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 ampelicida* (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. GRAMAJE[1], and S. DI MARCO[2]. [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]*CNR, 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. ALOI[1], F. REGGIORI[1], G. BIGOT[2], A. MONTERMINI[3], P. BORTOLOTTI[3], R. NANNINI[3], F. OSTI[5], L. MUGNAI[4], S. DI MARCO[5]. [1]*ISAGRO S.p.A. via Caldera 21, 20153 Milano, Italy.* [2]*PERLEUVE s.r.l., via Isonzo 25, 34071 Cormons (GO), Italy.* [3]*CONSORZIO FITOSANITARIO PROVINCIALE MODENA-REGGIO EMILIA, via Santi 14, 41123 Modena, Italy,* [4]*Department 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),* [5]*IBIMET-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 *Phaeomoniella 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. CUSTÓDIO[1], A. SPAGNOLO[2], C. RABENOELINA[1], C. CLÉMENT[2], A. GOMES[1] and F. FONTAINE[2]. [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 MARCO[1] and L. MUGNAI[2]. [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 (DISPAA), 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 *Phaeomoniella 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