71 av. Edouard Bourleaux, CS 20032, 33882 Villenave d'Ornon cedex, France. ²Chambre Régionale d'Agriculture du Languedoc-Roussillon - CS 30012 - 34875 LATTES, France. ³UMR AGAP, 2 Place Viala, F-34060 Montpellier, France. E-mail: lecomte@bordeaux.inra.fr

Esca is currently the most concerning grapevine disease in Europe. This trunk disease affects the longevity of vineyards and is characterized by the development of large inner necroses in the grapevine wood before partial or complete dieback of affected vines. In France, the overall average impact of trunk diseases is now estimated at about 13% of vines impaired in their production potential. This impact was only about 5% in 2003. Esca disease largely contributed to this overall impact. Different biotic and abiotic factors may explain this progression. Examination of plot by plot data has shown very high variability of damage between plots of the same cultivars, with the same age in similar environments. In some cases, the range of variability of damage strongly suggested the influence of cultural factors. For example, Cabernet Sauvignon in the Bordeaux area in 2007, with a vineyard planted in 1982 showing overall impact of 3% of esca-affected vines while another vineyard planted in 1990 exhibited an impact of 80%). Among them, two factors were identified as putative key influences: the training system and the quality of pruning. A survey was undertaken from 2007 by UMR SAVE Bordeaux to attempt to identify comparable plot situations forming pairs with the same variety, the same age and similar soil and climatic environments but varying according training decisions. The study

was based on observations in Aquitaine in late summer. Ratings were applied for all esca symptoms, on leaves and on the trunks. Several selected examples showed that training systems leading to long arms were generally less affected than the forms with very short arms or without arms. The quality of the pruning system may also play a major role. Higher vine densities/ha, decided by grower choice or by the rules imposed by the appellations (and not accompanied by a minimum distance between vines), led growers to modify their training system over time, towards simplification of the vine trunk structure that was too great. The study also indicated that the foliar symptoms of esca are relevant clues to reveal the presence of the disease but are not always reliable indicators of impact. Real prospects in terms of reduced damage seem possible only by effective training decisions.

This research is presently integrated in a national project entitled "Assessment of the impact of cultural practices or environmental factors for the control of grapevine trunk diseases". This project is financed via grants from CasDAR (Account of Specific Affection for Agricultural and Rural Development) and CNIV (National Committee of the Interprofessions of Wines with Label of origin and with Geographical Indication).

WINETWORK: a network for the exchange and transfer of innovative knowledge between European wine-growing regions to increase the productivity and sustainability of the sector. F. FONTAINE¹ and E. SERRANO². ¹Université de Reims Champagne-Ardenne, Unité de Recherche Vigne et Vins de Champagne, Laboratoire Stress, Défenses et Reproduction des Plantes, Bât. 18, BP 13039, 51687 Reims cedex 2, France. ²Institut Français de la Vigne et du Vin, V'Innopôle Sud-Ouest, 81310 Lisle sur Tarn, France. E-mail: florence.fontaine@univ-reims.fr

Building on a methodology for innovation-driven research previously developed and tested, the WINETWORK project has the ambition to stimulate collaborative innovation in the wine sector. The project will implement a methodology that has been successful in promoting demand-driven innovations in previous regional and European projects. This approach is mainly based on the interactions between a network of facilitator agents, several regional technical working groups and one European scientific working group. A participatory approach is used to translate research results and practical knowledge into technical datasheets that are used to prepare materials adapted to end-users. A bottom-up approach is also used to identify a demand-driven innovation agenda. In the WINETWORK project, the approach will be implemented in ten regions from seven countries representing more than 90% of EU wine production. The main topic addressed in the network concerns the control and the fight against diseases

that jeopardise the future production potential of the EU (Grapevine Trunk Diseases and Flavescence Dorée). As these diseases represent threats to the economic viability of the entire sector, these topics have been previously identified as a priority by winegrowers, scientists and decision-makers. As many winegrowers are testing innovative and sustainable approaches to fight these diseases, it is very beneficial to capture these ideas and to share them between EU countries. Innovative practices will be synthesized, tailored and translated to become fully accessible to innovation support services and to

winegrowers. The project will then deliver a large reservoir of existing scientific and practical knowledge related to sustainable vineyard management. It will also provide a methodology that will support all agriculture sectors to enhance innovation driven research. WINETWORK involves 11 partners of excellence representing the entire innovation chain, from science to farmers.

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