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Abstracts

Abstracts of oral and poster presentations given at the 8th International Workshop on Grapevine Trunk Diseases, Valencia, Spain, 18–21 June 2012

The 8th International Workshop on Grapevine Trunk Diseases was held in Valencia, Spain, on June 18–21 2012. The meeting was attended by 120 participants and 103 papers were presented either as oral or poster presentations in four sessions: Pathogen Detection and Characterization, Epidemiology, Host-Pathogen Interaction and Disease Management. A special session was dedicated on implications of trunk diseases for grapevine nurseries with five invited presentations, followed by several oral and poster presentations. A field trip to the Utiel-Requena wine-producing area was undertaken on June the 20th, including visits to vineyards and a winery.

The workshop is the 8th organised by members of the International Council on Grapevine Trunk Diseases (www.icgtd.org), a subject matter committee of the International Society for Plant Pathology (www.isppweb.org).

Special session - Invited lectures
IMPORTANCE AND IMPACT OF FUNGAL TRUNK PATHOGENS IN GRAPEVINE NURSERIES

Assessment of quality of plants in French grapevine nurseries with regard to fungi involved in wood diseases. P. LARIGNON. *Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France. E-mail: philippe.larignon@vignevin.com*

An investigation was conducted in three French regions (Burgundy, Provence-Alpes-Côte d'Azur, Midi-Pyrénées) between 2000 and 2010 to assess the quality of nursery plants with regard to fungi involved in grapevine wood diseases. Quality plants were defined as plants without these fungi. In France, the major wood disease pathogens are: *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, associated with esca disease and Petri disease and *Neofusicoccum parvum*, *Diplodia seriata*, and other species of *Botryosphaeriaceae* (less frequently encountered French vineyards), involved in Botryosphaeria dieback and various species of *Phomopsis* involved in different diseases (Phomopsis decline, Phomopsis leaf and cane spots). The causal agents of Black foot disease were not included in this study because the disease is of low importance in France and *Cadophora* species (involved in Petri disease) were not included because their pathogenicity was demonstrated only recently (Gramaje D. *et al*., 2011. *Phytopathol. Mediterr*. 50,112–126). The microbiological analyses were done according the method of Larignon and Dubos (Larignon P. and Dubos B. 1997. *Eur. J. Plant Pathol*. 103, 147–157). Isolations were made from woody tissues collected from the graft, graft union and from four levels in the rootstock. In this investigation the surfaces were not examined, but if this criterion was considered, the plant quality would be lesser. A study by Larignon *et al.* (Larignon P. *et al*., 2009. *Phytoma*. 622-623, 46–48) showed that these fungi can be detected on bark by using molecular tools.

In Burgundy, 25 samples of 50 plants were collected from 25 nurseries in 2009 and 2010. Infection rates ranged between 0% and 94%. Twelve samples were free from pioneer esca-associated fungi. Two samples were highly infected with these fungi: 61% of plants showed the presence of *P. chlamydospora* and 22% that of *P. aleophilum* for one sample and 34% of plants with *P. chlamydospora* and 4% with *P. aleophilum* for the other sample. Botryosphaeriaceae spp. were isolated from all the samples, but the percentage of plants with these fungi varied between 4% and 82%. Eleven samples were free from *D. seriata* and three were without *N. parvum*. The percentage of plants with *Phomopsis* spp. was also variable, from 0% to 90%.

In Provence-Alpes-Côte d'Azur, 12 samples of 50 plants were collected in 2005 and 2006 from different nurseries (Larignon P. *et al*., 2008. Champignons associés aux maladies du bois: une enquête en pépinières**.** *Rhône en V. O.* 3, 26–31). The value of the plant quality varied between 0 and 40%. Only one sample was free from pioneer esca-associated fungi. Two samples were highly infected

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with *P. chlamydospora* (34% each) and *P. aleophilum* (4% each). For Botryosphaeriaceae, all the samples showed the presence of *D. seriata* and *N. parvum*. The percentage of plants with *D. seriata* varied between 2% and 68.5% and between 1.8% and 63.5% for *N. parvum*. *Phomopsis* spp., were isolated from all the samples. Infection rates varied between 1.8% and 88%.

In Midi-Pyrénées, ten samples of 100, 200 or 300 plants were collected from eight nurseries in 2005 (Viguès V. *et al*., 2007. *Phytoma*. 609, 20–23). The value of the plant quality varied between 30% and 75%. No sample was free from one or both of the two pioneer esca-associated fungi, or from Botryosphaeriaceae or *Phomopsis* spp. One was without *P. chlamydospora* and another without *P. aleophilum*. In the most heavily infected samples, *P. chlamydospora* and *P. aleophilum* occurred in 19% and 6.4%, respectively. The percentage of plants with Botryosphaeriaceae varied between 6% and 77.3%, and the percentage with *Phomopsis* spp. varied between 0.3% and 45%.

Globally, the plant quality was very variable between the samples examined. Several hypotheses could explain these differences: the quality of rootstock and scion cuttings, variations in nursery practices and processes (hydration, cold storage, grafting, callusing etc.) and whether or not the vines were grown in pots with potting mix or in field nursery soils. Several studies of scion and rootstock mother vines in French nurseries have isolated the fungi involved in grapevine wood diseases from this propagation material (Larignon P. *et al*., 2006. *Phytoma*. 592, 14–17). Between 1996 and 2002, 3840 scion cuttings (Cabernet-Sauvignon) were analyzed. *P. chlamydospora* was isolated from 0.44% of scions, *P. aleophilum* from 0.89%, and *D. seriata* from 2.3%. No data were recorded for *N. parvum* and *Phomopsis* spp. Between 2000 and 2004, microbiological analyses made on 1150 rootstock cuttings of different varieties collected from 12 nurseries showed that *P. chlamydospora* was isolated from 1.04% of rootstock cuttings, *P. aleophilum* from 0.17%, *D. seriata* from 2.17%, *N. parvum* from 2.87% and *Phomopsis* spp. from 4.78%. Another study (Larignon P. *et al*., 2009. *Phytoma*. 622–623, 46–48) using molecular tools (PCR) showed that these fungi also occur on the surfaces of scion or rootstock cuttings. Surfaces from 2177 cuttings collected from 14 vineyards were sampled. The rate of detection varied between samples between 2% and 46% for *P. chlamydospora*, 0% and 26% for *P. aleophilum*, 1% and 28.1% for Botryosphaeriaceae. All the samples were taken from vineyards for which the sanitary status was not established. A preliminary study presented in this workshop (Viguès *et al*., 2012. *Phytopathol. Mediterr.* 51, 444) showed that there was no correlation between the sanitary status of the rootstock and scion mother fields, characterized by the mortality rate or/and diseased plant rate, and the quality of propagation material. The fungi involved in woody grapevine diseases were as much isolated from propagation material collected from vineyards with a high sanitary status as that with a low sanitary status.

It is also important to note that an effective control treatment applied early in the nursery process can be cancelled out later by another step. For example, hot water treatment (50°C, 45 min) was found to be a very effective control for several fungi (*P. chlamydospora*, *D. seriata*, *Phomopsis* spp.) in scion and rootstock cuttings, but reinfection occurred when the cuttings were planted in the field nursery and at the end of the growing season the quality of the treated plants (14.3%) was similar to that of control (18%). However, hot water treatment of the finished plants gave very good results and a very high quality score (92%).

Hot water treatment (50°C, 45 min) of the finished plants is the only practice that has shown promising results among the chemical, biological and technological control methods that were tested in France (Viguès V. *et al*., 2010. *Phytopathol. Mediterr.* 49, 130–131). However, another study presented in this workshop (Larignon *et al*., 2012. *Phytopathol. Mediterr.* 51, 449) showed that green-grafting technique can provide plants in nursery free of fungi involved in grapevine wood diseases. In conclusion, even if high quality cutting samples are found a process for producing high quality plants has not been demonstrated. Further studies are necessary to achieve this goal.

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Fungal trunk pathogens in Spanish grapevine nurseries: a survey of current nursery management practices in Spain. D. GRAMAJE[1], J. GARCÍA-JIMÉNEZ[2] and J. ARMENGOL[2]. [1]*Department of Crop Protection, Institute of Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain.* [2]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.*

During the last two decades, the incidence of young vine decline caused by fungal trunk pathogens increased dramatically in Spain, affecting as many as 40–60 % of vines in new established vineyards and causing important economic losses. The same situation has been reported from most grape-growing regions in the world. Thus, numerous phytopathological studies focused on the description of disease symptoms, on the isolation and identification of the causal agents, and on the epidemiology and control of these pathogens. These investigations led to the conclusion that planting material used in young vineyards is

already infected, either systemically from infected mother vines or by contamination during the propagation process. Similar studies on the incidence of fungal trunk pathogens in nurseries carried out by Spanish researchers identified many potential sources of inoculum during the propagation process including rootstock mother fields (soil and planting material), post storage hydration tanks, grafting tools, callusing media and open-root field nurseries (soil and planting material).

Since 1996, our research group in collaboration with other research institutions in Spain, has published numerous technical reports and research articles, and has given presentations at conferences for grapevine nurseries. But, unfortunately, this has not effected any improvement in propagation practices in nurseries and thus the level of fungal infections in nursery vines at the end of the propagation process remains high.

For this reason, we decided to undertake a survey of Spanish grapevine nurseries. The main objective was to assess the current knowledge of grapevine trunk diseases among nursery operators and determine current propagation practices and control measures using questionnaires covering all aspects of the propagation cycle. The questionnaire was divided into three main sections: 1) Fungal trunk pathogens, 2) Plant propagation practices and 3) Management strategies.

In general, the majority of respondents had a basic knowledge of young vine decline and other trunk diseases. However, an improved, but incomplete, understanding of microbial ecology (causal agents) and sanitation was evident in most responses. All nurseries used hydration, and some of them usually soaked cuttings in water for more than 4 days. Registered source blocks are inspected for disease status at least once a year in all cases. Most of the nurseries still sell a significant percentage of second quality vines to grape growers. Approximately 50% of the respondents did not use any individual sanitary practices (hot water treatment [HWT] or fungicides during the different stages of the propagation process). The majority of respondents reported that they knew about hot-water treatment, but had never used it. The reliability and efficacy of HWT was questioned by most nursery operators who argued that significant losses are still being attributed to HWT, that it is an ineffective treatment for eliminating trunk disease pathogens, the logistics involved in these treatments are too difficult and the lack of studies in commercial batches are barriers to adoption of HWT. There is also an unmet demand for further training in nursery management practices, fungal trunk pathogen infections and disease control. Nursery operators also requested more information to be transmitted via seminars or technical workshops. This study has clarified and improved our understanding of current propagation practices in Spain and the main purpose now is to incorporate the results into practical propagation procedures or guidelines for nurseries to produce high quality vines.

Grapevine trunk diseases and propagation practices: an Australian perspective. H. WAITE[1], M. WHITELAW-WECKERT[1,2], P. TORLEY[1] and W.J. HARDIE[1]. [1]*National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia.* [2]*Research Plant Pathologist, NSW Department of Primary Industries, National Wine and Grape Industry Centre, Locked Bag 588, Wagga Wagga, NSW 2678, Australia. E-mail: hwaite@csu.edu.au*

Growing demand for Australian wine in the early 1990's, led the Australian Wine Foundation to develop *Strategy 2025*. In this 30-year plan, grape production needed to increase from about 0.65 MT/yr to around 1.65 MT/yr by 2025. To achieve this required an increase in vineyard area from 70,000 ha in 1995 to 115,000 ha by 2025 (1,500 ha/yr). Buoyant economic conditions coupled with tax incentives for planting vineyards resulted in a rapid increase in vineyard area with a total of 165,000 ha by 2005. The *Strategy 2025* 30-year goal was exceeded by 50,000 ha in less than ten years.

The essential role of nurseries in vineyard development was not considered in *Strategy 2025* and nurseries were unprepared for the increased demand. To produce sufficient vines in a short period, nurseries introduced streamlined production methods. In particular, bench grafting largely replaced field grafting. Nurseries also introduced hot water treatment (HWT) of propagating material to control the phytoplasma disease Australian grapevine yellows. However, large scale vine failure occurred (characterised by losses of cuttings and poor establishment of vines) which were attributed to HWT. Early research improved the design and management of equipment used for HWT, including timing HWT to coincide with maximum dormancy of cuttings and vines. However, losses continued, and both the vine nurseries and growers became reluctant to use HWT except when required by quarantine regulations.

Meanwhile, Petri disease organisms and other fungal pathogens were identified as major causes of young vine decline (YVD) (also characterised by poor establishment of vines). The most commonly isolated pathogens from affected vines included Botryosphaeriaceae spp., *Cylindrocarpon destructans*, *Phaeomoniella chlamydospora* and *Phomopsis viticola*.

By the early 2000s it was known that HWT was an effective control for trunk disease pathogens, but HWT was not routinely used to treat cuttings entering the propagation chain because of continuing concerns about vine failure. Nursery practices contributing to cross contamination, physiological stress and ultimately vine failure included poor nursery sanitation, prolonged hydration of cuttings (sometimes exceeding 12 hours), and poor cold storage conditions. Nurseries began to treat water, reduce hydration times and improve cold storage conditions, but consistent standards and operating procedures were not developed and implemented and YVD

continued to occur. In the mid 2000s planting began to decline following a downturn in the wine industry and nurseries began to go out of business.
At this time research and improvements in nursery practices stalled and grapevine trunk diseases continued to be a significant cause of YVD in Australia. A recent survey of Australian nurseries identified inconsistent propagation protocols, an incomplete understanding of microbial ecology and epidemiology and continued reluctance to use HWT as barriers to improving nursery practices.
Reports of YVD continue, even in hot water treated vines. Further research has implicated nursery soils and contaminated water as sources of re-infection following HWT. There were also reports that nurseries sometimes deviate from standard HWT protocols which may have reduced antimicrobial effectiveness. However, the efficacy of the standard HWT regime (50°C for 30 min) may need to be reviewed.
The adoption of standard operating procedures incorporating HWT and good nursery sanitation are the key to YVD prevention in Australia. However, the decline in new vineyard plantings and consequent downturn in sales make it difficult for nurseries to invest in new equipment and infrastructure, or to participate in the development of national standards and SOPs. In addition there are no data quantifying the economic losses caused by failed planting material, or the effects of YVD on yield and grape quality. The impact of YVD on nurseries and the wine industry is not well understood and because nurseries are seen as peripheral to the wine industry, the issues of YVD and vine quality are not often addressed in industry forums.
The problems of trunk disease transmission in planting material and YVD have parallels with the issues of virus transmission in propagating material that were a major problem for nurseries and wine industry more than 30 years ago. By raising awareness of the impact of viruses on the health of grapevines and developing effective methods for their control in propagating material, virologists were able to significantly improve propagation success, vine health and wine quality. There is an opportunity for pathologists to draw on the history of virus control for lessons that can be applied to the current issues associated with trunk diseases in planting material and YVD for the benefit of both the nurseries and the wine industry.

Examples of plant material compromised by fungal pathogens. L. MORTON. *Viticulturist, PO Box 5607, Charlottesville, VA 22905. E-mail: luciemorton@gmail.com*

For every hectare of grapes they intend to plant, growers will receive thousands of plants.(5,000 plants for a 2 m × 1 m spacing). Field grown, dormant bench grafts are the most common form of grafted vine delivered to growers. In my experience, the quality of bench grafts varies greatly. This occurs for many reasons; from weather conditions in the growing fields to nursery practices and growers looking for a "bargain" with discounted lower grade plants.
In California, there are professionals who earn a living helping growers select strong, healthy plant material by inspecting plants at the nursery. Included in this service are destructive examinations of internal plant tissues, pathological tests for grapevine trunk diseases, and virus testing. This adds considerably to the cost of the plant material. However, it is considered a worthwhile expense when it contributes to a uniform stand of healthy plants with improved prospects for a longer lifespan.
As a viticulturist overseeing new vineyard establishments, I make sure that all plant material is examined before it is planted. This is very important both for the protection of the grower and the nursery. Plants from every lot are tested for physical integrity with special attention to the graft union. Vines with deep/hollow disbud wounds, incomplete graft unions, necrotic streaks, and poor rooting are examined internally.
Healthy live grape tissue is creamy-colored with greenish or beige tinges and pith is yellowish-brown. Some light browning can be expected from abiotic factors. Dark brown necrotic tissue should be absent or very discreet. Black coloration is almost always a sign of disease: black measles (esca), black goo (Petri), black dead arm (*Botryosphaeria* sp.), black foot (*Cylindrocarpon* sp.). Therefore vines with obvious black coloration in the graft union, pith, xylem tissue, or root crown should generally be rejected.
Pre-planting field inspection is of great value as it culls out obviously compromised and sick plants. It does not, of course, guarantee pathogen-free plants. Continued research will show whether pathogen-free plants are even possible. Awareness of the presence of trunk disease pathogens in mother vines and cuttings used in propagation is a first step to implementing control measures. Equally important is the awareness that apparently clean and healthy young vines need continued protection from infections after planting.

Current situation of fungal grapevine trunk disease pathogens in South African grapevine nurseries. F. HALLEEN[1,2] and L. MOSTERT[1]. [1]*Department of Plant Pathology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa.* [2]*Plant Protection Division, ARC Infruitec-Nietvoorbji, Private Bag X5026, Stellenbosch, 7599, South Africa. E-mail: halleenf@arc.agric.za*

As in most other major grape growing regions of the world, the prominence of grapevine trunk disease pathogens came to the fore after the rapid expansion of the grapevine industry during the 1990's. In South Africa the number of wineries increased from 245 in 1990 to 355 in 2000 and to 582 in 2011. At the same time there was a dramatic and sudden shift from the pro-

duction of predominantly white cultivars (84% of the total number of hectares under vines in 1990) to red cultivars (54% white and 46% red in 2004) as the international markets opened for South African wines. The demand for plant material placed a tremendous strain on the propagation process resulting in the production of nursery vines of a lower quality, as well as a market for uncertified vines. The number of complaints of poor establishment of new vineyards increased and the blame was placed on infected nursery material. Investigations into the source of this infection identified rootstock mothervines as a primary source for Petri disease fungi *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp., as well as Botryosphaeriaceae and *Phomopsis viticola*. A closer look at the propagation process revealed that infections could occur during all the major propagation processes, i.e. hydration of cuttings before cold storage and grafting, as well as during grafting and callusing. This was confirmed when *Pa. chlamydospora* was detected throughout the entire propagation process by means of molecular techniques. However, the situation in South African nurseries became even more complicated with the identification of black foot disease pathogens *Cylindrocarpon* and *Campylocarpon* in nursery soils. Research then focused on efforts to improve the phytosanitary status of nursery vines. Hot water treatment (HWT; 50°C for 30 min) of grafting material was identified as the most important tool to limit and/or reduce Petri disease pathogen infection in grapevine nurseries. Additional efforts focused on finding suitable treatments for use during all the various nursery processes. After several years of research, an integrated management strategy was recommended to nurseries. These strategies include pruning wound protection and sanitation in mother blocks, incorporation of chemicals and/or biological control agents in hydration and soak water during all stages of preparation and grafting of propagation material, HWT of propagation material prior to grafting, as well as HWT of dormant nursery plants. If the entire grapevine trunk disease complex is taken into consideration, a protocol where graft material is soaked in a benomyl (could be substituted with carbendazim) suspension prior to cold storage with HWT and a Sporekill soak before grafting, followed by a Trichoderma soak after grafting and once more before planting significantly reduced *Pa. chlamydospora* and Botryosphaeriaceae spp. incidences and consistently reduced *Phaeoacremonium* spp. incidences. None of the protocols tested consistently reduced *Pleurostomophora richardsiae* incidences. *Pleurostomophora richardsiae* occurred in 29% of diagnostic samples analysed by the Diagnostic Clinic of ARC Infruitec-Nietvoorbij during the 2010–2011 season (17% in 2009–2010), mostly associated with poor graft unions. The occurrence of *Pleurostomophora richardsiae* in South African nursery vines should therefore be of some concern especially since pathogenicity studies showed that this fungus was just as pathogenic as most *Phaeoacremonium* species. Statistics from the Diagnostic Clinic collected over the last twelve seasons remained more or less the same; Petri and black foot disease pathogens were isolated from the majority (50–80%) of diseased vines. The majority of these vines were also very young, again reflecting on the situation within grapevine nurseries where infections occur. The dynamics within the South African grapevine industry is changing all the time. For example, during the 2006 season 82.5% of new plantings during that particular year were white cultivars following a period of a white wine shortage. During the last couple of seasons the demand for rootstock Ramsey increased dramatically, placing a huge strain on current supply. In conclusion, markets and consumer preferences are always changing and therefore there will always be a demand for plant material. From a phytosanitary point of view the challenge is to convince local nurseries to implement and maintain strategies to eliminate or reduce trunk pathogen infections. A relative new development in the South African context was the discovery of Aster yellows (phytoplasm) in two production areas which resulted in a recommendation to nurseries to change the HWT regime to 50°C for 45 min. At this stage the effect on grapevine trunk disease pathogens is unknown.

Workshop Presentations
PATHOGEN DETECTION AND CHARACTERIZATION

Endophytic microflora of woody tissue of healthy and trunk diseased-grapevines. E. BRUEZ[1,2], J. VALLANCE[1,2], J. GERBORE[1,2], P. LECOMTE[1], L. GUERIN-DUBRANA[1,2] and P. REY[1,2]. *[1]INRA, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), F-33140 Villenave d'Ornon, France. [2]Université de Bordeaux, ISVV, UMR1065 SAVE, Bordeaux Sciences Agro, F-33140 Villenave d'Ornon, France. E-mail: emilie.bruez@bordeaux.inra.fr*

Microbial communities colonizing the woody tissue of grapevines that have or have not expressed foliar symptoms of Esca/Black Dead Arm diseases, were studied by using both microbiology and molecular biology techniques (Single Strand Conformation Polymorphism [SSCP] and DNA sequencing). Our sampling design was made in order to determine the diversity of microbial communities and the evolution of the microflora over a growing season. Fungal and bacterial communities were particularly abundant in the apparently healthy woody tissue in comparison with the necrotic tissue. Within the non-necrotic tissue, a shift in fungal communities was observed when spring, summer, autumn and winter-samples were compared. Only fungal communities from summer and autumn samples tended to be similar. As

far as the bacterial microflora is concerned, an evolution was observed over the growing season. Most of the bacterial communities from symptomatic and asymptomatic plants were different, particularly during the winter. Moreover, fungal and bacterial microflora that colonizes the various necroses was markedly different from that of the healthy tissue. Finally, our study indicates that potentially pathogenic and potentially beneficial fungi colonize the healthy wood of relatively young grapevines (10 years old) and that a competition between them likely occurs within the trunk. On the contrary, in mature vines (15–25 years old), wood is always necrotic, and only a few fungal species colonize these damaged areas. The factors responsible for this huge change and the possible role of bacteria in this process will be discussed.

Botryosphaeriaceae species involved in Botryosphaeria dieback in Sicily. V. MONDELLO, S. LO PICCOLO, G. CONOGLIARO, A. ALFONZO, L. TORTA and S. BURRUANO. *Department DEMETRA, University of Palermo, Viale delle Scienze 4, 90128 Palermo, Italy. E-mail: santella.burruano@unipa.it*

Botryosphaeriaceae species have been recognized as important grapevine pathogens worldwide. The occurrence and development of the Botryosphaeria dieback and the involved symptoms (late sprout or bud mortality, delayed growth, spur dieback and leaf chlorosis), previously reported in a Western Sicilian vineyard, as well as the recovery of fungi associated with the disease, were studied for three years. The trend of Botryosphaeria dieback was almost similar in 2008 and 2009, different in 2010. Moreover, the occurrence and development of each observed symptom varied from year to year and in the same year from survey to survey, depending on climatic conditions. The high temperature and the rainfall frequency of distribution during the spring/summer period, seems to have affected the vegetative and reproductive growth of the host as well as that of the botryosphaeriaceous fungi. Our results indicate that the phase of vegetative growth of the host corresponds to a greater expression of *Botryosphaeria* dieback symptoms, with delayed growth as the main symptom. Morphological identification confirmed by molecular analysis of the internal transcribed spacer regions (ITS1-5.8S-ITS2) of rRNA gene, showed *Diplodia seriata*, *Lasiodiplodia theobromae* and *Neofusicoccum parvum* to be associated with the grapevine trunk symptoms. Although the three species have been previously reported on grapevine in Italy, only *L. theobromae* has been reported as etiological agent of grapevine decline in Sicily. The satisfaction of Koch's postulates by all assayed *Botryosphaeriaceae* species, even if preliminary, seems to confirm also in Sicily their pathogenic role in Botryosphaeria dieback.

Characterization of fungal pathogens associated with *Botryosphaeria* canker of grapevines in China. W. ZHANG, Y. XIE, Z. Y. WANG, C.H. JIA, J.Y. YAN and X.H. LI. *Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China. E-mail: jiyeyan@gmail.com, lxh1962@yahoo.com.cn*

Botryosphaeria canker is becoming an important problem to the table- and winegrape industry in China. In the past 3 years, field surveys conducted throughout the main grape-growing provinces in China confirmed the presence of this disease. Over 362 diseased samples were collected from 20 provinces. In total, Botryosphaeria canker was found in 18 provinces. More than 3000 isolates were purified and at least four different Botryosphaeriaceae species were identified associated with the cankers. Morphological studies along with multigene phylogenetical analyses confirmed the identification of *Botryosphaeria dothidea*, *Diplodia seriata*, *Lasiodiplodia theobromae* and *Neofusicoccum parvum* associated with different symptoms. Koch's postulates were conducted in 25 of the main grapevine cultivars planted in China. The distribution of Botryosphaeriaceae spp. showed significant geographic variation. This research highlights the importance that Botryosphaeria canker has on grapevine health in growing regions in China where, due to different climatic conditions, interspecific hybrid cultivars are predominantly grown.

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Pathogenicity of Botryosphaeriaceae species from New Zealand nurseries. R. BILLONES-BAAIJENS, E.E. JONES, H.J. RIDGWAY and M.V. JASPERS. *Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 84, Lincoln University, Lincoln. New Zealand, 7647. E-mail: Regina.Billones@lincoln.ac.nz*

Pathogenicity of 114 isolates representing seven *Botryosphaeriaceae* species isolated from different New Zealand grapevine nursery plant material was investigated. The parallel experiments showed that all species were able to produce lesions on Sauvignon Blanc excised green shoots and one-year-old rooted canes. However, pathogenicity varied significantly among species, isolates within a species and nursery source. Overall, *Neofusicoccum parvum* was shown to be the most pathogenic in both tissue types, followed by *N. luteum* and *N. australe,* while *Diplodia mutila* and *D. seriata* species were highly pathogenic on canes but not on green shoots. Isolates of the prevalent species, *N. luteum* and *N. parvum,* showed high variability of pathogenicity in both assays; an evaluation of the factors associated with pathogenicity showed that the lesion lengths were significantly affected by experimental batch, which reflected inherent host and environmental

factors over time. Except for *D. seriata* which appeared to be necrotrophic, most isolates of the six other *Botryosphaeriaceae* species were shown to move endophytically beyond the lesions. These endophytic abilities of some *Botryosphaeriaceae* isolates observed in this study has a great implication for the New Zealand grapevine industry since the young, symptomless but infected young vines can be unwittingly sold to grape growers.

Microscopic studies of three Botryosphaeriaceae species growing in the presence of grapevine wood. J.A. OBRADOR-SÁNCHEZ and R. HERNÁNDEZ-MARTÍNEZ. *Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada, Carretera Ensenada-Tijuana No. 3918, Zona Playitas, C.P. 22860, Ensenada, Baja California, Mexico.*

Several species of Botryosphaeriaceae are cosmopolitan fungi known to be associated with the Botryosphaeria dieback of grapevine. To characterize the growth patterns of these pathogens in the presence of grapevine wood, three isolates that differed in their level of virulence were studied namely *Lasiodiplodia theobromae* UCD256Ma (high), *Diplodia seriata* BY06-3 (moderate) and *Diplodia corticola* SASI12-3 (low). Growth rate, percent of spore germination, number of hyphae and mycelium biomass were compared by growing conidia onto Vogel´s minimal medium supplemented with or without grapevine wood. The presence of wood in the medium increased all the studied parameters for all the species; however, *L. theobromae* presented the highest values. Analysis of germinating conidia stained with FM4-64 and calcofluor using confocal and epifluorescence microscopy, shed light on some cellular characteristics of the isolates and revealed the absence of lipids and chitin on the cell wall of pigmented conidia. Epifluorescence and scanning electron microscopy of grapevine trunks inoculated with conidia at 12, 24 or 48 h revealed the use of tracheae as the main infection entry for the hyphae, as well as some differences in the growth and branching patterns of hyphae among the species.

Botryosphaeria dieback: the current status of Botryosphaeriaceae species infecting grapevines. J.R. ÚRBEZ-TORRES[1] and W.D. GUBLER[2]. *[1]751B Coronation Avenue, Kelowna, British Columbia V1Y7A4, Canada. [2]Department of Plant Pathology, University of California Davis, Davis, California 95616, USA. E-mail: jrurbez@gmail.com*

During the last decade, scientists have focused on elucidating the role that Botryosphaeriaceae species play in grapevine diseases with special emphasis on grapevine trunk diseases. This study aims to summarize the work that has been conducted on Botryosphaeriaceae species as grapevine trunk disease pathogens and to present some of the research areas that need to be strengthened to fully understand the association between these fungi and their host. Since the early 2000s, much has been written about the association between these fungal taxa and grapevine dieback, which finally led to the recognition of Botryosphaeriaceae species as one of the most important grapevine pathogens worldwide. As a result of these studies, 21 different botryosphaeriaceous fungi are currently known to occur on grapes with several species being considered among the most virulent fungi causing grapevine disease symptoms, including leaf spots, fruit rots, shoot dieback, bud necrosis, vascular discoloration of the wood, and perennial cankers. Much progress has been made in the development of novel diagnostic and detection techniques as well as in understanding the biology and epidemiology of Botryosphaeriaceae species in vineyards, which has contributed to the development and implementation of several disease management strategies. These latest novel findings have clarified much of the confusion that has governed Botryosphaeriaceae species on grapevines for many years. Consequently, the disease name Botryosphaeria dieback is finally proposed here to describe the wide range of different grapevine trunk disease symptoms caused by species in the Botryosphaeriaceae family.

Association of fungal pathogens with Red blotch foliar symptoms in California vineyards. H.G. STANGHELLINI, L. ABDELSHAHID, Z. MORALES and J. MONIS. *Eurofins STA Laboratories, 7240 Holsclaw Rd. Gilroy, CA 95020, USA. E-mail: juditmonis@eurofinsus.com*

During the 2011 fall season a new syndrome, Red blotch, was reported in California vineyards. The foliar symptoms are described as red, pink, crimson orange or purple leaves with patchy or blotchy coloration with green, yellow, purple, or red veins in red-fruited grape varieties. In many cases, poor vigor and reduced fruit quality were reported to be associated with the blotchy red foliar symptoms. Many growers have confused the foliar symptoms and suspected virus infection. Our laboratory tested many samples from vineyards that exhibited "red blotchy" foliar symptoms. All tests (ELISA and RT-PCR) for the detection of viruses associated with decline and/or leaf roll disease were negative. In contrast, fungi were isolated from these symptomatic vines. In all instances the plant material showed typical fungal cankers and streaking in the wood. Additionally, most samples had constrictions caused by grafting tape or had twisted and/or "J" shaped roots. The isolated fungal pathogens were identified using taxonomic reference guides and subjected to sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA. The work confirmed the presence of *Bionectria* spp., *Cylindrocarpon* spp., *Phaeoacremonium* spp., *Verticillium* spp., *Phoma* spp. and other fungal species. We speculate that the stress caused by

constrictions and/or malformed roots intensified the effects of the pathogenic fungi present in the diseased vines. Future work will require pathogenicity assays to confirm the association of the characterized fungal species with blotchy red foliar symptoms and differential susceptibility among cultivated grapevine varieties.

Pathogens associated with canker diseases in California table grape vineyards. P.E. ROLSHAUSEN[1], S. VASQUEZ[2] and C. GISPERT[3]. *[1]Department of Plant Pathology and Microbiology, Riverside, CA 92521, USA. [2]Viticulture Farm Advisor, Fresno, CA 93702, USA. [3]Viticulture Farm Advisor, Riverside CA 92201, USA. E-mail: philrols@ucr.edu*

The California table grape industry needs to maintain a high level of production in order to keep its dominant position in the worldwide marketplace. Most studies on wood diseases in California vineyards have been focused on wine grape production, while little is known on their impact in table grape production. We conducted a survey in 2010–2011 in the Coachella valley and San Joaquin valley, two table grape production areas with different climates and viticulture practices. For example, grape growers in the Coachella valley use overhead sprinkler irrigation in the winter to improve chilling units and overcome the mild winters that the region encounters. We sampled from symptomatic vineyards (n=17), and subsequently recovered fungi from diseased wood. Fungi were identified with morphology and by sequence homology of the PCR amplified ITS ribosomal DNA with specimen sequences posted in GenBank database. The vineyards ranged from 2 to 35 years old and 94% were infected with esca disease while 65% were infected with Botryosphaeria canker. We identified at least 12 different fungal species residing in symptomatic wood. *Togninia minima* and *Phaeomoniella chlamydospora* were commonly found in both locations. In addition, each production area had unique pathogens. Vineyards in Coachella valley were also infected with *Phaeoacremonium parasiticum*, *Lasodiplodia theobromae*, *L. crassispora*, and *Eutypella* sp. Vineyards in the San Joaquin valley were infected with *Diplodia seriata* and *Phomopsis* sp. These findings will impact the strategy that needs to be implemented in order to manage these diseases efficiently and maintain fruit quantity and quality.

Diversity of *Ilyonectria* species in a young vineyard affected by black foot disease. P. REIS, A. CABRAL, T. NASCIMENTO, H. OLIVEIRA and C. REGO. *Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal. E-mail: crego@isa.utl.pt*

Fungi of the *Ilyonectria* genus are the main causal agents of black foot disease of grapevine. These pathogens cause necrosis in the basal end of the rootstock, leading to the early decline and the death of young vines in nurseries and young vineyards. In the present study, 33 isolates of the genus *Ilyonectria*, obtained from a vineyard located in the Alentejo region of Portugal, were characterized. This vineyard has been established with planting material originated from three different nurseries. To assess the inter- and intra-specific variability among these isolates, morphological, cultural and biomolecular characteristics were evaluated. Morphological, cultural and molecular data (RAPD. ISSR and histone H3 nucleotide sequence) allowed the identification of *I. liriodendri*, *I. macrodidyma*, *I. europaea*, *I. estremocensis*, *I. torresensis*, *"Cylindrocarpon" pauciseptatum* and *I. vitis* (represented by one isolate distinct from all the others). A greenhouse experiment was conducted to establish the pathogenicity of the 30 most representative isolates by inoculation of rooted plants of the cultivar Touriga Nacional. As indicated by the symptoms produced and by the percentage of reisolation, pathogenicity was confirmed for all isolates. The highest disease severity was achieved in plants inoculated with isolates of *I. torresensis*, the most common species found in the survey representing more than 50% of the isolates obtained. Within *I. torresensis* isolates originated from each of the three nurseries, both *MAT1-1-1* and *MAT1-2-1* genes were identified. This finding suggest that active recombination within *I. torresensis* may be occurring.

Contribution for a better understanding of Petri disease and other grapevine trunk diseases in the Portuguese Dão wine region. J.SOFIA[1,3], C. REGO[2], T. NASCIMENTO[2] and M.T. GONÇALVES[3]. *[1]Direcção Regional de Agricultura e Pescas do Centro, Estação de Avisos do Dão, 3504-504, Viseu, Portugal. [2]Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal. [3]Centre for Functional Ecology, Department of Life Sciences, University of Coimbra.PO BOX 3046, 3001-401. Coimbra. Portugal. E-mail: jorsofia@gmail.com*

Petri disease, one of the most important trunk diseases of grapevine, is responsible for significant losses by promoting premature decline and dieback in vineyards worldwide, including the Portuguese Dão wine region. It has been noticed that the local viticulturists' knowledge on Grapevine Trunk Diseases and Petri disease in particular, is quite incomplete. The real importance of those problems is based mostly on the individual perception rather than on a methodical evaluation of the situation. In order to get a full picture of the situation of those diseases, a leaflet with color pictures has been produced and issued to viticulturists, accompanied by a simple survey. The results of this survey represent a first perception of the real situation of Petri disease in the Dão wine region, namely its economic impact and relevance for the local wine industry. During that period, several

samples of wood collected from Petri diseased symptomatic grapevines throughout the entire region were processed, and isolates of *Phaeomoniella chlamydospora* were obtained. To assess the intra-specific variability among these isolates, morphological, cultural and biomolecular characteristics were evaluated. An experiment was conducted to establish the pathogenicity of selected *P. chlamydospora* isolates by inoculation of pruned canes of the cultivar Touriga Nacional, one of the most important of the Dão wine region.

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

Grapevines affected by esca have been found in all major vine-growing areas in South Africa. During a survey of vineyards displaying symptoms of esca, several basidiomycetes were isolated from grapevine wood rot. A preliminary study identified ten taxa in the Hymenochaetales on the basis of ITS phylogeny. Efforts are now underway to characterise all ten taxa, though characterisation is hampered by the scarcity of fruiting bodies in the field. Spore-trapping studies were conducted in two Stellenbosch vineyards during June to November in 2010 and 2011 to determine when spore-release occurs. Data indicate a wide variation in spore release patterns between basidiocarps. Some, though not all, fruiting bodies' spore-release patterns coincided with rainfall data, though spores were found to be present in small amounts throughout the trapping period. The trapping period coincided with the time of winter pruning and spring suckering practices, which may provide entry ports to the organism considering the availability of spores throughout this time.

One fungus one name: implications for the grapevine trunk disease community. P.W. CROUS. *CBS-KNAW Fungal Biodiversity Institute, Uppsalalaan 8, 3584CT Utrecht, Netherlands. E-mail: p.crous@cbs.knaw.nl*

The International Code of Nomenclature for Algae, Fungi and Plants (ICN) that was accepted at the 18th International Botanical Congress in Melbourne, Australia (2011), heralded the end of Article 59, and dual nomenclature for pleomorphic fungi. In the subsequent "One Fungus = Which Name" meeting held in Amsterdam in April 2012, the Nomenclature Committee for Fungi (NCF) and the International Committee for the Taxonomy of Fungi (ICTF) requested users to organize committees under the NCF and ICTF, and present lists of protected names for genera in common use. One such community is the International Council on Grapevine Trunk Diseases, specifically dealing with genera of pleomorphic fungi infecting grapevines. Some examples that need further debate include *Togninia/Phaeoacremonium, Fusicoccum/Botryo-sphaeria, Neonectria/Cylindrocarpon, Diaporthe/Phomopsis, Trichoderma/Hypocrea, Eutypa/Libertella, Valsa/Cytospora, Phyllosticta/Guignardia, Botrytis/Botryotinia, Erysiphe/Oidium, Elsinoë/Sphaceloma*. I herewith propose that we initiate this discussion and write a community-based paper to argue for the retention of genera in common use, to provide such genera with a protected status.

Genetic diversity of *Eutypa lata* from vineyards, orchards and riparian areas in California. R. TRAVADON[1], K. BAUMGARTNER[2] and P.E. ROLSHAUSEN[3]. [1]*Department of Plant Pathology, University of California, One Shields Avenue, Davis, CA 95616, USA.* [2]*United States Department of Agriculture, Agricultural Research Service, Davis, CA 95616, USA.* [3]*Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA. E-mail: rtravadon@ucdavis.edu*

The epidemiology of Eutypa dieback has been studied primarily in vineyards. *Eutypa lata* has a broad host range, but the contribution of alternative hosts to disease spread from such hosts to vineyards is unclear. To determine levels of gene flow among pathogen populations from vineyards, *Prunus* orchards and riparian areas in California, we gathered 216 isolates from six host species (grapevine, apricot, cherry, almond, pear, willow) at four locations (350 km between most distant locations). The incidence of *E. lata* ranged from 30–50% in *P. armeniaca* (apricot) orchards and vineyards, and from 4–24% in *Salix* spp. (willow) in riparian areas. Isolates were genotyped with a combination of nine microsatellite loci. Bayesian analyses did not reveal any clustering of genotypes based on location or host, suggesting that the 216 isolates are part of one genetically-homogenous population. Of the 216 isolates, 214 had unique genotypes, confirming that ascospores are the main infection propagules on all hosts. No differences in measures of genetic diversity were found among locations. In contrast, populations from willow were significantly less diverse than those from grapevine (mean number of alleles per locus=2.6 vs 3.1; P=0.007). Both the low incidence and the low genetic diversity of populations from willow suggest that the pathogen population size is low in riparian areas. However, genetic differentiation among all samples was low and non-significant (F_{st}=0.008; P=0.12). Our findings suggest that *E. lata* ascospores are dispersed bilaterally between *Prunus* orchards and vineyards, whereas infections in riparian areas more likely originate from *Prunus* and grapevine.

Validation of Simple Sequence Repeat (SSR) markers from genomic sequence of *Phaeomoniella chlamydospora* strain UCD-C67 from California. F. PEDUTO[1], H. KIM[2], R.G. COTA-SIECKMEYER[2], W.D. GUBLER[1] and B.H. BLUHM[2]. [1]*Department of Plant Pathology, University of California Davis, One Shields Avenue, Davis, CA 95616, USA.* [2]*Department of Plant Pathology, University of Arkansas, 217 Plant Sciences Building, Fayetteville, AR 72701, USA. E-mail: fpeduto@ucdavis.edu*

Twenty *P. chlamydospora* isolates, randomly selected from a UC Davis collection of strains gathered in California between 1997 and 2004, were screened for pathogenicity on grapevine cuttings cv. Cabernet sauvignon/101-14. Among these, isolate UCD-C67 induced foliar necrosis resembling some of the symptoms described for Esca disease and was selected for further analysis. From a draft genome sequence of this strain we identified 146 potential SSR loci containing at least seven repeats. Because of the availability of flanking sequences, 49 SSRs were selected and PCR primers were designed for each locus. Of these, 47 generated specific and reproducible amplicons from the sequenced *P. chlamydospora* isolate and were further tested for polymorphism on a selection of *P. chlamydospora* strains. Among those tested, 12 SSRs produced the highest number of polymorphisms and were finally evaluated on all 57 *P. chlamydospora* isolates comprised in the UC Davis collection referred above. All loci showed allelic diversity and were polymorphic with 4 to 13 alleles per locus. These markers will be useful for future analysis of the genetic structure of *P. chlamydospora* population and will provide valuable information of genetic diversity.

Biology of *Togninia minima:* genetic and virulence diversity, and mating type distribution in Spain. D. GRAMAJE[1,2], J. ARMENGOL[1] and H. J. RIDGWAY[3]. [1]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.* [2]*Department of Crop Protection, Institute of Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain.*[3]*Plant Pathology Research Group, Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 84, Lincoln 7647, New Zealand. E-mail: dgramaje@ias.csic.es*

The genus *Phaeoacremonium* includes a total of 35 species, and 25 have been isolated from grapevines. Of those, *Phaeoacremonium aleophilum* (teleomorph *Togninia minima*) appears to be the most widely distributed species, as well as the most common in grapevines. In this study, fifty eight single-spore *Togninia minima* isolates were recovered from the rootstock of plants that showed symptoms of Petri disease and esca from 2001 to 2008 in Spain. These isolates were studied by means of mating type distribution, UP-PCR analysis and virulence assays. For mating type experiments, isolates were paired by pipetting conidial suspensions onto GWA in all possible combinations, including self-pairing. After 28 days, perithecia were observed on wood surfaces and in the agar. Analysis of clone-corrected data sets showed equal frequencies of both mating types in the entire Spanish population, however there were regional differences. In the Ciudad Real region, equal frequencies of mating type were evident at both inter-vineyard and intra-vine spatial scales, whereas unequal mating type distribution was detected in Valencia and Zaragoza regions, and at the same spatial scales. This is the first study on the distribution of *T. minima* mating types at spatial scales varying from vineyards to regions. A total of 49 polymorphic UP-PCR markers were obtained with the seven UP-PCR primers used. Isolates clustered into four statistically defined groups with percentages of similarity among isolates >82%. The high number of unique genotypes observed within the Spanish population, combined with a near-equal distribution of mating types, suggested that sexual reproduction probably does occur. Gene and genotype diversities across the subpopulations were similar and ranged from 0.24 to 0.27 and from 27 to 37%, respectively. The detection of genetically identical isolates within and among subpopulations indicates that an asexual reproductive component should not be excluded. Contrast analysis among groups defined by UP-PCR analyses showed significant differences in the virulence of *T. minima* isolates.

Black foot disease: Mating type structure of the *Ilyonectria liriodendri* and *I. macrodidyma* species complex. A. CABRAL[1], L-H. ZWIERS[2], C. REGO[1], H. OLIVEIRA[1] and P.W. CROUS[3,4]. [1]*CEER-Biosystems Engineering, Instituto Superior de Agronomia, Technical University of Lisbon, Lisboa, Portugal.* [2]*CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.* [3]*Microbiology, Department of Biology, Utrecht University, Utrecht, The Netherlands.* [4]*Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Wageningen, The Netherlands. E-mail: anacabral@isa.utl.pt*

The majority of the causal agents of black foot disease of grapevines are members of the genus *Ilyonectria*. The recent increase in incidence and severity of this disease, along with the large genetic diversity observed among its causal agents, led to the need to better understand the occurrence of sexual recombination in such species. This in turn requires a thorough knowledge of the mating types in these fungi. In the present study, the entire mating-type loci of *I. liriodendri* and of *Ilyonectria* spp. from the *I. macrodidyma* species complex were obtained and validated by comparison to sequences from closely related species. The idiomorph structure of species belonging to the *I. macrodidyma* species complex matched those of other heterothallic hypocrealean fungi. In contrast, the

organization of the mating-type locus in *I. liriodendri* was completely different. Two types, A and B, could be distinguished. Both types contained *MAT1-2-1* and *MAT1-1-1*, but type B also contained *MAT1-1-2* and *MAT1-1-3*, making it genetically homothallic. However, all *I. liriodendri* isolates (both type A and B) tested were self-sterile and functionally heterothallic, a behaviour termed "pseudo-heterothallism". This could represent an intermediate evolutionary step from heterothallism to homothallism in *I. liriodendri*. *MAT1-1-1* and *MAT1-2-1* specific primers were generated and employed for the analysis of the population structure of 238 isolates representing 23 species from the *macrodidyma*, *radicicola* and *pauciseptatum* clusters for the presence of *MAT1-1-1* and/or *MAT1-2-1*. All isolates tested appeared to be heterothallic.

Characterization of *Phomopsis* species recovered from wood cankers in eastern North American vineyards. K. BAUMGARTNER[1], P.T. FUJIYOSHI[1], R. TRAVADON[2], L.A. CASTLEBURY[3] and P.E. ROLSHAUSEN[4]. *[1]United States Department of Agriculture, Agricultural Research Service, Davis, CA 95616, USA. [2]Department of Plant Pathology, University of California, Davis, CA 95616, USA. [3]United States Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705, USA. [4]Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA. E-mail: kbaumgartner@ucdavis.edu*

In eastern North American vineyards, Phomopsis cane and leaf spot (causal fungus *Phomopsis viticola*) is a destructive foliar disease, but it is also associated with wood cankers, along with other fungi. To determine the association between the typical foliar symptoms of the disease and the wood-canker symptom, we recovered and identified *Phomopsis* isolates from wood cankers in 23 vineyards with Phomopsis cane and leaf spot, in eight northeastern US states and Quebec, Canada. Identification of 29 *Phomopsis*/*Diaporthe* isolates was based on morphology and phylogenetic analyses of the rDNA internal transcribed spacer region (ITS), and gene-coding regions elongation factor subunit 1-alpha and actin, in comparison with 100 type specimens and known isolates. From the wood cankers, we identified *P. viticola*, *P. fukushii*, and *D. eres*. Spore inoculations to wounded, woody stems of potted *Vitis labruscana* 'Concord' and *V. vinifera* 'Chardonnay' showed that *D. eres* and *P. fukushii* were pathogenic (mean lesion lengths of 7.4 and 7.1 mm, respectively, at one year post-inoculation), but significantly less so than *P. viticola* (mean lesion length of 13.5 mm). Our findings of *Phomopsis*/*Diaporthe* species causing wood cankers in all 23 vineyards suggest a frequent co-occurrence of foliar symptoms and wood cankers, but the latter is not always due to *P. viticola*. If *P. viticola, P, fukushii*, and *D. eres* directly infect woody tissues, then controls for preventing the foliar symptoms of Phomopsis cane and leaf spot may not prevent wood cankers.

Phomopsis dieback: 40 years of ignoring a grapevine trunk disease. J.R. ÚRBEZ-TORRES[1], F. PEDUTO[2] and W.D. GUBLER[2]. *[1]751B Coronation Avenue, Kelowna, British Columbia V1Y7A4, Canada. [2]Department of Plant Pathology, University of California Davis, Davis, California 95616, USA. E-mail: jrurbez@gmail.com*

In the early 1900s, a grapevine disease named dead-arm was described in the north-eastern USA and associated with *Phomopsis viticola*. Symptoms were characterized as shoot dieback, lack of spring growth, chlorotic leaves, fruit shriveling, cane bleaching, wood necrosis, and wedge-shaped cankers. However, the discovery of *Eutypa lata* as the causal agent of grapevine dieback in both Australia and California in the early 1970s led to the conclusion that *E. lata* and not *P. viticola* was the causal agent of dead-arm symptoms on grape. Since then, *P. viticola* has been only associated with Phomopsis cane and leaf spot disease of grapevines. However, it came to our attention to revise the status of *P. viticola* as a potential grapevine trunk disease pathogen after observing that it was the third most prevalent fungus isolated from grapevine cankers during recent field surveys conducted throughout grape-growing regions in the USA. Morphological studies, along with multigene sequence analyses of the ITS1-5.8S-ITS2 region and part of the translation EF1-α gene, confirmed the presence of *P. viticola* in grapevine cankers. Pathogenicity tests with *P. viticola* as single inoculum were completed in both *Vitis vinifera* and American hybrid cultivars and confirmed that the fungus is capable of causing vascular symptoms first described in the early 1900s, including dark-streaking of the wood, wedge-shape cankers, shoot dieback, and cane bleaching. This study presents sufficient evidences to consider *P. viticola* as a grapevine trunk disease pathogen, and thus; the disease name Phomopsis dieback is proposed here to describe the grapevine trunk disease symptoms caused by *P. viticola*.

Identification and pathogenicity of Botryosphaeriaceae species isolated from grapevines in Croatia. J. KALITERNA and T. MILICEVIC. *Department of Plant Pathology, Faculty of Agriculture, University of Zagreb, Svetosimunska 25, 10000 Zagreb, Croatia. E-mail: josko.kaliterna@gmail.com*

To date, 21 Botryosphaeriaceae spp. have been reported worldwide from grapevine, primarily associated with grapevine trunk diseases. In Croatia, these phytopathogenic fungi have not been fully studied and very little is known about their occurrence and pathogenicity on grapevine. In order to identify the Botryosphaeriaceae spp. associated with grapevine in Croatia, infected grapevine wood samples were collected from vineyards in different grape-growing regions, and pure fungal isolates were obtained. Fungal isolates were identified to the species level based on their morphological char-

acters, while molecular identification was based on the analysis of the internal transcribed spacer region (ITS1-5.8S-ITS2) and part of the translation elongation factor 1-alpha gene (EF1-α), using primers ITS4/ITS5 and EF1-728F/EF1-986R, respectively. To test the pathogenicity of the identified Botryosphaeriaceae species, *in vitro* inoculations of green shoots and lignified canes of Croatian grapevine cultivars Graševina and Škrlet were conducted. This study showed the presence of Botryosphaeriaceae spp. in the genera *Diplodia, Botryosphaeria*, *Neofusicoccum* and *Dothiorella*, from which the most prevalent species were *Diplodia seriata* and *Botryosphaeria dothidea*. All fungal species identified in this study showed pathogenicity on grapevine but virulence varied. There were no significant differences between pathogenicity trials with green shoots and lignified canes. This research shows Botryosphaeriaceae spp. to occur in all Croatian grape-growing regions and the significant role that these fungi play in the etiology of Grapevine trunk diseases in Croatia.

Transformation of *Lasiodiplodia theobromae* by restriction enzyme-mediated integration. J.Y. YAN, A.F. LIU, X. ZHANG, W. ZHANG, H. YAN, J.H. LIU and X.H. LI. *Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing, 100097, China. E-mail: lxh1962@yahoo.com.cn*

Pathogenicity genes are known to play a key role in the process of fungi infecting plants. In an attempt to identify potential pathogenicity genes in *L. theobromae*, mutants were generated. *L. theobromae* JZB 0300251, a highly virulent isolate, was selected for transformation. A simplified and efficient genetic transformation of *L. theobromae* was established using pKNTG, a plasmid carrying green fluorescent protein (GFP), which was provided by Dr. Youliang Peng of China Agricultural University. Using fluorescence confocal microscopy, visualization of GFP-expressing transformants of *L. theobromae* was performed in inoculated Red Globle grapevine cuttings. This is the first successful genetic transformation of *L. theobromae*. In addition, restriction enzyme-mediated integration was also developed in *L. theobromae* using pUCATPH, a plasmid carrying a hygromycine marker. H*ind* III and K*pn*I restriction enzymes were used in the procedure. To date, 1369 transformants were generated and screened for pathogenicity or virulence mutants.

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Occurrence and distribution of fungi associated with grapevine trunk diaseases in Castilla-La Mancha Region (Spain). P.M. IZQUIERDO CAÑAS[1,2] L. GARCÍA MARTÍN[1], E. GARCÍA ROMERO[1] and J.L. CHACÓN VOZMEDIANO[1]. *[1]Instituto de la Vid y el Vino de Castilla-La Mancha (IVICAM), Crta. Toledo-Albacete s/n, 13700 Tomelloso (Ciudad Real), Spain. [2]Fundación Parque Cientifico y Tecnológico de Albacete, Paseo de la Innovación 1, 02006 Albacete, Spain. E-mail: pmizquierdo@jccm.es*

Castilla-La Mancha is the region in Spain with the largest area for grapevine cultivation with approximately 520,000 hectares. The aim of this study was to examine the occurrence and distribution of grapevine trunk pathogens in this region. The study presents the results of isolates obtained from 258 grapevine samples received at the Castilla-La Mancha Institute of Vine and Wine (IVICAM) from 2008 to 2011, which evidenced typical symptoms of decay in the field and which were obtained from vines in different provinces in the region. A total of 2,072 fungal isolates were obtained and identified as belonging to the family Botryosphaeriaceae, the genera *Cylindrocarpon*, *Phaeoacremonium* and *Phomopsis*, and the species *Phaeomoniella chlamydospora*. The highest proportion of pathogenic fungi was identified in rootstock samples. Botryosphaeriaceae isolates were the most abundant and found in all parts of the plant, mainly at the graft union. *Cylindrocarpon* spp. were found mainly in roots, while *Phaeoacremonium* spp. and *Pa. chlamydospora* mainly affected rootstocks. *Phomopsis* spp. were isolated in a much smaller proportion, but mainly at the graft union. The number of isolates of *Phaeoacremonium* spp. and *Pa. chlamydospora* increased significantly in 2011 compared with the previous three years. In terms of their distribution in the different provinces of Castilla-La Mancha region, there was a higher incidence of *Cylindrocarpon* spp, *Phaeoacremonium* spp and *Phaeomoniella chlamydospora* in samples from the province of Ciudad Real (dry area) and *Phomopsis* spp. in the province of Cuenca (wet and cold area).

Identification of fungi associated with grapevine trunk diseases by fragment length analysis of internal transcribed spacer regions. M. FERNÁNDEZ GONZÁLEZ[1,3], A. MENA MORALES[2] and P.M. IZQUIERDO CAÑAS[2,3]. *[1]Instituto de Investigación Científica Aplicada de Castilla La Mancha (IRICA),Universidad de Castilla La Mancha. Avda. Camilo José Cela, s/n. 13071 Ciudad Real, Spain. [2]Instituto de la Vid y el Vino de Castilla La Mancha (IVICAM), Ctra. Toledo-Albacete, s/n. 13700, Tomelloso (Ciudad Real), Spain. [3]Fundación Parque Científico y Tecnológico de Albacete, Paseo de la Innovación 1, 02006 Albacete, Spain. E-mail: pmizquierdo@jccm.es*

Grapevine trunk diseases caused by fungal pathogens are responsible for significant economic losses to the wine industry worldwide. The aim of the present study was to develop a sensitive and rapid method for the identification of pathogenic relevant fungi isolated in vineyards of Castilla La Mancha. Fluorescent polymer-

ase chain reaction (PCR)-fragment length analysis of the internal transcribed spacer (ITS) regions 1 and 2 and the D2 hypervariable region of the rDNA were used. The most frequently isolated fungi from grape wood tissue were *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum, Cylindrocarpon liriodendri, C. macrodidymum, Neofusicoccum parvum, N. luteum*, *Phomopsis* spp. and *Phoma* spp. Fungi isolated less frequently were *Botryosphaeria dothidea, Cadophora luteo-olivacea*, *Diplodia seriata*, *Dothiorella sarmentorum, D. iberica, D. viticola, Macrophomina phaseolina* and *Phaeoacremonium iranianum.* These fungal species included many of the fungi commonly reported from other grape-growing countries, some of them causing Petri disease, black foot and esca. Type strain of other important pathogenic species such as *Eutypa lata, Stereum hirsutum, Fomitiporia mediterranea, Cylindrocarpon destructans* and *Phomopsis viticola* were also studied. The majority of the fungi had unique species-specific PCR products ranging from 212–370 bp (ITS1), 332–428 bp (ITS2) and 320–325 bp (D2); however, few fungi demonstrated fragments of equal length or nearly equal length. These included *Cylindrocarpon macrodidymum/ pauciseptatum; Cylindrocarpon destructans/ liriodendri* and *Neofusicoccum parvum/ luteum.* We conclude that size analysis of PCR-amplified ITS1, ITS2 and D2 regions rDNA is a rapid and reliable method to identify significant fungal trunk pathogens in grapevine.

Identification and pathogenicity of Botryosphaeriaceae species associated with grapevine decline in Spain (Galicia, Madrid and Sevilla). C. PINTOS, V. REDONDO, O. AGUÍN, M. CHAVES, C. RIAL and J.P. MANSILLA. *Estación Fitopatolóxica Do Areeiro, Deputación Pontevedra, Subida a la Robleda s/n. 36153 Pontevedra, Spain. E-mail: cristina.pintos@depo.es*

Between 2009 and 2012 a total of 115 plants with symptoms of grapevine decline from 59 vineyards were received in the laboratory of Phytophathological Station located in Pontevedra (Northwest Spain). Vineyards were located in six wine producing areas of Spain, principally Galicia, including six wine grape (Albariño, Loureira, Mencía, Merlot, Syrah, Tempranillo) and one table grape (Crimson) cultivars. One hundred fifty six isolates were obtained from symptomatic grapevines (over five year age). Identity of isolates was based on morphological and cultural characteristics. These identifications were confirmed by sequencing of ITS and β-tubulin gene regions. The incidence of the main phytophatogenic fungi isolated was Botryosphaeriaceae spp. (48.7%), *Phomopsis viticola* (28.2%), *Cylindrocarpon* spp (10.3%) (*C.lirio dendri* (8.4%) and *C. macrodidymum* (1.9%), *Phaeoacremonium aleophilum* (5.8%) and *Phaeomoniella chlamydospora* (5.1%). In this study, *Neofusicoccum parvum* reached 33.3% of total identified fungi and was the most prevalent Botryosphaeriaceae species isolated (68.4%). In addition *Diplodia seriata* and *Botryosphaeria dothidea* were found in 14.5% and 9.2% of the Botryosphaeriaceae isolates respectively, whereas the 7.9% remaining isolates included *Diplodia corticola, Diplodia mutila, Neofusicoccum australe, Neofusicoccum luteum* and *Neofusicoccum mediterraneum.* Pathogenicity of these eight Botryospaheriaceae species was tested on grapevine canes after seven weeks and in one-year old grapevine plants growing in pots after 6 months. Vascular necroses were measured. One way ANOVA showed significant differences ($P<0.0001$) in mean lesion length. As a result in our study *N. mediterraneum*, detected for first time in grapevine in 2010, was considered the most virulent species whereas *D. seriata* was the least aggressive in both pathogenicity assays.

Molecular identification of *Neofusicoccum parvum*/ *Neofusicoccum ribis* complex species from vineyards in Spain. O. AGUÍN, V. REDONDO, A. ARES, C. PINTOS and J.P. MANSILLA. *Estación Fitopatolóxica Do Areeiro, Deputación Pontevedra, Subida a la Robleda s/n. 36153 Pontevedra, Spain. E-mail: olga.aguin@depo.es*

Neofusicoccum parvum is a cosmopolitan, polyphagous fungal pathogen and one of the most virulent Botryosphaeriaceae species on grapevine. *Neofusicoccum ribis "sensu stricto"* was detected on grapevine for the first time in 2011. *Neofusicoccum parvum* and *N. ribis* are two closely related species. In fact, the variability and overlapping in the morphological and culture characteristics of both species makes difficult their identification based only on morphological criteria. Until recent years, molecular identification of *Neofusicoccum* species has been only based on the analysis of single locus sequence data, which grouped *N. parvum* and *N. ribis* in the same clade, the *Neofusicoccum parvum*/*Neofusicoccum ribis* complex. Recent molecular studies have aided to clarify boundaries between these two species by using a multiple loci approach, which revealed the existence of eight cryptic species in the complex: *N. batangarum*, *N. cordaticola*, *N. kwambonambiense*, *N. occulatum*, *N. parvum*, *N. ribis*, *N. umdonicola*, and a *Neofusicoccum* sp. karanda. The aim of this study was to re-evaluate the identity of a collection of thirty single-conidial strains isolated from grapevines, which had been previously identified as *Neofusicoccum parvum*. DNA was extracted from mycelia and four gene regions were sequenced: internal transcribed spacer of the rDNA (ITS), β-tubulin (BT), translation elongation factor 1-α (EF) and RNA polymerase subunit II (RPB2). Six unique nucleotides across four loci that characterize *N. parvum* were observed: ITS position 380 (C), BT position 86 (T), EF positions 53 (C) and 54 (A) and RPB2 positions 379 (T) and 511 (T). All isolates analyzed in this study were identified as *N. parvum*, thus confirming the initial identification.

Fungal pathogens associated with grapevine trunk diseases in Sardinia (Italy). A. DEIDDA, B.T. LINALDEDDU, B. SCANU, G. SERRA and S. SERRA. *Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università degli Studi di Sassari, via E. De Nicola 1, 07100 Sassari, Italy. E-mail: salvase@uniss.it*

The area given over to grapevine in Sardinia is about 18,866 ha, making it one of the most important crops on the island. Over the last years, an increasing spread of trunk diseases has been observed in young vineyards. Since 2009, a field survey has been carried out to study the fungal species involved in the aetiology of grapevine trunk diseases. Twenty-one diseased vines ranging in age from 3 to 11 years (cv. Cannonau, Vermentino, Monica and Merlot) showing reduced vegetative growth, foliar symptoms, defoliation, dieback of shoots, stem, or even whole plant, apoplexy, were uprooted and transferred to the laboratory for analysis. Woody chips were aseptically cut from the margin of symptomatic tissues along the whole plant and plated onto malt extract agar. Botryosphaeriaceae were the most common fungal species isolated from all necrotic woody tissues. Four species (*Diplodia seriata*, *Lasiodiplodia pseudotheobromae*, *Neofusicoccum australe* and *N. parvum*) were identified based on cultural and micro-morphological features of the isolates, and sequencing of the internal transcribed spacer region (ITS1-5.8S-ITS2) of rDNA. Other trunk pathogens, such as *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp. and *Fomitiporia* spp., were isolated with less frequency. Pathogenicity of Botryosphaeriaceae was assessed by inoculation of excised green grapevine shoots of cv. Vermentino and Cannonau under controlled laboratory conditions. *N. australe* and *L. pseudotheobromae* were the most virulent species. Our results emphasize the involvement of several species of Botryosphaeriaceae in the aetiology of grapevine trunk diseases. Further studies are in progress in order to verify the distribution of Botryosphaeriaceae species in Sardinian vineyards. To our knowledge, this is the first report of *L. pseudotheobromae* on grapevine.

Molecular and morphological characterization of *Eutypa* isolates associated with Eutypa dieback and esca of grapevine in Algeria. A. BERRAF-TEBBAL[1], Z. BOUZNAD[2] and A.J.L. PHILLIPS[3]. *[1]Département de Biologie, Faculté des Sciences Agro-Vétérinaires, Université Saad Dahleb, 09000 Blida, Algeria. [2]Département de Botanique, Laboratoire de Phytopathologie et Biologie Moléculaire, Ecole Nationale Supérieure d'Agronomie (ENSA), El-Harrach, Algeria. [3]Centro de Recursos Microbiologicos, Departamento de Ciências da Vida, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, Portugal. E-mail: berraf.a@hotmail.fr*

Eutypa dieback and esca are very destructive grapevine decline diseases that occur in most countries where grapevine is cultivated. They are one of the main factors limiting both vineyard longevity and productivity. *Eutypa lata* has been the most studied as the agent of Eutypa dieback of grapevine. Recent studies have shown that additional *Diatrypaceae* are contributing to grapevine dieback. However, it is difficult to distinguish between species of *Eutypa* based only on morphology. This study was carried out for molecular identification and phylogenetic analysis of 48 *Eutypa* spp. recovered from vines with mild or severe forms of esca and with symptoms of Eutypa dieback sampled in the main production areas of the northern Algeria. Isolates were characterized and grouped according to their microsatellite-primed PCR (MSP-PCR) profiles. Representative isolates from each group including, when possible, isolates from Eutypa dieback and esca symptoms were subsequently selected for a molecular phylogeny based on the rDNA ITS region (ITS1-5.8S-ITS2). DNA sequences were compared with those available in GenBank using Neighbor-joining (NJ) and Maximum-parsimony (MP) analyses. The phylogenetic trees of the ITS region revealed that the *Eutypa* isolates clustered with *Eutypa lata* reference sequences with a bootstrap support of 99%. Morphological study showed colonies characterized by a fast growing and white cottony mycelium on PDA, turned dark gray with age producing numerous pycnidia containing long filiform conidia. In the present study, only *Eutypa lata* was found to be associated with esca and eutypa dieback symptoms. This finding confirms the involvement and the importance of *Eutypa lata* in the decline of the vine in Algeria.

Occurrence of grapevine fungal trunk pathogens and viruses from young declining vines in Castilla-La Mancha. M.L. LERMA, P. CASTILLO and R.M. MUÑOZ. *Servicio de Diagnóstico y Asistencia Fitosanitaria (SEDAF), Instituto Técnico Agronómico Provincial de Albacete, Avda. Gregorio Arcos s/n. 02006 Albacete, Spain. E-mail: mll.itap@dipualba.es*

In the period 2002–2011 a total of 206 samples of symptomatic grapevines (each with one, two or three plants) obtained from young vineyards in Castilla-La Mancha were analyzed for fungal trunk pathogens and viruses. Fungal isolation was performed in representative plants of each sample. Fragments of roots, basal and medium areas of rootstocks, graft unions and scions were analyzed. In addition, all plants (401 in total) were analyzed by ELISA for the presence of viruses listed in Commission Directive 2005/43/EC on the marketing of material for the vegetative propagation of the vine: GFLV, GLRaV-1, GLRaV-3, ArMV and GFkV (GFkV was analyzed only until 2006; later this virus was replaced by ArMV). Grapevine fungal trunk pathogens were detected in most of the samples (70%), 22% were infected with at least one virus and pathogens were not detected on 24% of the samples. *Phaeomoniella chlamydospora* and *Cylindrocarpon* spp. were the two most frequently isolated fungi.

Phaeomoniella chlamydospora was detected mainly in the basal end of the rootstocks and *Cylindrocarpon* spp. in the roots. GFKV and GLRaV3 were the most frequently detected viruses, followed by GFLV. Syrah was the most frequently analyzed variety, of which 70% were positive for fungal trunk pathogens and 5% were positive for viruses. Regarding the rootstocks, 110 R was the most common in our study, being 78% of the samples positive for fungal trunk pathogens and 17% positive for viruses.

A non-destructive method to locate internal wood symptoms of Esca disease in grapevine plants. A.T. VAZ[1], S. MONTEIRO[1], H. OLIVEIRA[1] and R. BOAVIDA-FERREIRA[1,2]. [1]*Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda 1349-017 Lisboa, Portugal.* [2]*Instituto de Tecnologia Química e Biológica, New University of Lisbon, 2780-157 Oeiras, Portugal. E-mail: rbferreira@itqb.unl.pt*

Esca of grapevine comprises a number of distinct diseases caused by at least three fungi, *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Fomitiporia mediterranea* acting alone, in combination or in succession. The first fungi are responsible for vascular necroses, which appear as dark wood streaking, while *F. mediterranea* (and/or other wood-rotting basidiomycetes) induces white rot, or "amadou". The earlier onset of esca could be attributed to the production of rooted cuttings infected by *Pa. chlamydospora* and/or *P. aleophilum* (and by other fungi) in nurseries. In these materials the wood symptoms appear as dark streaking. Once planted in the vineyard, infected propagating material develops Petri disease or decline, also characterized by wood dark streaking, slow dieback and black goo. As the vines age, foliar symptoms appear as tiger stripes, the wood shows dark streaking, but these plants are frequently still free from basidiomycete fungi. Later, they can develop white rot, caused by *F. mediterranea* (or other wood rotting fungi). Tracing these grapevine trunk diseases is a difficult and destructive process. For all these reasons, it is of paramount importance to develop methodologies capable of non-destructive screening and detection of the initial stages of esca, either in nurseries or in vineyards. In the present work, a non-destructive method is described which identifies and locates, within each plant, the presence of healthy and esca-infected tissues at the various stages of its development. Several non-destructive techniques were applied to study the development of esca symptoms, but only X-ray computerized tomography showed promising results.

Characterization of fungi associated to Esca disease in Baja California, Mexico. L.G. MORALES-PEDRAZA, C. VALENZUELA-SOLANO and R. HERNÁNDEZ-MARTÍNEZ[1]. [1]*Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada, Carretera Ensenada-Tijuana No. 3918, Zona Playitas, C.P. 22860, Ensenada, Baja California, Mexico.* [2]*INIFAP, Campo Experimental Costa de Ensenada, Ensenada, BC, 22800 México. E-mail: ruhernan@cicese.mx*

Esca disease complex is one of the most destructive diseases of grapevine worldwide. *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. have been identified as two of the principal pathogens within of the complex. Recently, vines showing Esca symptoms have been observed in Baja California. The objective of this work was to determinate the presence of these fungi in our region. A total of 23 putative *P. chlamydospora* and *Phaeoacremonium* spp. isolates were obtained. Isolates were identified by their morphological and genetic characteristics. Molecular markers used were, for all isolates, the internal transcribed spacer (ITS) and a partial sequence of the β-tubulin; for the putative *Phaeoacremonium* isolates, a partial sequence of the actin gen; and for *P. chlamydospora*, a partial fragment of the translation elongation factor 1-α (EF1-α). Analyses resulted in the identification of 9 isolates of *Phaeoacremonium aleophilum* and 14 of *P. chlamydospora*. To evaluate the ability of some isolates to cause lesions, green tissue of 1 year old Cabernet Sauvignon vines were infected. After 30 days, it was clear that the *P. chlamydospora* isolates produced more necrotic lesions than the *P. aleophilum* isolates. This work reports the presence of *P. chlamydospora* and *P. aleophilum* in grapevines showing young Esca in Baja California.

A novel *Fomitiporia* species associated with esca in South African vineyards. M. CLOETE[1], M. FISCHER[2], F. HALLEEN[1,3] and L. MOSTERT[1]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa.* [2]*Julius-KühnInstitut (Bundesforschungsanstalt für Kulturpflanzen), Geilweilerhof, Germany.* [3]*Plant Protection Division, ARC Infruitec-Nietvoorbji, Private Bag X5026, Stellenbosch, 7599, South Africa. E-mail: halleenf@arc.agric.za*

During recent surveys of esca related pathogens in South African vineyards, several unidentified basidiomycetes were isolated from white rot occurring in diseased vines. A new *Fomitiporia* species, *Fomitiporia capensis*, is described based on fruitbody morphology and combined ITS and LSU phylogeny, where it forms a clearly delineated and well-supported clade. Morphologically, *Fomitiporia capensis* is characterised by perennial, resupinate basidiomata of up to 5 mm thick with a pore surface which is yellowish brown to rusty brown in colour and 4–6 mm in size with thick dissepiments. Spores are globose-ovoid, dextrinoid and sometimes quite thick-walled with a distinct apiculus. Setae are almost entirely absent. The hyphal system is dimitic with golden brown skeletal hyphae and hyaline generative hyphae with simple septa and no clamp connections. *Fomitiporia*

capensis was found to occur widely as vegetative mycelium throughout the Western Cape Province, though fruiting bodies were scarce in comparison. A vineyard with fruiting bodies was also found in Mpumalanga in the north east of the country. Fruiting bodies were found growing on the underside of the cordon of living vines displaying external symptoms typically associated with esca, or general decline and dieback symptoms together with internal white rot.

Grapevine trunk disease pathogens associated with sucker or spring wounds in South African vineyards. G. MAKATINI[1], C. MUTAWILA[1], F. HALLEEN[1,2] and L. MOSTERT[1]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa.* [2]*Plant Protection Division, ARC Infruitec-Nietvoorbji, Private Bag X5026, Stellenbosch, 7599, South Africa. E-mail: lmost@sun.ac.za*

Grapevine trunk diseases are considered to be a major contributor to grapevine decline in wine and table grapes and have increased in occurrence over the last two decades. Winter pruning wounds are believed to be major portals of entry for trunk disease pathogens although a recent study has shown that sucker or spring wounds may also pose as possible ports of entry for *Eutypa lata*. However, the role of sucker wounds as infection portals to other pathogens within the trunk disease complex has not been investigated. A survey was carried out to determine the incidence of trunk disease pathogens in sucker wounds of one- and two-year-old wood in the main grape growing areas of the Western Cape Province of South Africa during autumn of 2011. Grapevine canes showing clear sucker wounds were selected from both wine (Chenin blanc and Cabernet Sauvignon) and table grapes (Crimson Seedless and Thompson Seedless). Isolations were made from 130 sucker wounds from wood symptoms (browning and streaking) originating from sucker wounds, or from apparently healthy tissue just below the wound. Wine grape cultivars had a higher pathogen incidence than table grape cultivars. *Phomopsis viticola*, species of Botryosphaeriacae and *Phaeomoniella chlamydospora* were the most commonly isolated fungi at incidences of 43, 35 and 28% from wine grape cultivars and 24, 14 and 5% from table grapes, respectively. Other pathogens isolated included species of *Phaeoacremonium* and *Eutypella*. The infection of sucker wounds by trunk pathogens was further ascertained by artificial inoculation of *P. chlamydospora* and *E. lata* on sucker wounds on actively growing one-year-old grapevine canes in a glasshouse trial. *P. chlamydospora* and *E. lata* were re-isolated from these wounds after 98 days. These results confirm that sucker wounds are naturally infected by trunk disease pathogens and thus also serve as portals for trunk pathogen infections.

Occurrence of grapevine trunk pathogens in nurseries and vineyards in the northern and southern coast of Peru. L.A. ÁLVAREZ[1], D. TAMAYO[2], C. CASTILLA[1,2], J. MUNIVE[2], C. AGUSTÍ-BRISACH[3], D. GRAMAJE[3] and J. ARMENGOL[3]. [1]*Departamento de Sanidad Vegetal, Universidad Nacional San Luis Gonzaga de Ica, Fundo Arrabales, Panamericana Sur km 299, Ica - Peru.* [2]*Vivero Los Viñedos SAC. Panamericana Norte km 508,5 Viru, La Libertad - Peru.* [3]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. E-mail: lalvarezb@cip.org.pe*

In Peru, the impact of fungal trunk diseases of grapevine has increased in the last years, together with the growth of the table grape areas. A survey of symptomatic plants collected from vineyards and nurseries were performed from 2009 to 2011 across the coastal valleys of the country. A total of 622 symptomatic plants were processed to determine the fungal pathogens associated with trunk diseases. *Lasiodiplodia theobromae* was isolated as unique pathogen in 236 samples (38%); *Campylocarpon pseudofasciculare*, *Ilyonectria novozelandica* and *Cylindrocladiella peruviana* were isolated from 44 plants (7% of the total samples) associated with black foot disease symptoms. Fungi associated with Petri disease: *Phaeoacremonium parasiticum*, *P. krajdenii* and *Phaeomoniella chlamydospora* were isolated from 9% of the total samples (56 plants), *P. parasiticum* being the main species isolated. *L. theobromae* and the pathogens associated with black foot and Petri disease were found in 286 plants (46% of the samples). This is the first report of *P. krajdenii*, *C. peruviana*, *Campyl. pseudofasciculare* and *Ilyonectria novozelandica* associated with grapevine in Peru.

Characterization of Spanish isolates of *Phaeacremonium aleophilum*, etiological agent of Petri disease. V. GONZÁLEZ, M.L. TELLO and P. ANDRÉS. *Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Agroalimentario (IMIDRA), Finca "El Encín". Apdo. 127, Ctra. N II, Km 38,200. 28800 Alcalá de Henares (Madrid), Spain. E-mail: vicente.gonzalez.garcia@madrid.org*

Petri disease is considered as one of the most important grapevine trunk diseases (GTD) in terms of incidence and geographic distribution, being responsible for the decay and death of young vine plants worldwide. One of the etiological agents associated with this disease is *Phaeoacremonium aleophilum*, a fungus belonging to the extended group of mitosporic Ascomycetes, mostly producing asexual reproductive structures without any differentiated conidiogenic structure and possessing a teleomorphic phase identified as *Togninia minima* (Calosphaeriales). Although other members of the genus have been identified as grapevine pathogens, *P. aleophilum* is considered to be the main species of the genus involved in some GTD in Spain. The aim of the present study is to provide several

criteria to characterize a collection of Spanish isolates of this fungus. The possibility of having alternative acceptable criteria for the recognition of different variants of this pathogen could facilitate the location of non-pathogenic or hypovirulent isolates within the species, or contribute to the knowledge of the biology of this fungus, especially in relation to their dispersion, mating system or the type of ecological range occupied in the vine agroecosystem. Some of the criteria adopted have involved the management and integration of data provided by cytological (nuclear staining), ecological (pathogenicity, soil survival capability), genetic markers (microsatellites) or reproductive (mating systems) methods. Results have allowed the characterization and recognition of different groups based on criteria such as DNA fingerprinting, nuclear status, virulence or persistence in natural soils.

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Development of a monoclonal antibody TAS-ELISA assay for detection of *Phaeomoniella chlamydospora*. F. CARDOSO[1], T. NASCIMENTO[2], C. REGO[2] and H. OLIVEIRA[2]. *[1]UEI Parasitologia Médica, IHMT-New University of Lisbon, Rua da Junqueira, 100, 1349-008 Lisboa, Portugal. [2] CEER-Biosystems Engineering, Instituto Superior de Agronomia, Technical University of Lisbon, 1349-017 Lisboa, Portugal. E-mail: fcardoso@ihmt.unl.pt*

Phaeomoniella chlamydospora is the main causal agent of Petri disease and esca, two of the most destructive diseases of young and mature grapevines, respectively. Although planting material is frequently infected by this pathogen, no regular screening against this fungus is done, mainly due to the lack of fast, accurate and inexpensive methods for large-scale application. In the present study, we developed a monoclonal antibody-based triple antibody sandwich (TAS) enzyme-linked immunosorbent assay (ELISA) able to detect specifically *Pa. chlamydospora* from the wood of artificially inoculated grapevine plants. A polyclonal preparation, previously prepared in rabbit, was used as a capturing reagent and the Mab, obtained in Balb/c mice, as a second antibody for TAS. To obtain the MAb, three cell fusions were done between splenocytes and Sp2/AG -14 cell partner, using PEG. The hybridomas were screened against 10 different isolates of *Pa. chlamydospora* and two stable hybridomas, both of the IgM isotype, were selected. TAS-ELISA was used to test the Mab against 20 fungal species commonly isolated from grapevine wood tissues and no cross-reactions were observed. Further, positive results were achieved when the TAS-ELISA assay was used to detect *Pa. chlamydospora* from the wood of artificially inoculated grapevine plants, six-months after inoculation. This new TAS-ELISA assay could be especially useful for large-scale application in nurseries to ensure *Pa. chlamydospora*-free stocks, and thus contributing to the production of healthier propagation material.

Identification of *Cylindrocladiella parva* and *C. peruviana* associated with black-foot disease of grapevine in Spain. C. AGUSTÍ-BRISACH[1], S. ALANIZ[2], D. GRAMAJE[3], A. PÉREZ-SIERRA[1], J. ARMENGOL[1], E. LANDERAS[4] and P.M. IZQUIERDO[5]. *[1]Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain. [2]Departamento de Protección Vegetal, Facultad de Agronomía, Universidad de la República, Garzón 780 CO 12900, Montevideo, Uruguay. [3]Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain. [4]Laboratorio de Sanidad Vegetal, Consejería de Agroganadería y Recursos Autóctonos del Principado de Asturias. C/ Lucas Rodríguez, 4-bajo, 33011 Oviedo, Spain. [5]Instituto de la Vid y el Vino de Castilla-La Mancha (IVICAM), Ctra. Toledo-Albacete s/n, 13700 Tomelloso, Ciudad Real, Spain. E-mail: caragbri@upvnet.upv.es*

From 2007 to 2009 *Cylindrocladiella*-like isolates were obtained from grapevine roots showing characteristic symptoms of black-foot disease in different locations in Spain. Three representative isolates were selected to confirm their identity: isolate CPa1 collected from cv. Albarín Tinto and isolate CPa2 collected from cv. Carrasquín, both grafted onto 110-R rootstock and from Cangas de Narcea (Asturias, northern Spain), and isolate CPe523 collected from cv. Syrah grafted onto 161 49 C rootstock from Villagarcía del Llano (Cuenca, Central Spain). Using morphological and molecular methods, isolates CPa1 and CPa2 were identified as *Cylindrocladiella parva*, and isolate CPe523 was identified as *C. peruviana*. Pathogenicity tests were conducted on one-month-old grapevine seedlings with inoculum produced on wheat seeds. Symptoms developed in all plants by 20 days after inoculation and consisted in reduced vigour, interveinal chlorosis and necrosis of the leaves, necrotic root lesions with a reduction in root biomass, and occasionally plant death. Mean shoot dry weights of inoculated plants (0.25, 0.16 and 0.28 g for isolates CPa1, Cpa2 and CPa523, respectively) were significantly lower (P<0.05) than noninoculated controls (0.74 g). Mean root dry weights of inoculated plants (0.28, 0.16 and 0.29 g for isolates CPa1, Cpa2 and CPa523, respectively) were also significantly lower (P<0.05) than noninoculated controls (0.68 g). This is the first report of *C. parva* and *C. peruviana* associated with black-foot disease of grapevine in Spain as well as other countries in Europe.

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Detection and quantification of *Ilyonectria* spp. associated with black-foot disease of grapevine in nursery soils using nested multiplex PCR and quantitative PCR. C. AGUSTÍ-BRISACH[1], J. ARMENGOL[1] and L. MOSTERT[2]. [1]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.* [2]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa.E-mail: caragbri@upvnet.upv.es*

Soils of three open-root and three rootstock mother fields from grapevine nurseries located in Valencia province (eastern-central Spain) were sampled in July 2011. Ten soil samples were collected in each field, and three subsamples (10 g each) were taken from each soil sample. These soil subsamples were lyophilized overnight, crushed to a powder and DNA extracted using ZR Soil Microbe DNA MiniPrep™. A nested polymerase chain reaction (PCR) combined with a multiplex PCR approach was used for the detection of *Ilyonectria* spp. using species-specific primers. Among the 180 soil DNA samples analyzed, *Ilyonectria* spp. were detected in 172 of them. *Ilyonectria macrodidyma* complex was the most frequently detected, being identified in 141 subsamples from all the fields evaluated. However, *I. liriodendri* was detected in only 16 subsamples, but was present in all open-root field nurseries and in two rootstock mother fields. In addition, quantitative real-time PCR (qPCR) assays were done to assess the levels of *I. liriodendri* and *I. macrodidyma* complex DNA in the soil samples. The presence of *Ilyonectria* spp. DNA with qPCR correlated with the fields found positive with the nested multiplex PCR. DNA concentrations of *Ilyonectria* spp. ranged from 0.001 to 190.5 pg μL^{-1}. In general, samples from rootstock mother fields showed the highest DNA concentrations. These molecular techniques are powerful tools for specific, sensitive detection and quantification of *Ilyonectria* spp. associated with black-foot disease. Our results show the prevalence of these pathogens in nursery soils in Spain.

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Fungal species associated with trunk diseases of table grapes in Northeastern Brazil. K.C. CORREIA[1], M.P.S. CÂMARA[1], M.A.G. BARBOSA[2], R. SALES Jr.[3], C. AGUSTÍ-BRISACH[4], D. GRAMAJE[5], J. GARCÍA-JIMÉNEZ[4], P. ABAD-CAMPOS[4], J. ARMENGOL[4] and S.J. MICHEREFF[1]. [1]*Universidade Federal Rural de Pernambuco, Departamento de Agronomia, 52171-900 Recife, Pernambuco, Brazil.* [2]*Embrapa Semiárido, 56300-970, Laboratório de Fitopatologia, Petrolina, Pernambuco, Brazil.* [3]*Universidade Federal Rural do Semi-Árido, Departamento de Ciências Vegetais, 59625-900 Mossoró, Rio Grande do Norte, Brazil.* [4]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.UPV.* [5]*Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain. E-mail: sami@depa.ufrpe.br*

In 2009, 54.577 t of table grapes were exported from Brazil, being the main fresh fruit export from this country. Most of these table grapes are produced in the Northeastern region, where 10,000 ha are cultivated. This is a tropical region, thus the management systems for grapevine production are adapted to the specific environmental conditions of a tropical viticulture. Samples of table grape plants showing symptoms of trunk diseases were obtained from grapevine nurseries, young plantations and vineyards in three different areas: the São Francisco Valley (Bahia and Pernambuco states), the Assú Valley, (Rio Grande do Norte state) and the Siriji Valley (Pernambuco state). These samples were subjected to fungal isolation. Fungal isolates were identified tentatively by means of morphological features and confirmed by sequencing the ITS1-5.8S-ITS2, β-tubulin and EF1-α regions of DNA. The sequences obtained were then blasted in GenBank. The following fungal species were found: *Botryosphaeria mamane*, *Campylocarpon fasciculare*, *C. pseudofasciculare*, *Lasiodiplodia crassipora*, *L. parva*, *L. pseudotheobromae*, *L. theobromae*, *Neofusicoccum parvum*, *Phaeoacremonium aleophilum*, *P. parasiticum* and *Phaeomoniella chlamydospora*. All of them are reported for the first time on grapevine in Brazil, with the exception of *L. theobromae*, which was the only species present in all production areas. This study shows the high diversity of fungal species associated with trunk diseases of table grapes in Northeastern Brazil.

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Comparison of fungi associated to grapevine trunk diseases in Castilla y León and in Douro. L. MARTÍN[1], D. FLORES[1], M.T. MARTÍN[1], T. NASCIMENTO[2] and C. REGO[2]. [1]*Instituto Tecnológico Agrario de Castilla y León, Zamadueñas, Ctra. Burgos km 119, 47071 Valladolid, Spain.* [2]*Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal.*

Symptomatic plants from Castilla y León (Spain) and Douro (Portugal) regions were analyzed and a collection of isolates were obtained. Different species associated with grapevine trunk diseases were identified. Botryosphaeriaceae isolates were the most frequent in both re-

gions; *Diplodia seriata* was the most abundant in Castilla y León while *Neofusicoccum parvum* was the dominant species in Portugal. Five different species of *Phaeoacremonium* were identified in Castilla y León being *P. aleophilum* the most frequent. *Phaeomoniella chlamydospora* represented 15% of isolates in Castilla León. Four species of *Ilyonectria* were found with *I. macrodydima* and *I. liriodendri* as the most representative in both regions. *Ilyonectria* spp. are found almost exclusively in young plants, *Stereum hirsutum* only in adult/old vines. *Ilyonectria* spp. were commonly isolated from the root and root inclusion/rootstock. Botryosphaeriaceae fungi, *Phaeoacremonium* spp. and *Pa. chlamydospora* were isolated from all parts of the plant. From plants showing external eutypa dieback symptoms, *Eutypa lata* was isolated in a small percentage. No other species of *Eutypa* were identified. However, Botryosphaeriaceae, *Pa. chlamydospora*, *Pm. aleophilum* were isolated in large and quite comparable percentages as it was obtained form plants with esca symptoms. This result indicated that eutypa dieback symptoms could be due to other phyto-pathogenic fungi and not only to *Eutypa lata*.

Occurrence and distribution of fungi associated with grapevine decline in Kurdistan region-Iraq. R.A. HALEEM[1], S.K. ABDULLAH[2] and J.M.S. JUBRAEL[3]. [1]*Plant Protection Department, Faculty of Agriculture and Forestry, University of Duhok, Iraq.* [2]*Department of Biology, Faculty of Science, University of Zakho, Iraq.* [3]*Scientific Research Center, University of Duhok, Iraq.E-mail: raed.haleem@uod.ac*

A survey was carried out in five locations representing the main grapevine production areas of Duhok governorate-Iraq during 2008–2009 in four seasons, winter (Dec. – Feb.), spring (Mar. – May), summer (Jun. – Aug.), and fall (Sept. – Nov.). Decline symptoms on grapevines included plants that failed to thrive normal, with reducing shoot growth and chlorotic interveinal areas that later became necrotic. In a cross section of grapevine arms, the internal wood tissues were frequently dark brown to black with a wedge-shaped necrotic sector. Severe symptoms were noted during spring to summer almost in all locations. Canes (bark and wood), buds, trunks or arms (bark and wood), and roots were sampled for fungal isolation. Fungal pathogens belonging to 18 genera were isolated from green growth and roots of the grapevine samples during the four seasons. *Cylindrocarpon destructans, Neofusicoccum parvum* and *Togninia minima* were the main trunk pathogens found in all grape-growing regions. These three species were reported for the first time in Iraq. *N. parvum* was isolated with a high frequency (98–100%) from wood of arms and canes in Bajelor and Badi locations during summer and fall. *Togninia minima* was found commonly in Zawita by 37–75% of isolation in the wood of arms and canes during fall. *Cylindrocarpon destructans* was the most dominant root rot fungus and was frequently isolated from almost all locations particularly in Badi (41–75% of isolations).

PCR - based identification and pathogenicity of *Cylindrocarpon destructans*, the causal agent of grapevine black-foot disease in Iraq. R.A. HALEEM[1], S.K. ABDULLAH[2] and J.M.S. JUBRAEL[3]. [1]*Plant Protection Department, Faculty of Agriculture and Forestry, University of Duhok, Iraq. Department of Biology, Faculty of Science, University of Zakho, Iraq.* [3]*Scientific Research Center, University of Duhok, Iraq. E-mail: samer_abdalh@yahoo.com*

Symptoms of black-foot disease of grapevine caused by *Cylindrocarpon destructans* include reduced vigor with small-sized trunks, reduction in root biomass, black discoloration and brown to dark streaks in the wood, mainly at the base of the rootstock. According to morphological and cultural characteristics of *Cylindrocarpon*, all isolates from infected roots of declined plants in Iraq were tentatively identified as *C. destructans*. For accurate identification of *C. destructans* isolates on the basis of PCR technique, ten isolates of the fungus were selected from different locations. These isolates were subjected to species-specific PCR assay. Total genomic DNA was isolated from pure cultures of the isolates. The average DNA yields ranged between 1.5–6.7 μg mL^{-1} with a purity 1.6–1.8. The specific primers for *C. destructans* (400 bp) were used to amplify the ITS region of rDNA containing ITS1, ITS2 and the intervening 5.8 rRNA genes. All isolates were confirmed as *C. destructans*. This fungus is reported for the first time in Iraq during this study. Pathogenicity of *C. destructans* was tested under greenhouse conditions. One–year-old dormant rooted cuttings of two grape cultivars, Reshmew and Taefi, were inoculated by dipping their roots for 30 min in conidial suspensions (1×10^6 conidia mL^{-1}). Infected vines showed reduced vigor with small leaves, interveinal chlorosis and necrosis. Other symptoms included a reduction in root biomass and root hairs. Root mass reduction reached to 0.41 on cv. Taefi after five months from inoculation with significant difference compared with the control treatment. *C. destructans* caused a significant decrease in the fresh and dry weight of grapevine shoots and roots.

EPIDEMIOLOGY

Studies on the role of pruning wounds in infection by *Phaeocremonium aleophilum* and *Diplodia seriata* in France. P. LARIGNON. *Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France. E-mail: philippe.larignon@vignevin.com*

Experiments were conducted in a vineyard close to Nîmes during 4 consecutive years. The cultivar was Sauvignon grafted on SO4 rootstock, planted in 1989 and cordon-trained. Fifteen percent of the vines showed characteristic symptoms of esca/black dead arm in 2005, 12% in 2006, 26% in 2007 and 20% in 2008. After each pruning date (February 9th 2005, February 15th 2006,

February 22th 2007, February 4th 2008), 40 pruned one-year-old canes left on the vines were collected randomly every week until June 23th 2005, June 7th 2006, May 16th 2007 and April 30th 2008. Comparative microflora studies of pruned and unpruned canes carried out during this period showed that *Phaeoacremonium aleophilum* and *Diplodia seriata* were more frequently isolated from pruned than unpruned canes, suggesting that these fungi were able to penetrate via pruning wounds. Average infection percentages of pruned canes in the four years were respectively 2.6, 4.4, 10, 6.7% for *P. aleophilum* and 21.5, 13.1, 21.9, 14.6% for *D. seriata*. Average infection percentages of unpruned canes in the four years were respectively 0 in the first three years, and 0.4% for *P. aleophilum* and 12.5, 3.6, 3.3 and 4.6% for *D. seriata*. Contaminations were observed immediately after pruning in 2007, six weeks after pruning in 2005 and 2006, and 8 weeks after pruning in 2008, but always after the period of bleeding (except for 2007 where contaminations took place a week before bleeding). Comparison of these results to weather data showed that the conditions for the contamination of pruning wounds by these two fungi would be a period of average temperature above 10°C with a maximum temperature of at least 16°C, accompanied by a rainy period.

Natural infections of pruning wounds by fungal trunk pathogens in mature grapevines in Catalonia (NE Spain). J. LUQUE[1], G. ELENA[1], F. GARCÍA-FIGUERES[2], J. REYES[3] and G. BARRIOS[4]. *[1]Dep. Patologia Vegetal, IRTA Cabrils. Ctra. de Cabrils km 2, E-08348 Cabrils, Spain. [2]Laboratori de Sanitat Vegetal. DAAM Generalitat de Catalunya. Ctra. de Cabrils km 2, E-08348 Cabrils, Spain. [3]Oficina Alt Penedès, DAAM Generalitat de Catalunya. Plaça Àgora s/n, E-08720 Vilafranca del Penedès, Spain. [4]Serveis Territorials de Tarragona, DAAM Generalitat de Catalunya. Avinguda Catalunya 50, E-43002 Tarragona, Spain. E-mail: jordi.luque@irta.cat*

Little information is available about the rates of spontaneous infections caused by fungal trunk pathogens under natural conditions in Spain. This study aimed at determining the pathogenic mycoflora infecting pruning wounds in two mature vineyards with different levels of trunk diseases. In each vineyard in mid-fall, about one hundred vines were pruned leaving 4–6 buds. Three months later, 250 of the pruned canes were randomly chosen in each vineyard and were taken to the laboratory for pathogen isolation and identification. All the vines were then pruned leaving two buds. Sampling for pathogen isolation and identification from these canes was repeated three months later. The main fungal pathogens identified during this study were, in increasing order of abundance, *Eutypa lata*, *Neofusicoccum parvum*, *Cryptovalsa ampelina*, *Phaeomoniella chlamydospora* and *Diplodia seriata*. The latter species was by far the most frequently isolated, with percentages between 2 and 68%, depending on the vineyard but mainly on the pruning season. *Phaeomoniella chlamydospora* was isolated in a range between 0.4 and 12% while the rest of the taxa were detected in fewer than 3% of isolations. No relevant differences were observed between the high- and the low-diseased vineyards. However, a strong seasonal effect was observed for *D. seriata* and *P. chlamydospora*, with higher isolation percentages in spring (after the late pruning) than in winter (after the early pruning). Since traditional viticultural practices in Spain recommend late pruning, a shift in these practices would be advisable, by pruning as early as possible, to avoid such higher rates of infections when pruning late in the season.

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Susceptibility of grapevine tissues to *Neofusicoccum luteum* conidial infection. N.T. AMPONSAH, E.E. JONES, H.J. RIDGWAY and M.V. JASPERS. *Lincoln University, Faculty of Agriculture and Life Sciences. P.O. Box 84, Lincoln University, Canterbury, New Zealand. E-mail: Marlene.Jaspers@lincoln.ac.nz*

This study investigated the ability of *Neofusicoccum luteum* to infect wounded shoots, trunks, pruned cane ends, leaf surfaces, buds, berries and roots and its further progression into stem tissues. All tissue types were susceptible to infection except roots, with highest incidences in trunks (100%), cane ends (100%), shoots (92%) and buds (88%), indicating that in New Zealand, *N. luteum* is primarily a wood and stem pathogen. In trunks there were no external trunk symptoms although *N. luteum* could be reisolated from 60–70 cm up the trunk after 4 months, by which time the pathogen had progressed into side shoots which became necrotic. Wounded and non-wounded buds became infected; most were killed, with pathogen progression downwards into the supporting shoots. Berries wounded and inoculated from pre-bunch closure were susceptible to *N. luteum* infection, with isolation incidence increasing over the season and peaking at harvest, when infected berries became mummified and produced pycnidia that oozed many conidia. The pathogen was able to progress from berries into bunch stems and supporting canes. Results from this research have indicated that *N. luteum* infection can occur in all aerial grapevine tissues and progresses to young stem tissues where it causes wood necrosis. This research shows that growers should remove mummified berries from vineyard trash and ensure that pruning and trimming times do not coincide with rainy periods when conidia are released and dispersed. Furthermore, the susceptibility of buds to *N. luteum* infection indicates that fungicide sprays before bud burst in spring might reduce bud infection.

Foliar symptoms are not related to presence of fungal species in wood in the Eutypa dieback syndrome. A. MURUAMENDIARAZ and F.J. LEGORBURU. *NEIKER-Tecnalia, Basque Institute for Agriculture Research and Development, Apdo 46, E-01080 VITORIA/GASTEIZ, Basque Country, Spain. E-mail: jlegorburu@neiker.net*

Grapevine trunk pathogens cause distinct foliar symptoms in spite of not colonizing the leaves. There is a three-way relationship among foliar symptoms, wood symptoms and the pathogens that requires investigation to delimit the different diseases. In previous work, we only found a weak relationship between the intensity of foliar symptoms in the Eutypa dieback syndrome and the frequency of *Eutypa lata* isolation by non-destructive, blind wood sampling. To further characterize the three-way relationship in this disease, 18 thirty-year-old vines were selected from a single vineyard to represent the full range of foliar symptom intensities. The selected vines were uprooted and the trunks were cut in transversal sections every 10 cm. The cut surfaces were photographed and studied using image analysis software. Wood chips representative of the different kinds of symptoms were plated onto PDA + streptomycin. While the relationship between wood symptoms and the fungi isolated was as expected (*Fomitiporia mediterranea*-soft decay, *Eutypa lata*-dark necrosis, *Phaeomoniella chlamydospora*-brown dots), stronger foliar symptoms were not associated with presence of a particular fungus or wood symptom, but to a wider perimeter of affected trunk, independently of the kind of wood symptom. While the role of *E. lata* as the causal agent of Eutypa dieback is beyond doubt, since foliar symptoms can consistently be induced by artificial inoculation, blind isolation from wood samples seems not to be the best way to prove it.

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Esca development in France over the last decade. P. LECOMTE[1,2], G. DARRIEUTORT[3,1], P. PIERI[4], P. REY[2,1] and M. FERMAUD[1,2]. *[1]INRA, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), F-33140 Villenave d'Ornon, France. [2]Univ. Bordeaux, ISVV, UMR1065 SAVE, Bordeaux Sciences Agro, F-33140 Villenave d'Ornon, France. [3]Univ. Bordeaux, ISVV, Vitinnov, F-33170 Gradignan, France. [4]INRA, ISVV, UMR1287 Ecophysiologie et Génomique Fonctionnelle de la Vigne (EGFV) F-33140 Villenave d'Ornon, France. E-mail: lecomte@bordeaux.inra.fr*

Over the last decade, the Esca disease in vines has become of great concern for winegrowers in France. According to French data issued from the "National Survey of Wood Diseases", its progression was slow but uniform from 2003 to 2008, while incidence of Eutypa dieback tended to regress. Although it is difficult to determine and separate accurately the factors responsible for esca (Lecomte *et al*., 2011. Integrated Protection and Production in Viticulture, IOBC/wprs Bulletin, 67, 171–180), the following hypotheses may individually or jointly account for the increase: *(i)* the ban on the use of sodium arsenite in 2001, *(ii)* the marked (strong warming) summer heat waves or long periods of drought, *(iii)* changes in cultivation practices, *e.g.* plant material quality, late planting, increased density and over-cropping. In this survey, two patterns of leaf symptoms, previously described as esca and black dead rm (BDA), were recorded. As it was difficult to differentiate the two leaf patterns, the field assessors grouped and named those types of symptom as "esca-BDA". In fact, observations made in the Bordeaux region, in various European and Mediterranean countries, convincingly showed that the esca syndrome included the BDA leaf symptoms (Lecomte *et al*., 2012. Plant Disease 96, 924–934). Furthermore, these observations pointed out that the foliar symptoms of esca are mostly associated with a vascular disorder that has longitudinal wood discoloration in the outer xylem just under the bark ("stripe"). This disorder was found in vines showing wilting or drying zones on the foliage. The process of development of this peculiar wood symptom is still unknown (Lecomte *et al*., 2012. *Plant Disease* 96(7), 924–934). Nevertheless, it may be hypothesized that esca disease appears as the consequence of the development of large inner necrosis into the grapevine wood leading to the sudden occurrence of one, or several, stripe(s), and then to a large variability of foliar symptoms according to damage severity. Another new insight is that, in our survey, we did not distinguish two forms of the diseases (apoplectic *versus* mild form) but a gradual increase in severity of symptoms. Additionally, leaf symptoms did not always begin with discoloration that became dried and necrotic as it is commonly described. Previous descriptions were not complete as drying zones becoming necrotic may form as soon as the first symptoms are appearing. Many questions about esca aetiology are raised, including the role of toxins and/or *Botryosphaeriacea* species involved in the early development of stripes, of the frequency of Bot longitudinal cankers that originate from stripes, and the relevance of temperature, in relationship with water availability and inoculum pressure within the plant, as a determining factor to trigger the foliar symptoms and their development.

Statistical analysis of the grapevines mortality associated with foliar expression of Esca or Eutypa dieback. L. GUÉRIN-DUBRANA[1,2], J.C. LABROUSSE[1,2], S. BASTIEN[1,2], P. REY[1,2] and A. GÉGOUT-PETIT[3]. *[1]INRA, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), F-33140 Villenave d'Ornon, France. [2]Université de Bordeaux, ISVV, UMR1065 SAVE, Bordeaux Sciences Agro, F-33140 Villenave d'Ornon, France. [3]Université de Bordeaux, IMB*

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Esca and Eutypa dieback are the two main wood diseases in France. Their widespread distribution in vineyards leads to grapevine decline and to a loss of productivity. However, little is known about the temporal dynamics of these diseases on a plant scale, and also the relationships between foliar expression of the diseases and vine death is relatively unknown. To investigate these relationships, we surveyed the vines of six vineyards cv. Cabernet Sauvignon in the Bordeaux region, by recording foliar symptoms, dead arms and dead plants from 2004 to 2010. In 2008, 2009 and 2010, about 5% of the asymptomatic vines died but the rate of death was higher for vines which had previously expressed Esca foliar symptoms and varied between the vineyards. The use of a logistic regression statistical model demonstrated that mortality of Esca-affected vines was always associated with foliar symptom expression in the year preceding vine death. One or two other earlier years of expression were frequently additional risk factors. Presence of Eutypa dieback symptoms was also a risk factor for death, greater or equal to that observed with Esca. A study of the internal necroses of vines expressing Esca or Eutypa dieback is discussed in the light of these statistical results.

Survival and persistence of *Phaeomoniella chlamydospora* in vineyard soils; effect of temperature, moisture and initial inoculum concentration. M. L. TELLO, V. GONZÁLEZ and P. ANDRÉS. *Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Agroalimentario (IMIDRA), Finca "El Encín". Apdo. 127, Ctra. N II, Km 38,200. 28800 Alcalá de Henares (Madrid), Spain. E-mail: vicente.gonzalez.garcia@madrid.org*

Phaeomoniella chlamydospora is able to infect grapevine plants grown in the greenhouse by penetrating through their roots. Due to this mode of infection it has been suggested that natural soils could play a role in the spread and progression of the pathogen, together with the fact that these soils could allow for the persistence and survival of this fungus for long-term periods. The present laboratory study explores the capability for persistence of five selected *P. chlamydospora* isolates, artificially inoculated in several natural vineyard soils of different textures and organic material content. The main parameters assayed were related with the evolution of inocula through a 36-months bioassay under several controlled conditions of moisture and temperature. In addition, the infective capability of these artificially inoculated soils was evaluated after 12 months of experiments by planting vine seedlings in them. Results demonstrated that *P. chlamydospora* is able to survive up to 36 months and maintain its infective properties for at least 12 months in natural soils with low content of organic matter. The influence and interaction of the several factors studied, as well as the evolution of inoculum rates across time are discussed. It is concluded that, under certain conditions, soil could act as a reservoir of this fungus, prior to its infection through the plant roots. Results also showed that from these artificially inoculated soils, the pathogen could penetrate and infect grapevine seedlings. Finally, these results suggested that soil should be considered as another possible inoculum source for dispersal of *P. chlamydospora*.

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Detection and identification of black-foot and Petri disease pathogens in natural soils of grapevine nurseries and vineyards using bait plants. C. AGUSTÍ-BRISACH[1], D. GRAMAJE[2], J. GARCÍA-JIMÉNEZ[1] and J. ARMENGOL[1]. [1]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.* [2]*Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, APDO. 4084, 14004 Córdoba, Spain. E-mail: caragbri@upvnet.upv.es*

The presence and diversity of black-foot and Petri disease pathogens in natural soils of grapevine nurseries and vineyards was investigated using bait plants. Seedlings of rootstock 41-B, and cvs. Bobal and Palomino were used to detect these pathogens in soils of grapevine nurseries and vineyards. In greenhouse experiments, seedlings were planted in pots which were filled with soil samples collected from ten commercial vineyards. The seedlings were analyzed by fungal isolation from roots and xylem vessels nine months after planting. In field experiments, seedlings were planted in a rootstock mother field and in an open-root field nursery, and analyzed at nine or twenty-one months after planting. Black-foot pathogens: *Ilyonectria alcacerensis*, *I. macrodidyma*, *I. novozelandica* and *I. torresensis* were frequently isolated from grapevine seedlings grown in all soils evaluated, whereas the causal agents of Petri disease: *Cadophora luteo-olivacea*, *Phaeoacremonium aleophilum*, *Pm. parasiticum* and *Phaeomoniella chlamydospora* were only isolated from nursery soils, with a low incidence. *Ilyonectria alcacerensis*, *I. novozelandica* and *I. torresensis* were isolated for the first time from grapevines in Spain, and *Pm. parasiticum* and *Ca. luteo-olivacea* were detected for the first time in grapevine nursery soils. Moreover, this is the first study to demonstrate that natural soilborne inoculum of Petri disease pathogens can infect grapevine roots and reach the xylem vessels. Our results confirmed the soil as an important inoculum source for black-foot and Petri disease pathogens.

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The effect of grapevine and rootstock cultivars on esca symptom expression. S. MUROLO and G. ROMANAZZI. *Department of Environmental and Crop Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131 Ancona, Italy. E-mail: g.romanazzi@univpm.it*

Previous investigation of grapevine cultivars and rootstocks have revealed different degrees of susceptibility to esca disease. The aim of this study was to determine the presence of esca foliar symptoms on a range of grapevine cultivars and rootstocks. The incidence of symptomatic and dead plants was recorded for 78 *Vitis vinifera* cultivars, 100–200 vines each, in an experimental vineyard in the Marche region (central-eastern Italy). Among the white cultivars, Riesling renano, Sauvignon blanc, Elbling and Manzoni bianco had the highest incidence (40–50%) of symptomatic plants, while the cvs. Chiapparù, Grechetto, Fiano and Pinot bianco had the lowest incidence (0–5%). Among the red cultivars, Traminer aromatico and Rebo had the highest incidence (46 and 41%, respectively) of symptomatic plants, with the lowest recorded for cvs. Brugentile, Nebbiolo and Lacrima (0–5%). There was no apparent difference in average values between the red (18%) and white (19%) cultivars. Furthermore, the incidence of esca disease symptoms on seven cultivars which were grafted onto different rootstocks was recorded. Cvs. Fiano, Riesling renano and Sauvignon blanc grafted onto SO4, a rootstock known to be less adapted to limited water conditions, showed higher disease incidence than seen when the same varieties were grafted onto 1103P, which is drought tolerant. This data confirmed previous results that reported grapevine cultivars and rootstocks varied in expression of esca symptoms. These results may assist in the choice of materials for new plantings, in order to reduce esca disease symptoms in the vineyard.

Wood decay of almond trees on the island of Mallorca (Spain); new insights on the epidemiology of fungal trunk pathogens. D. GRAMAJE[1], C. AGUSTÍ-BRISACH[1], A. PÉREZ-SIERRA[1], E. MORALEJO[2], D. OLMO[3], L. MOSTERT[4], U. DAMM[5] and J. ARMENGOL[1]. *[1]Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. [2]Departamento de Biología (Área Botánica), Universitat de les Illes Balears,Carretera Valldemossa km 7,5, 07122 Palma de Mallorca, Spain. [3]Laboratori de Sanitat Vegetal, Millora Agrària, Conselleria d'Agricultura,Medi Ambient i Territori, Govern Balear, C/d'Eusebi Estada 145, 07008 Palma de Mallorca, Spain. [4]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa.[5]CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherland. E-mail: jarmengo@eaf.upv.es*

In Spain, as well as in other countries, *Prunus* trees are often planted adjacent to vineyards, and known to have some wood pathogens in common with grapevines. In recent years, a severe decline of almond trees has been observed in several orchards on the island of Mallorca (Balearic Islands, western Mediterranean Sea). A study was therefore undertaken to determine whether almond trees were inhabited by known grapevine trunk disease pathogens and whether they could act as alternative hosts. Between August 2008 and June 2010, wood samples from branches of almond trees showing internal necroses or brown to black vascular streaking were collected in the Llevant region on the island of Mallorca. Several fungal species were subsequently isolated from the margins between healthy and symptomatic tissues. Five species of *Botryosphaeriaceae* (namely *Botryosphaeria dothidea*, *Diplodia olivarum*, *D. seriata*, *Neofusicoccum australe* and *N. parvum*), *Eutypa lata*, *Phaeoacremonium iranianum* and *Phomopsis amygdali* were identified based on morphology, culture characteristics and DNA sequence comparisons. *Neofusicoccum parvum* was the dominant species, followed by *E. lata*, *D. olivarum* and *N. australe*. First reports from almond include *D. olivarum* and *Pm. iranianum*. Two species were newly described, namely *Collophora hispanica* sp. nov. and *Phaeoacremonium amygdalinum* sp. nov. These results indicated that almond trees can serve as potential inoculum sources for trunk disease pathogens, which may have great implications in the epidemiology and control of these diseases.

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Olive trees, a potential alternative host for grapevine trunk disease pathogens. J.R. ÚRBEZ-TORRES[1], F. PEDUTO[2] and W.D. GUBLER[2]. *[1]751B Coronation Avenue, Kelowna, British Columbia V1Y7A4, Canada. [2]Department of Plant Pathology, University of California Davis, Davis, California 95616, USA. E-mail: jrurbez@gmail.com*

During a study conducted between 2008 and 2009 to elucidate the etiology of olive twig and branch dieback in California, 16 fungal species known to be involved in grapevine trunk diseases were isolated from symptomatic wood of olive trees. The species were identified by means of morphological characters and multigene sequence analyses of the internal transcribed spacer region, a partial sequence of the β-tubulin gene, part of the translation elongation factor 1-α gene, and a partial sequence of the small subunit mitochondrial rDNA. These species included *Diatrype oregonensis*, *Diatrype stigma* and *Eutypa lata* associated with Eutypa dieback of grapevines; *Botryosphaeria dothidea*, *Diplodia mutila*, *Diplodia seriata*, *Dothiorella iberica*, *Lasiodiplodia theobromae*, *Neofusicoccum luteum*, *Neofusicoccum vitifusiforme*, and *Neofusicoccum mediterraneum* associated with Botryosphaeria dieback of grapevines; *Phaeomoniella chla-*

mydospora and *Phaeoacremonium aleophilum* associated with both Petri disease and Esca disease of grapevines; *Ilyonectria macrodidyma* associated with black foot of grapevines; and the basidiomycetous fungi *Schizophyllum commune* and *Trametes versicolor* associated with wood decay of grapevines. Perithecia and/or pycnidia for most of the species were also found on symptomatic olive twigs and/or branches. Although all 16 fungal species were shown to be pathogenic in olive trees, virulence varied among fungal taxa. Olive trees are traditionally grown next to vineyards throughout grape-growing regions in the Mediterranean regions. Furthermore, in California, olive trees are planted along road sides separating vineyards. These findings suggested that olive trees may be an overlooked, yet important source of inoculum for grapevine trunk disease pathogens and may have important implications in the epidemiology, and consequently the management of these diseases. Additionally, these pathogens lead to shortened life of olive trees in California.

Arthropods disseminate Petri and other grapevine trunk disease pathogens. P. MOYO[1], F. HALLEEN[1,2], F. ROETS[3], E. ALLSOPP[2] and L. MOSTERT[1]. *[1]Department of Plant Pathology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa. [2]Plant Protection Division, ARC Infruitec-Nietvoorbji, Private Bag X5026, Stellenbosch, 7599, South Africa. [3]Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa. E-mail: halleenf@arc.agric.za*

Although Petri disease has been studied for several years in all major grape growing countries, several questions remain unresolved regarding the epidemiology of the pathogens involved, specifically with regards to dissemination. Insects have been observed in association with *Phaeomoniella chlamydospora* sporulation in Australian grapevines, but to date no study has investigated the role of these insects as possible spore vectors. The objectives of this study were (1) to determine if specific arthropods are associated with declining grapevines, (2) to determine the presence of trunk pathogens on them, and (3) to determine whether these arthropods can transfer pathogens to susceptible pruning wounds. Destructive vine sampling, trunk and cordon traps, manual searches, pruning wound traps and emergence cages were found to be effective in collecting arthropods from diseased vines. During the 2010 and 2011 seasons (early winter to early summer), 10,660 arthropods were collected. From these, 5,677 water samples were obtained by washing fungal spores from the arthropods. An agar plate technique was used to obtain fungal cultures from water samples which were thereafter identified based on morphology and sequencing of the relevant reference gene for each fungal taxon. Total genomic DNA was extracted from a subset of water samples in order to screen for *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* using PCR with genus and species-specific primers. Identified pathogens included *Phaeoacremonium* spp. (*P. aleophilum*, *P. alvesii*, *P. parasiticum* and *P. sicilianum*), *Phaeomoniella chlamydospora*, Botryosphaeriaceae (*Diplodia seriata, Neofusicoccum parvum, N. australe, Botryosphaeria stevensii* and *Spencermatinsia viticola*), Diatrypaceae (*Eutypa lata*, *Eutypella* spp. and *Cryptovalsa ampelina)*, Diaporthales (*Phomopsis viticola* and *Diaporthe helianthi*) and Basidiomycetes. Numerous arthropod species were found to carry pathogen spores. Several of these were associated with infected pruning wounds. A study is currently in progress with selected arthropods and a DsRed-Express transformed *Phaeomoniella chlamydospora* isolate to confirm whether arthropods can inoculate vines through pruning wounds.

International viticultural consultant's view of grapevine trunk diseases and their impact on clients. R. SMART[1], L. MUGNAI[2] and C. LANE[3]. *[1]Smart Viticulture, 31 North Corner, Newlyn TR185JG, UK. [2]DiBa, Protezione delle piante, P.le Cascine 28, 50144 Firenze, Italy. [3]The Food and Environment Research Agency Sand Hutton, York YO41 1LZ, UK. E-mail: vinedoctor@smartvit.com.au*

The senior author consults widely, and has recently discovered trunk disease in many vineyards in many countries. The most widespread incidence is in UK, where 97% of vineyards inspected have symptomatic vines. The most frequently confirmed pathogen is *Botryosphaeria parva*, and it has been recorded as spreading rapidly by pruning wound invasion. Fortunately the disease is generally of low incidence in most vineyards. Infection rates as assessed by dead, replanted and symptomatic vines can however exceed 50%, and with all non-symptomatic vines inspected showing trunk staining. Old vineyards show differences in incidence among different varieties with Rondo having the highest incidence. Madeline Angevine, Muller Thurgau, Chardonnay, Pinot Noir and Regner had intermediate incidence and Bacchus and Seyval Blanc were apparently less affected. Mild wet winters are thought to facilitate spread. Severe damage was also seen in Thailand's tropical vineyards where the vines have two growth cycles and so two pruning operations per year. Vine death associated with *Botryosphaeriaceae* species was very common in vineyards older than 10 years, and there was evidence of rapid spread by pruning wound infection. In New Zealand the distribution of *Botryosphaeria* fungi could be determined with infrared imagery which allowed new infection foci to be determined. Substantial vineyard variability was associated with Botryosphaeria symptoms in Ningxia, China, where the disease was recently described. There appeared a strong interaction with winter freeze injury, as

has been shown for other woody plants in the US mid west. In Australia, Belgium, China, Denmark, Mexico, New Zealand, Thailand and the UK there were considerable problems with new plantings of grafted vines. *Cylindrocarpon*, *Phaeoacreomonium*, *Phaeomoniella* and *Botryosphaeriaceae* species have been implicated in vine deaths and ill thrift. In the majority of vineyards observed, vignerons were unaware of trunk disease presence and its implications, which was very disturbing. Local research on pruning wound treatment is needed.

The minimum inoculum potential of *Diplodia seriata* and *Phaeomoniella chlamydospora* needed for the artificial infection of grapevine pruning wounds. G. ELENA[1], F. GARCÍA-FIGUERES[2] and J. LUQUE[1]. [1]*Dep. Patologia Vegetal, IRTA Cabrils; Ctra. de Cabrils km 2, E-08348 Cabrils, Spain.* [2]*Laboratori de Sanitat Vegetal; DAAM Generalitat de Catalunya; Ctra. de Cabrils km 2, E-08348 Cabrils, Spain. E-mail: georgina.elena@irta.cat*

The minimum inoculum potential of *Diplodia seriata* and *Phaeomoniella chlamydospora* required to infect pruning wounds was determined through the inoculation of conidial suspensions at different estimated concentrations. Two independent experiments for each pathogen were conducted in 2011 and 2012 on five-year old potted vines of cv. Tempranillo grafted onto 110R. For each pathogen, a serial dilution was conducted to obtain a decreasing range of conidial suspensions from 1×10^5 conidia·mL^{-1} to 2.5×10^2 conidia·mL^{-1}. Dormant canes were cut at 6–7 buds in winter (January), and a 40 μL drop of the inoculum was placed on eachwound. The inocula corresponded to 4000 conidia for the most concentrated suspension, and to 2000, 1000, 100 and 10 conidia per wound in the subsequent dilutions. An additional control treatment was inoculated with 40 μL of sterile water. The experiments were performed as a randomized complete block design with 20 replicates. Four months later, canes were collected for the measurement of vascular necrosis and the reisolation of pathogens. The percentage of cane infections was calculated from the frequencies of fungal reisolations. For both pathogens, 10 conidia were the minimum inoculum potential required to infect pruning wounds, although mean percentages of cane infection were below 50% at this concentration. Infection by *D. seriata* reached 100% of inoculated canes when 1000 conidia or higher were inoculated per wound, while for *P. chlamydospora* the mean infection percentage ranged between 80 and 95 % at the highest concentrations. For both pathogens, the length of vascular necrosis increased as did conidial concentration.

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Pruning wound susceptibility to infection by *Eutypa lata* ascospores over time follows an exponential decay model. A. MURUAMENDIARAZ and F.J. LEGORBURU. *NEIKER-Tecnalia, Basque Institute for Agriculture Research and Development, Apdo 46, E-01080 VITORIA/GASTEIZ, Basque Country, Spain. E-mail: jlegorburu@neiker.net*

It is known that grapevine wound susceptibility to fungal infection diminishes as winter progresses and with the time elapsed since pruning. Field experiments were set up in two different vineyards over two winters. Vines were pre-pruned in early, mid- and late winter and inoculated with *Eutypa lata* ascospores either on the same day or one, two, three and four weeks afterwards. Two weeks after inoculation, cane samples were taken to the laboratory and plated onto PDA + streptomycin. The diminution of susceptibility both over the winter and with time after pruning date could be modelled by experimental decay, and these factors were independent from each another. These models provide quantitative measures of wound susceptibility diminution that allowed comparison between experiments. For instance, the two years were significantly different but the two vineyards were not. On the other hand, the residues of the model can be used to quantify other factors affecting the susceptibility and/or infection pressure, such as weather conditions. As a drawback, the logarithmic transformation necessary to fit the model impeded the addition of the natural infection effect.
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Kiwifruit trees an alternative host and potential source of inoculum of grapevine trunk disease pathogens in Chile. J. AUGER, I. PÉREZ and M. ESTERIO. *Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile. Zip Code 8820808, Santiago, Chile. E-mail: jauger@uchile.cl*

Chile, an important wine, table grape and kiwifruit producer in the world, should constantly diversify the species and/or varieties of fruit-bearing species or increment cultivated areas with these fruit trees. Grapevine trunk diseases are a very serious problem from nursery to mature plants. Several pathogens that cause grapevine trunk diseases were shown not to be host specific, and had been also described from other hosts such as kiwifruit, olive, pome and stone fruits. In Chile, grapevines are frequently cultivated in close proximity to kiwifruit trees; furthermore, kiwifruit replants are often established in soils that previously grew grapevine. To study the extend in which kiwifruit trees are inhabited by known grapevine trunk disease pathogens, fungi

were isolated from dieback, canker and wood necrosis symptoms in commercial kiwifruit orchards. Identification was based on morphological and cultural characteristics and DNA sequence data (5.8S rDNA, ITS-1, ITS-2). Species belonging to *Botryosphaeriaceae*, *Phaeoacremonium* and Basidiomycetes were isolated from kiwifruit trees wood symptoms. Species of *Phaeoacremonium* were identified as *Ph. aleophilum*, *Ph. rubrigenum* and *Ph. inflatipes*. *Cadophora luteo-olivacea* was recovered from symptomatic vegetal material of kiwifruit trees. This species had not been previously isolated from grapevines in Chile. Further examinations will include pathogenicity trials on grapevine and kiwifruit material. The findings suggested that kiwifruit may be an overlooked yet important source of inoculum for certain grapevine pathogens and may have implications in the epidemiology and control of these diseases, or kiwifruit trees could be susceptible to be infected by these pathogens.

Monitoring symptoms of fungal trunk diseases in a vineyard in Manchuela (Spain): a six year study. R.M. MUÑOZ, M.L. LERMA and P. CASTILLO. *Servicio de Diagnóstico y Asistencia Fitosanitaria (SEDAF), Instituto Técnico Agronómico Provincial de Albacete, Avda. Gregorio Arcos s/n. 02006 Albacete, Spain. E-mail: rmg.itap@dipualba.es*

A mature goblet trained vineyard of cv. Bobal located in the Manchuela wine region was monitored for foliar symptoms annually from 2004 to 2009; these symptoms were associated with fungal trunk diseases and consisted of red-brown rounded or irregular spots between the veins or along the leaf edges. In the period 2007–2009, dead arms were also detected and recorded. The percentage of symptomatic vines and the overall rate of disease were lowest in 2005; later both parameters rose annually except in 2008 when a slight decrease was observed. Annual mortality in vines varied between 0 (2004, 2005) and 0.28% (2007). The cumulative mortality at the end of the study (2009) reached 0.70%, whereas the cumulative incidence of disease reached 8.62%. A total of 34% of symptomatic plants with foliar symptoms showed dead arms in later years and 43% of them did not display foliar symptoms afterwards. Overall, the average percentage of symptomatic vines increased from 37.62% (foliar symptoms) to 48.02% (foliar and dead arm symptoms). A high correlation was found between annual percentage of plants which had showed symptoms in the past and annual rainfall (October- September); however no correlation was found between annual rainfall and annual percentage of new symptomatic vines, annual percentage of symptomatic vines or overall rate of disease. Lower annual rainfall was related with lower symptom incidence in 2005.

Remote sensing by UAV (Unmanned Aerial Vehicles) for the detection of spatial distribution and development of grapevine trunk diseases. S.F. DI GENNARO[1], M. BENANCHI[2], J. PRIMICERIO[1], E. FIORILLO[1], A. MATESE[1], F.P. VACCARI[1], L. GENESIO[1], S. DI MARCO[3], L. MUGNAI[2] and G. SURICO[2]. *[1]IBIMET-CNR,Via G. Caproni 8, 50145 Firenze, Italy. [2]DiBA, Protezione delle piante, P.le Cascine 28, 50144 Firenze, Italy. [3]IBIMET-CNR, Via Gobetti 101, 40139 Bologna, Italy. E-mail: f.digennaro@ibimet.cnr.it*

The availability of new tools to detect and monitor vine health status was under evaluation in a vineyard heavily affected by grapevine leaf stripe disease (GLSD). The vineyard, located in Tuscany, had been surveyed for foliar symptoms each year from 2003 to 2012; each vine was mapped and classified for foliar symptom appearance and disease severity. During summer 2011 an unmanned aerial vehicle system ("VIPtero") was used to investigate the correlation between Normalised Differential Vegetation Index (NDVI) and GLSD foliar symptoms. The acquired high resolution multi-spectral images (0.056 m/pixel) were also analyzed for the detection of vines that had shown symptoms in the previous years, but were asymptomatic in 2011. All sample vines site locations were accurately measured with a Differential-GPS to extract single plant reflectance from the georeferenced multispectral images. Moreover during the last winter the vigour of the surveyed vines was measured by cane lengths, number of canes produced and total dry mass weight. The preliminary results obtained in the pilot experiment, showed a high level of correlation between NDVI and GLSD symptoms. The method has demonstrated both the ability to discriminate symptomatic from asymptomatic plants, but also to detect within the sample of asymptomatic plants, those that had shown GLSD symptoms in the past, suggesting the opportunity to have an innovative early detection tool for GLSD. The work offers a significant potential in terms of research perspectives and operational applications to improve the mapping of spatial distribution and development of GLSD.

The French Grapevine Trunk Wood Diseases Survey: overview of the data obtained over the last decade. J. GROSMAN[1], B. DOUBLET[2], P. LECOMTE[3,4], L. GUERIN-DUBRANA[4,3] and P. REY[4,3]. *[1]DRAAF-SRAL Rhône-Alpes, 165 rue de Garibaldi BP 3202 69401 LYON CEDEX 3 France. [2]DRAAF-SRAL Champagne-Ardenne, France. [3]INRA, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), F-33140 Villenave d'Ornon, France. [4]Université de Bordeaux, ISVV, UMR1065 SAVE, Bordeaux Sciences Agro, F-33140 Villenave d'Ornon, France. E-mail: patrice.rey@agro-bordeaux.fr*

After the ban of sodium arsenite in 2001, the National Grapevine Trunk Diseases Survey was established in

France in order to monitor the development of several grapevine diseases, in particular Eutypa dieback, Esca decline and Black Dead Arm (BDA). For the period 2003 to 2008, it was reported that 50 to 83% of the plots showed symptoms of Esca/BDA, the lowest value being obtained in 2003 (50%) and the highest in 2005 (83%), with an average of about 70%. For Eutypa dieback, the percentages were lower, varying from 30 to 53%. Over this period, the number of vines showing foliar symptoms of Esca/BDA was not very high but increased steadily from 1.04 to 3.23%. However, three points should be considered to obtain an overview of the situation: *(i)* From one year to another the same vines did not necessarily express foliar symptoms, so that when we considered a period of several years, the number of "vines which had displayed foliar symptomatic at least once" was much higher. *(ii)* Certain varieties were severely damaged, for instance about 10% of Sauvignon vines were infected in Aquitaine. *(iii)* It was recently estimated that around 11% of the French vineyard was unproductive due to these three diseases. In the Loire-et-Cher vineyard, the economic impact of the Esca/BDA alone has recently been estimated at 5-6 million euros per year. Everywhere, these grapevine trunk diseases are a matter of great concern for winegrowers, and have a definite impact on the economy of every vineyard region in France.

Cultural practices affecting esca and Botryosphaeria dieback prevalence of vineyards in the Alsace region of France. P. KUNTZMANN[1], J. BARBE[1], M. MAUMY-BERTRAND[2] and F. BERTRAND[2]. *[1]Institut Français de la Vigne et du Vin, Biopole, 28 rue de Herrlisheim, 68000 Colmar, France. [2]IRMA, Université de Strasbourg, 7 rue René-Descartes, 67084 Strasbourg, France. E-mail: philippe.kuntzmann@vignevin.com*

The Alsace region of France is much concerned by the declining of grapevines due to esca and Botryosphaeria dieback (Bot. dieback). There are few data concerning the impact of cultural practices on disease development. That's why esca and Bot. dieback prevalence data from a survey network of 82 vineyards over 8 years were analyzed in relation to cultural practices and agronomical variables. We considered cultural practices such as: the soil management, the application of sodium arsenite, the farming mode, the claimed AOC and the frequency of late harvest. Regarding agronomical variables, we considered for example the soil classification, the variety, the type of selection for planting material, the rootstock, the planting density, the vigour and the vegetative expression. The statistical analysis after preliminary analysis by principal component analysis and multiple correspondence analysis, followed a Partial Least Squares regression. This study showed that esca and Bot. dieback prevalence was correlated to the harvest dates and especially to the vineyard's frequency of late harvest for the production of sweet wines, particularly for the Gewurztraminer variety, among the set of different variables considered.

HOST-PATHOGEN INTERACTION

Defence responses in green stems, cordon and trunk of *Vitis vinifera* L. cv. Chardonnay in response to esca proper and apoplexy. M. MAGNIN-ROBERT[1], E. ABOU-MANSOUR[2], A. SPAGNOLO[1], L. MERCIER[3], C. CLEMENT[1] and F. FONTAINE[1]. *[1]Université de Reims Champagne Ardenne, UFR Sciences Exactes et Naturelles, Unité de Recherche Vignes et Vins de Champagne - EA 4707, Laboratoire de Stress Défenses et Reproduction des Plantes, Moulin de la Housse - Bâtiment 18, BP 1039, 51687 Reims Cedex 2, France. [2]Université de Fribourg, Département de Biologie des Plantes, 3 rue Gockel, 1700 Fribourg, Suisse. [3]Moët & Chandon, 20 Avenue de Champagne, 51200 Epernay, France. E-mail: florence.fontaine@univ-reims.fr*

Esca proper (E) is the proposed term to indicate the coexistence of two different syndromes of the grapevine trunk dieback, named esca and grapevine leaf stripe disease (GLSD), on the same plant. Apoplexy (A), consisting in the sudden wilt of partial or complete part of the crown, is regarded as the severe form of GLSD and/or esca proper. E and A, which incidence has increased in the last 10–15 years, may severely affect vineyard throughout the world. Information about the response of *Vitis vinifera* to these diseases is scarce, particularly on grapevine organs other than leaves. In the present work, to improve our knowledge in grapevine (cv. Chardonnay) responses to E and A, the perturbation of any targeted metabolism was observed in green shoot, cordon and trunk from E- or A-affected plants. Targeted genes and metabolites associated with defense and stress responses were investigated by qPCR and HPLC analysis, respectively. Foliar expression of E- and A-affected plants was accompanied by an increase of PR-protein- and oxidative stress-related gene expressions in their green shoot, cordon and trunk. Moreover, an up-regulation of the phenylpropanoid pathway-related gene expression and an accumulation of phenolic compounds were observed. Significant differences were noted for several gene expressions in green shoot, cordon and trunk according to the expressed form, E or A. Results indicate that (i) green shoot, cordon and trunk are able to react during foliar expression of E and A and (ii) various levels in defence responses are triggered upon onset of their symptoms.

Physiological changes in green stems of *Vitis vinifera* L. cv. Chardonnay in response to esca proper and apoplexy revealed by proteomic analysis. A. SPAGNOLO[1], M. MAGNIN-ROBERT[1], T.D. ALAYI[2], C. CILINDRE[1,3], L. MERCIER[4], C. SCHAEFFER-REISS[2], A. VAN DORS-

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Among all grapevine trunk diseases, esca proper and apoplexy represent a threat for viticulture worldwide. In order to retrieve further information about the mechanisms activated in apoplectic and esca proper-affected plants, a two-dimensional gel electrophoresis based analysis was conducted on green stems from 26-year-old standing vines. Symptomatic (A and E) and asymptomatic (aA and aE) stems from both apoplectic (A) and esca proper-affected (E) plants compared to control (without visual symptom for 10 years) stems were studied. Thirty-three differentially expressed proteins were identified by nanoLC-MS/MS and included into three groups conceptually defined as proteins involved in (i) metabolism and energy, (ii) stress tolerance, and (iii) defence response. Results clearly indicate that proteome variations occurred in apoplectic and esca proper-affected plants as compared to control plants, being therefore specifically related to the symptom appearance. However, remarkable quantitative differences were observed for several proteins according to the expressed syndrome (A or E). Consequently, variations in symptomatic stems of apoplectic plants (A) were likely different from those occurring in symptomatic stems of the esca proper-affected plants (E). In contrast, a similar response strategy seemed to be triggered in asymptomatic stems (aA and aE) of affected plants since no significant differences were detected among them. Therefore, it could be speculated that a diverse response (or effect) occurs in terms of protein expression upon onset of these different external symptoms. Overall, results suggest that esca proper and apoplexy symptom expression results in a different effect putatively caused by the same causal agent(s) on plant metabolisms.

Secondary metabolite production in esca-associated fungi and impact of fungicides on the biosynthesis rate. J. FISCHER[1], E. BIRNER[2], M. MERZ[2], J. RETHER[2], L. ANTELO[1], T. OPATZ[3] and E. THINES[1]. *[1]Institute of Biotechnology and Drug Research, Erwin-Schroedinger-Strasse 56, D-67663 Kaiserslautern, Germany. [2]BASF SE, 67114 Limburgerhof, Germany. [3]Johannes Gutenberg University Mainz, Department of Organic Chemistry, Duesbergweg 10-14, D-55128 Mainz, Germany. E-mail: thines@ibwf.de*

Esca is known as a destructive disease of grapevine caused by fungal pathogens that can behave as endophytes, including *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, and as wood decay, *Fomitipora mediterranea*. It has been suggested that phytotoxins are secreted by the fungi inducing disease development in the leaves and berries. Several toxins produced by esca-associated fungi have been reported previously. However, in order to characterize phytotoxic metabolites produced by the pathogenic fungi, the organisms were grown individually and in co-culture in submerged cultures. Several metabolites were identified by bioactivity-guided isolation and HPLC-MS as well as by NMR-analysis. The isolation and identification of the compounds were based on phytotoxic, cytotoxic and antimicrobial activities. Several fungal secondary metabolites were identified, which have not been reported yet for esca-associated fungi. Amongst the bioactive metabolites identified there were siderophores, e.g. triacetylfusigen as well as linoleic acid, methylemodin, phaeofuran and methoxycoumarin. The production rates of the bioactive secondary metabolites were analysed under stress conditions, including heat stress, salt stress or stress induced by sublethal concentrations of F500, a fungicide of the strobilurin class. It was found that the application of the fungicide at sublethal concentrations under salt stress conditions resulted in a significantly lower production rate of phytotoxic compounds.

Short-time kinetics of defence responses induced by *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora* in *Vitis vinifera* L. R. PIERRON[1,2], A. JACQUES[1] and S. COMPANT[2]. *[1]Université de Toulouse, Equipe Vins Viticulture et Œnologie, Dept. des sciences agronomiques et agroalimentaires, INP-EI Purpan, 75 voie du TOEC BP57611, 31076 Toulouse cedex 3, Toulouse, France. [2]Université de Toulouse, LGC UMR 5503 (CNRS/UPS/INPT), Dept. BIOSYM, INP-ENSAT, 1 Avenue de l'Agrobiopôle, 31326 Castanet-Tolosan, France. E-mail: romain.pierron@purpan.fr*

The epidemiology of fungal agents associated with Esca disease and defence mechanisms of grapevine wood are still poorly understood. However, the understanding of the grapevine-pathogen interactions is a pre-requisite to develop efficient biological control tools. A study was carried out to evaluate "woody cuttings" defence induction in *Vitis vinifera* L. challenged with two fungi associated with Esca disease. Four-weeks-old cuttings (5–6 leaves) of cv. Cabernet Sauvignon (clone 15) were inoculated with *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora*. Either spore solutions or mycelial plugs

were inoculated into 2–3 mm wounds made in the cuttings. Wood sections (0.5mm long) were collected 5, 12, 24, 72, and 120 h after inoculation with the phytopathogens. Induction of genes involved in jasmonic acid synthesis (*Lox*), salicylic acid synthesis (*Pal*), coding for chitinases (*Chit3b*), resistant protein (*PR10.3*), Glutamate synthase (*Glu*), and stilbene synthase (*Sts*) were then monitored by quantitative RT-PCR. Results showed that relative gene expressions of a wood section injured and inoculated with mycelium of either *Phaeoacremonium aleophilum* or *Phaeomoniella chlamydospora* were different compared to expression patterns of sections that have received mock inoculation. Plant perception of pathogens associated with Esca disease appears to be early and specific to microbes. This experiment provides new insights in molecular aspects of grapevine-pathogen interactions.

Effect of *Neofusicoccum parvum* and *Diplodia seriata* extra-cellular compounds on defence gene expression in *Vitis vinifera* cv. Chardonnay and cv. Gewurztraminer calli. M. RAMÍREZ-SUERO[1], J. CHONG[1], S. FARINE[1], F. KIEFFER-MAZET[1], F. PENSEC[1], I. GACOUGNOLLE[1], Y. LEVA[1], P. LARIGNON[2], P. KUNTZMANN[3], E. ABOU-MANSOUR[4], M. MAGNIN-ROBERT[5], F. FONTAINE[5] and C. BERTSCH[1]. [1]*Laboratoire Vigne Biotechnologie et Environnement EA 3391, Université de Haute-Alsace, UFR Pluridisciplinaire Enseignement Professionnalisant Supérieur, 33, rue de Herrlisheim, 68000 Colmar, France.* [2]*Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, France, Domaine de Donadille, 30230 Rodilhan, France.* [3]*Institut Français de la Vigne et du Vin, Pôle Alsace, France, 68000 Colmar, France.* [4]*Université de Fribourg, Département de Biologie, Unité Plantes, 3 rue Albert Gockel, 1700 Fribourg, Suisse.* [5]*Laboratoire de Stress, Défenses et Reproduction de Plantes URVVC EA 2069, Université de Reims Champagne-Ardenne, UFR Sciences Moulin de la Housse, BP 1039, 51687 Reims cedex 2, France. E-mail: christophe.bertsch@uha.fr*

Grapevine trunk diseases are the consequence of a complex of fungi. They attack the perennial organs of the vine eventualy causing the death of the plant. Over the past decade, the frequency of symptoms due to these fungi has considerably increased worldwide. Several fungi are associated with these diseases. In this study, we focused on pathogens associated with Botryosphaeria dieback. Three strains of *Neofusicoccum parvum* and 2 strains of *Diplodia seriata* from different geographical origins were studied. The effect of global extra-cellular compounds, fraction > 10kDa and several purified toxins (mellein from *D. seriata* and NpTox4 from *N. parvum*) were tested on grapevine calli of *Vitis vinifera* cv. Gewurztraminer and Chardonnay considered as sensible and less sensible to foliar symptom expression, respectively. Toxicity (evaluated by visual observations) and gene expression by Real-Time quantitative PCR at 1, 3 and 6 days post inoculation suggested a different pattern of defence gene expression between the two varieties of *V. vinifera*. Extracellular compounds, purified toxins and fraction > 10kDa, induced different patterns of responses. The optimized *in vitro* test allowed us to evaluate in a short time the direct effect of extra-cellular compounds and to determine the virulence of different strains of *N. parvum* and *D. seriata*. Our tests also allowed us to evaluate and understand the different susceptibility of *Vitis vinifera* varieties.

***Neofusicoccum parvum* a prolific source of secondary metabolites.** E. ABOU-MANSOUR[1], J-L DÉBIEUX[1], C.REGO[2] and P. LARIGNON[3]. [1]*Université de Fribourg, Département de Biologie, Unité Plantes, 3 rue Albert Gockel, 1700 Fribourg, Suisse.* [2]*Institut Supérieur d'Agronomie, Tapada da Ajuda, 1349-017 Lisbone, Portugal.* [3]*Institut Français de la Vigne et du Vin Pôle Rhône-Méditerranée, France, Domaine de Donadille, 30230 Rodilhan, France. E-mail: eliane.abou-mansour@unifr.ch*

Thirteen strains of the fungus *Neofusicoccum parvum* were isolated from different grapevine cultivars in the field and in nurseries. Organic extracts of each strain were analyzed by LC-UV-DAD and screened for their ability to produce diverse secondary metabolites. One strain was selected for further chemical studies and led to the isolation and characterization of eleven metabolites. Structures were elucidated by spectroscopic analyses including 1D and 2D NMR and HRMS, and by comparison to literature data. Isolated compounds belonged to three different chemical groups, including five compounds related to dihydrotoluquinones, two compounds to pyranones and four compounds to dihydroisocoumarins. We report here the structure identification of the isolated compounds and the preliminary phytotoxic activity on *Vitis vinifera* leaf discs assays.

Phenotypic evaluation and differential gene expression of *Lasiodiplodia theobromae* on grapevine wood. L.L. LÓPEZ-ZAMBRANO[1], J.L. STHEPHANO-HORNEDO[2] and R. HERNÁNDEZ-MARTÍNEZ[1]. [1]*Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada, Carretera Ensenada-Tijuana No. 3918, Zona Playitas, C.P. 22860, Ensenada, Baja California, Mexico.* [2]*Universidad Autónoma de Baja California, Facultad de Ciencias, Baja California, México. E-mail: ruhernan@cicese.mx*

Lasiodiplodia theobromae is one of the causal agents of Botryosphaeria dieback of grapevine; it is considered a highly virulent species and it was found in vineyards of Baja California, Mexico. Previous studies have shown that *L. theobromae* produces enzymes able to hydrolyze specific substrates (cellulose, xylan, pectin, etc.), and also it is able to produce toxins. However, little is known on the behavior of this fungus at phenotypic and genotypic

level during its interaction with grapevine. Thus, the objective of this work was to evaluate the growth and gene expression of this fungus in the presence of wood, and to identify putative genes related with pathogenicity and virulence. *In vitro* assays performed in Vogel's minimal medium and Vogel's salts, with and without grapevine wood, revealed higher rate of branching, biomass, and growth in the presence of wood, indicating that wood induces a change in gene expression. Additionally, the study of differential gene expression with or without wood, using the technique of suppression subtractive hybridization, identified a partial sequence of 125 bp, showing 65% similarity to an arabinanase present in the genome of *Aspergillus fumigatus*. Arabinanases are hydrolytic enzymes, part of the hemicellulases, which, together with cellulases, pectinases and ligninases degrade the plant cell wall. This is the first work to indicate that *L. theobromae* produces an arabinanase which, together with other hydrolytic enzymes may be involved in cell wall degradation of grapevine.

Control of a fungus involved in grapevine trunk disease (Esca) by using the oomycete, *Pythium oligandrum*: analysis of the induced resistance. J. GERBORE[1,3], N. MAGNIN[1,2], E. BRUEZ[1,2], J. VALLANCE[1,2], D. GRIZARD[3], C. REGNAULT-ROGER[4] and P. REY[1,2]. *[1]INRA, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), F-33140 Villenave d'Ornon, France. [2]Université de Bordeaux, ISVV, UMR1065 SAVE, Bordeaux Sciences Agro, F-33140 Villenave d'Ornon, France. [3]BIOVITIS, 15400 Saint Etienne de Chomeil, France. [4]Université de Pau et des Pays de l'Adour, UMR CNRS 5254/IPREM-EEM, IBEAS, 64013 Pau, France. E-mail: jonathan.gerbore@bordeaux.inra.fr*

Colonization of the rhizosphere by *Pythium oligandrum* is associated with the induction of the plant defense systems, *e.g.* it allows controlling *Botrytis cinerea* infections on grapevine or tomato. *P. oligandrum* produces elicitin-like proteins such as oligandrin and two cell wall protein (coded "POD1" and "POD 2"). When applied on plants, the elictin-like proteins also induce plant resistance. In our study, control of *Phaeomoniella chlamydospora* (a pathogenic fungus involved in esca grapevine trunk disease) was achieved by using *P. oligandrum* strains isolated from a Bordeaux vineyard. The *P. oligandrum* strains were identified by rDNA ITS region sequencing and the elicitors genes (oligandrin and POD1) were compared. A greenhouse assay conducted in 2010 and 2011 pointed out that Cabernet Sauvignon cutting necrosis caused by *P. chlamydospora* were reduced in half when *P. oligandrum* colonised the root system of young vines. Plant responses were investigated at the root and trunk levels by using *Vitis vinifera* micro-array (Roche-Nimblegen® 12x135K - GEO: GPL13936). Results obtained from the various plant/ *P. oligandrum*/ *P. chlamydospora* interactions show that the F-box protein, previously mentioned in plant reaction to biotic stress, is involved. Results will be discussed by taking into account and by comparing the up and down regulated genes for each treatment.

Phytotoxic secondary metabolites produced by *Neofusicoccum australe*, a pathogen involved in a grapevine trunk dieback in Italy. A. ANDOLFI[1], S. BASSO[1], A. CIMMINO[1], A. EVIDENTE[1], B.T. LINALDEDDU[2], S. SERRA[2] and L. MADDAU[2]. *[1]Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e della Produzioni Animali, Università degli Studi di Napoli Federico II, via Università 100, 80055 Portici, Italy. [2]Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università degli Studi di Sassari, via E. De Nicola 9, 07100 Sassari, Italy. E-mail: andolfi@unina.it*

Fungal trunk diseases have become a growing threat to viticulture throughout the world. Recent studies revealed that species in the Botryosphaeriaceae family include some of the main pathogens involved in grapevine canker, dieback and decline in most grape-growing regions of Northern and Southern hemispheres. A field survey conducted in a grape-growing area located in northern Sardinia (Italy) detected *Neofusicoccum australe* as one of the main fungal pathogens associated with grapevine cordon dieback. The nature and appearance of wood symptoms caused by this pathogen suggested that phytotoxic metabolites could be involved in the host-pathogen interaction. To our knowledge, no data are available in the literature on the production of phytotoxic metabolites by *N. australe* strains involved in grapevine trunk diseases. Thus, a highly virulent isolate of *N. australe* was examined for its ability to produce *in vitro* phytotoxic secondary metabolites. For this purpose, the fungus was grown in liquid culture. The culture filtrate and the corresponding organic extract showed to be highly phytotoxic when assayed on host and non-host plants by cutting and leaf puncture assay. This communication reports the chemical and biological characterization of phytotoxic metabolites produced in liquid culture by *N. australe* isolated in Sardinia from grapevine and other host plants. Their role in the disease will be discussed.

Role of the manganese transporter MntH as a pathogenicity factor in *Xylophilus ampelinus*, the causal agent of bacterial grape blight. S. SEVILLANO[1], R. COBOS[1], P. GARCÍA-ANGULO[2], J.L. ACEBES[2], E. GARZÓN[1] and J.J.R. COQUE[1]. *[1]Instituto de Investigación de la Viña y el Vino (IIVV). Universidad de León. Campus de Vegazana s/n. 24071-León, Spain. [2]Área de Fisiología Vegetal. Facultad de Ciencias Biológicas y Ambientales.Universidad de León. Campus de Vegazana s/n. 24071-León, Spain. E-mail: jjrubc@unileon.es*

The Gram-negative bacteria *Xylophilus ampelinus* is the causal agent of bacterial blight of grapevines, a dis-

ease producing severe economic losses in European and South African vineyards. This can be considered a grapevine trunk disease since the bacteria are able to survive and multiply in the xylem vessels as biofilms, persisting in the plants in a latent state for years. The bacterial blight of grape is characterized by typical symptoms such as necrotic foliar spots, cankers on both stems and petioles, and bud death. These symptoms, which sometimes are confused with those of fungal excoriosis, can result in the fast decline of both young and adult plants. We have developed a strategy based on mutagenesis by using a Tn5 transposon derivative to obtain avirulent or virulence-reduced mutants of the bacterium. Virulence degree has been checked in an assay using leaves of grapevine plants obtained from *in vitro* cultures (Tempranillo variety). Genetic analyses of one of the avirulent clones obtained (clon 23) have lead us to identify a gene (*mnt*H) encoding a manganese transporter. Mn^{2+} ion is an important cofactor for a number of enzymes, contributes to protection against oxidative stress, and is required for virulence. Studies are in progress to confirm the signification of MntH transporter as a virulence factor. Our data confirm that the isolation and analysis of mutants obtained by random insertion of transposons are a good strategy to identify virulence factors in *X. ampelinus*.

A proteomic approach for the identification of pathogenicity factors in the fungus *Diplodia seriata* involved in black dead arm of grapevine. R. COBOS[1], C. BARREIRO[2], R.M. MATEOS[1], S. SEVILLANO[1], E. GARZÓN[1] and J.J.R. COQUE[1]. [1]*Instituto de Investigación de la Viña y el Vino (IIVV). Universidad de León. Campus de Vegazana s/n. 24071-León, Spain.* [2]*Servicio de Proteómica. Instituto de Biotecnología de León (INBIOTEC). 24008-León, Spain. E-mail: jjrubc@unileon.es*

Diplodia seriata is a phytopathogenic fungus that produces severe infections in both grapevines and fruit trees. In grapevines is recognized as one of the most prevalent pathogens involved in grapevine trunk diseases, especially isolated from plants affected by black dead arm. Despite its importance as a pathogen there is a lack of information on how the fungus develops the disease process. In an attempt to better understand this process, we used a proteomic approach to identify potential virulence factors. Between the secreted proteins identified we detected as putative pathogenicity factors glucosidases, peptidases, and specially the necrosis and ethylene-inducing proteins Nep1 and Nep2 that represents a well-known class of necrotic elicitors. Nep-like proteins are responsible of several responses in many host plants and include accumulation of ROS (reactive oxygen species) and localized cell death that could be involved in the typical sectorial injuries found in trunks and arms of affected plants. Putative cytoplasmic pathogenicity factors were also detected including cyclophylins, malate dehydrogenase, and mitochondrial peroxiredoxin 1 (PRX1). Cloning of some of the corresponding genes are currently in progress as well as the development of a genetic transformation system in order to analyze the putative role of these proteins as virulence factors.

Response of *Vitis vinifera* cell cultures by culture filtrate of *Eutypa lata* and *Trichoderma atroviride*: Expression of defence-related genes. C. MUTAWILA[1], C. STANDER[2], A. DE BEER[3], M. VIVIER[2], F. HALLEEN[1,4] and L. MOSTERT[1]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa.* [2]*Institute of Wine Biotechnology, Department of Viticulture and Oenology, University of Stellenbosch, 7600, South Africa.* [3]*Department of Biochemistry, University of Stellenbosch, 7600, South Africa.* [4]*Plant Protection Division, ARC Infruitec-Nietvoorbji, Private Bag X5026, Stellenbosch, 7599, South Africa. E-mail: lmost@sun.ac.za*

Cell suspension cultures of *Vitis vinifera* cv. Dauphine (derived from green grape berry callus) were used to study the early response of *V. vinifera* to *Eutypa lata*, a vascular pathogen, and *Trichoderma atroviride*, a biological control agent. Grapevine cell cultures were treated with culture filtrate (2.5%) and the expression of defence-related genes profiled using quantitative RT-PCR. Pathogenesis-related proteins class 2, 3, 4, 6 and chitinase class IV were upregulated by *E. lata* fungal broth. Secondary metabolism genes of the phenylpropanoid pathway, phenylalanine ammonia-lyase (PAL), Coumaroyl Co-A (CCo-A), and stilbene synthase (STS) were also upregulated while chalcone synthase (CHS) was down regulated. STS and CHS catalyse the same substrate (4-coumaroyl-CoA and malonyl-CoA) to produce either trans-resveratrol (stilbene pathway) or tetrahydroxychalcone (flavonoid pathway), respectively. It is interesting to note that upon elicitation the grapevine up-regulates the STS for the production of phytoalexins while down-regulating CHS. Efficacy of such defence response in preventing infection is dependent on the level and the time of initiation of response by the plant. The bio-control *T. atroviride* culture broth also triggered similar genes and could potentially be used to enhance plant defence before pathogen infection. However, more work needs to be done to ascertain the effect of enhanced gene expression on defence related phenotypes.

Extracellular enzymes of *Phaeoacremonium aleophilum* isolates. A. GÓMEZ-GARAY[1], L. MARTÍN[1], P. MUÑOZ[1], M.L. TELLO[2] and B. PINTOS[1]. [1]*Department of Plant Biology I: Plant Physiology, Faculty of Biology, Universidad Complutense de Madrid (UCM).Ciudad Universitaria s/n. 28040 Madrid.* [2]*National Institute for Agricultural and*

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Phaeoacremonium aleophilum causes wood-degrading processes during petri/esca diseases. The mechanisms involved in virulence on grapevine for this phytopathogenic fungus could comprise qualitative and quantitative differences in the production of enzymes that degrade the plant cell wall. Eleven isolates of *P. aleophilum* were tested by their growth on diverse media. Heterogeneity among isolates regarding their growth on PDA, pectin and xylan media was observed. Three isolates showed faster growth on pectin media than in PDA. Nevertheless, only one isolate showed slower growth on xylan media than in PDA. Wood requires high dosage of xylanase for efficient hydrolysis. In general, *P. aleophilum* isolates showed the mechanism to degrade hemicelluloses (a mechanical barrier to fungal penetration).

Characterization of *Phaeomoniella chlamydospora* isolates through growth on diverse media enriched with plant cell-wall components. B. PINTOS[1], L. MARTÍN[1], C. PARRA[1], M.L. TELLO[2] and A. GÓMEZ-GARAY[1]. *[1]Department of Plant Biology I: Plant Physiology, Faculty of Biology, Universidad Complutense de Madrid (UCM).Ciudad Universitaria s/n. 28040 Madrid. [2]National Institute for Agricultural and Food Research and Technology (INIA).Ctra. de La Coruña, km. 7.5. 28040 Madrid. E-mail: magom02@bio.ucm.es*

Phaeomoniella chlamydospora causes wood-degrading processes during petri/esca diseases. These processes must start with degradation of primary walls. Degradation of plant cell walls is usually rapid and extensive during infection and implicates cell wall degrading enzymes as key factors involved in pathogenicity (disease symptoms, tissue susceptibility, and relative virulence). Five isolates of *P. chlamydospora* showed diverse morphological characteristics when grown *in vitro* and different levels of virulence in plants. The growth of these isolates on diverse culture media was tested. Heterogeneity among isolates regarding their growth in PDA and pectin media was found. Growth inhibition was detected in the xylan medium after four weeks of incubation, probably due to inhibition of enzyme production and activity of the end-product of xylan hydrolysis, xylose.

Phytotoxic exopolysaccharides produced by Botryosphaeriaceae spp., involved in grapevine trunk disease in Italy. A. CIMMINO[1], A. ANDOLFI[1], L. MADDAU[2], T. CINELLI[3], L. MUGNAI[3], J. LUQUE[4], A EVIDENTE[1]. *[1]Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e della Produzioni Animali, Università degli Studi di Napoli Federico II, via Università 100, 80055 Portici, Italy. [2]Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università degli Studi di Sassari, via E. De Nicola 9, 07100 Sassari, Italy. [3]Dipartimento di Biotecnologie-Patologia Vegetale, Università di Firenze, 50144 Firenze, Italy. [4]Institut de Recerca i Tecnologia Agroalimentàries (IRTA), 08348 Cabrils, Barcelona, Spain. E-mail: alessio.cimmino@unina.it*

Recently, several Botryosphaeriaceae spp. have been associated with grapevine decline symptoms worldwide including dieback, cankers and characteristic wedge-shaped necrosis in arms and trunks. An increasing number of species including *Neofusicoccum parvum* have been shown to produce phytotoxic secondary metabolites *in vitro*, both lipophilic low molecular weight compounds and high molecular weight exopolysaccharides (EPSs), some of which appeared structurally different from previously reported botryosphaerans. Preliminary results on the chemical and biological characterization of these EPSs were previously reported and compared with EPSs produced by *Phaeomoniella chlamydospora*, a fungus associated with grapevine esca complex along with *Phaeoacremonium* spp. In this communication, the progress on the chemical and biological characterization of EPSs produced by *N. parvum* and *P. chlamydospora* will be reported, as well as the results on the characterization of the EPSs produced by *N. australe*.

***In planta* study of *P. chlamydospora* and *P. aleophilum* colonization and interaction patterns.** J. POUZOULET[1,2], A. JACQUES[1], X. BESSON[2] and N. MAILHAC[1]. *[1]Université de Toulouse, INP, EI-Purpan, Département «Sciences Agronomiques et Alimentaires», Equipe «Vins, Viticulture et Œnologie», 75 voie du TOEC, 31076 Toulouse Cedex 3. [2]LVVD, ZA du Landreau, 49610 Mozé sur Louet. E-mail: nathalie.mailhac@purpan.fr*

Esca is mainly caused by xylem-inhabiting fungi such as *Phaeomoniella chlamydospora (Pch)* and *Phaeoacremonium aleophilum (Pal)*. These fungi colonize perennial tissues of vine plants causing typical symptoms including gum exudates from infected vessels, black spots and xylem parenchyma death which, result in a central red-brown discoloration in the form of longitudinal wood streaking. Manifestations in the field of *Pch* and *Pal* infected plants can take different forms according to the age of the vine. "Tiger-stripes" foliar symptoms are characteristic. Nevertheless, *Pch* and *Pal* can be isolated from central brown discoloration of the trunk and arms, even if no symptoms are observed. In order to determine the colonization patterns and the nature of the interactions between these two fungi in lignified tissues, detection and quantification of both *Pch* and *Pal* were studied using quantitative real-time PCR approach on one month old *Vitis vinifera* cv. Cabernet Sauvignon inoculated cuttings. This work showed that *Pal* growth is not affected in co-inoculation with

Pch, whereas *Pch* growth is delayed. Macroscopic and microscopic observations at 45 days post-inoculation showed that *Pch* produces wood streaking, and strongly inhibits bark response to injury, whereas *Pal* does not. In co-inoculated plants, intermediate phenotype is observed. However, the study demonstrated that even if no macroscopic phenotype can be observed in *Pal* inoculated plants, its DNA coul be detected in wood samples from a remote distance from the inoculation site, and in higher concentrations than *Pch* (5 fold higher). Perspectives of these approaches are discussed.

GRAPEVINE NURSERIES AND TRUNK DISEASES

Young Vine Decline in the Riverina is caused by co-infection by *Botryosphaeri*a and *Ilyonectria* at different propagation stages. M.A. WHITELAW-WECKERT, L. RAHMAN, L.M. APPLEBY, A. HALL, A.C. CLARK, H. WAITE and W.J. HARDIE. *National Wine and Grape Industry Centre, NSW Department of Primary Industries, Charles Sturt University, Wagga Wagga, NSW, Australia. E-mail: mweckert@csu.edu.au*

During the last decade many wine grape-growers in the Riverina wine region of NSW, Australia have purchased diseased young grapevines. These plants typically fail in the first season or are developmentally retarded, low yielding, and die prematurely. This syndrome, Young Vine Decline (YVD), was most commonly reported in grafted grapevines. We have shown that the cause of YVD in the Riverina is the co-infection by *Botryosphaeria* spp. and *Ilyonectria spp*., infecting at different stages of the nursery propagation process. Although both *Botryosphaeria* and *Ilyonectria* alone cause the decline and death of young grapevines, co-infection leads to more severe disease symptoms. Co-infection is initiated when cuttings are taken from source rootstock plants infected by *Botryosphaeria*. Those cuttings, and others cross-contaminated during propagation, are then invaded by strains of *Ilyonectria macrodidyma* or *Ilyonectria liriodendri.* These Riverina strains were shown to produce phytotoxic brefeldin A. Consequently the rootstock stem below the graft union becomes internally infected with both *Ilyonectria* and *Botryosphaeria,* while usually the scion is initially uninfected. As a result, *Ilyonectria* disrupts root function and retards early plant development while *Botyrosphaeria* gradually invades the xylem. *Botryosphaeria* hyphae move basipetally to the roots and acropetally through the graft union to the scion, thus leading to eventual plant death. As *Botryosphaeria* infections are especially severe when the host plant has been subjected to water stress, we speculate that water-stress induced by brefeldin A-impaired root growth accounts for the particularly debilitating consequences of co-infection by these pathogens.

Occurrence of *Ilyonectria* spp. in the grapevine nursery propagation process in Spain. C. AGUSTÍ-BRISACH[1], D. GRAMAJE[2], J. GARCÍA-JIMÉNEZ[1] and J. ARMENGOL[1]. *[1]Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain. [2]Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, APDO. 4084, 14004 Córdoba, Spain. E-mail: caragbri@upvnet.upv.es*

Two commercial nurseries located in Comunidad Valenciana region (central-eastern Spain) were surveyed in 2010. In both nurseries, samples from pre-grafting hydration tanks, secateurs used for cutting buds, omega-cut grafting machines and peat used for callusing were collected. DNA from these samples was extracted and analyzed by multiplex nested-PCR using specific primers for *Ilyonectria* species. *Ilyonectria liriodendri* and *I. macrodidyma*-complex were detected in hydration tanks, secateurs, grafting machines and peat, *I. macrodidyma*-complex being the most frequent. In addition, ten cuttings each from five grapevine scion/rootstock combinations were collected from each nursery immediately after callusing, and again after one growing season in an open-root field nursery. Roots of these cuttings were analyzed for fungal isolation. *Ilyonectria liriodendri*, *I. novozelandica* and *I. torresensis* were isolated after callusing and after one growing season, showing the highest incidence in this latter sampling moment. *Ilyonectria novozelandica* was the most frequent species isolated. Moreover, DNA from roots of these cuttings was extracted and analyzed as described before. *Ilyonectria liriodendri* and *I. macrodidyma*-complex were also detected at both sampling times. The use of the multiplex nested-PCR technique improved the detection of *I. liriodendri* and *I. macrodidyma*-complex from cuttings in both nurseries. Our results demonstrate that hydration tanks, grafting machines, secateurs and peat for callusing are potential infection sources for *Ilyonectria* spp. in nurseries, and confirm that grapevine planting material is highly infected by *Ilyonectria* spp. after growing in open-root fields.

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Unveiling inoculum sources of Black foot pathogens in a commercial grapevine nursery. M. CARDOSO*, I. DINIZ*, A. CABRAL, C. REGO and H. OLIVEIRA. *CEER-Biosystems Engineering, Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda 1349-017 Lisboa, Portugal. E-mail: heloliveira@isa.utl.pt.* Both authors have contributed equally to this research.*

Black foot of grapevine is an important disease caused primarily by *Ilyonectria* species. These pathogens affect

grapevine nurseries and young vineyards, causing the decline and death of plants. In the nursery, the primary infections of the grafted cuttings are mainly attributed to soil-borne inoculum that infects the roots and the basal end of rootstocks during the rooting process. .Currently, the frequency of infected planting material is a major constraint for the nursery industry. The aim of this research was to detect possible sources of inoculum for *Ilyonectria* spp. and "*Cylindrocarpon*" *pauciseptatum* throughout the different nursery stages using classical and molecular techniques (nested-PCR and/or multiplex nested-PCR). Multiplex nested-PCR allowed the simultaneous detection of *I. liriodendri, I. macrodidyma sensu lato* and "*C.*" *pauciseptatum* and the identification of unknown sources of inoculum for these fungi. Sampling included plant material (rootstock and scion cuttings, grafted-cuttings and rooted graftlings), cutting tools, water from hydration tanks, well water, callusing medium and soil (from mother-fields, nurseries and plots in rotation). Air-samples (from cold-chambers and warehouses) were tested only by classical methods. Positive results were obtained for plant materials, cutting tools and soils (by both techniques). Water from hydration tanks, well water and callusing-medium tested positive for *Ilyonectria* fungi only by molecular techniques, whereas one air-sample (cold-chamber) tested positive with classical methods. Results of qPCR analysis applied to soil samples reveal that the compulsory field nursery rotation system used in Portugal (fields are used only once every 4 years in the crop rotation) unequivocally contributes to the *reduction* of soil-borne inoculum.

Study of grapevine trunk disease causal agent colonization in nurseries using quantitative real-time PCR approach. J. POUZOULET[1,2], M. TOMÁS[1], C. COUDERC[1], A. JACQUES[1], X. BESSON[2] and N. MAILHAC[1]. [1]*Université de Toulouse, INP, EI-Purpan, Département «Sciences Agronomiques et Alimentaires», Equipe «Vins, Viticulture et Œnologie», 75 voie du TOEC, 31076 Toulouse Cedex 3.* [2]*LVVD, ZA du Landreau, 49610 Mozé sur Louet. E-mail: nathalie.mailhac@purpan.fr*

Grapevine trunk diseases (GTD) represent a real threat to viticulture and are responsible for significant economic losses to the wine industry. Causal agents infect wounds made during vineyard management. At the nursery, that is suspected to be the first contamination step, they are present on mother vines cane surfaces and are spread in the multiplication process to infect plant material during soaking and stratification. They attack perennial organs, leading to extensive inner necrosis in trunk and arms. Trunk diseases appear several years after the first infection. GTD fungi have in common a slow growth and induce extremely complex and variable symptom expression, making diagnosis difficult. Previous methods to detect disease consisted of microbiological isolation of fungi from wood samples on semi-selective media. GTDs are fastidious and this method frequently leads to false negative results because pathogens are often overgrown by other microorganisms. In this study our goal was to develop a rapid detection and quantification tool to evaluate contamination and wood colonization patterns of the fungi. We have developed a real-time PCR approach in multiplex conditions. This work has shown that low inoculum pressure in soil can lead to random infection in wood. Evaluation of colonization patterns of naturally infected grafted plants at the nursery has shown that most positive cases were found at the base of the plants rather than at the graft point. The advantages and relevance of this technique for evaluating GTD infection and spatial distribution in nursery vines is discussed.

Soaking grapevine cuttings in water: a potential cause of cross contamination by micro-organisms. H. WAITE[1], D. GRAMAJE[2,3], M. WHITELAW-WECKERT[1], P. TORLEY[1] and W.J. HARDIE[1]. [1]*National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia.* [2]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.* [3]*Department of Crop Protection, Institute of Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain. E-mail: hwaite@csu.edu.au*

Grapevine nurseries routinely soak large quantities of cuttings in water for between 1 and 12 hours during propagation in the belief that itcompensates for dehydration. However, grapevine trunk disease pathogens have been isolated from soaking water thus indicating that cross contamination of the cuttings with bark-inhabiting microorganisms is likely. In this experiment cuttings of *Vitis vinifera* cv. Sunmuscat and *V. berlandieri* × *V. rupestris* rootstock cv. 140 Ruggeri were immersed in distilled, deionised water for varying periods (1, 2, 4, 8 and 16 hr). Samples of soaking water were cultured (25°C for 3 days) on a non-specific medium and specific media for fungi and bacteria. In addition, after soaking, the base of each cutting was debarked and trimmed by 3mm and three 3mm, contiguous, transverse slices of wood were cultured (25°C for 3 days) on potato dextrose agar. The soaking water for both cultivars became contaminated with fungi, yeasts and bacteria within the first hour. The number of fungi isolated from the wood slices of both cultivars soaked for one hour was significantly greater than those from non-soaked cuttings. The number of yeast and bacterial colonies growing from the soaked wood slices was accordingly also greater but only after the cuttings had been soaked for 2–4 hours. These results confirm that soaking cuttings is a potential cause of cross contamination during propagation and demonstrate contamination of cuttings occurs after rela-

tively short periods of soaking. Accordingly we suggest that alternative methods be employed to prevent dehydration of cuttings during processing.

Changes in protein expression and growth in hot water treated cuttings of *Vitis vinifera* cvs. Pinot Noir and Cabernet Sauvignon. H. WAITE[1], S. TAWFILIS[2], M. AYTON[3], M. WHITELAW-WECKERT[1], P. TORLEY[1] and J. HARDIE[1]. *[1]National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW, Australia 2678. [2]GE Healthcare Life Sciences, 3/310 Ferntree gully road, Nottinghill, Melbourne, Victoria, Australia, 3168. [3]School of Animal and Veterinary Science, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW, Australia 2678. E-mail: hwaite@csu.edu.au*

Grapevine nurseries report unacceptable losses of hot water treated cuttings of some *Vitis vinifera* cultivars, particularly Pinot Noir, that held in cold storage after hot water treatment (HWT). Previous research has shown variable cutting viability attributed to interactions between cultivar, HWT, storage conditions and duration of storage. To understand the molecular basis for the observed variability in plant viability, the effects of HWT and anaerobic cold storage conditions on growth and protein expression of cuttings of Pinot Noir (sensitive) and Cabernet Sauvignon (tolerant) were examined over time. Cuttings were sampled for protein analysis 1, 24, 48 and 72 hours after HWT and after 6 and 13 weeks of cold storage. Shoot growth after 6 and 13 weeks cold storage was measured weekly for 11 weeks and shoot length was measured in midsummer and at leaf fall. Preliminary results have revealed significant differences between the proteomes of the two cultivars before HWT; a probable reflection of the different genotypes. Changes in protein expression after HWT in both cultivars appear to persist for more than 72 hr following HWT and are likely to be due in part to the expression of heat shock proteins that maintain functional protein integrity and thus cell function under heat stress. Growth patterns of cuttings after cold storage varied with initial treatment and duration of storage, thus pointing to more sustained changes in protein expression that may be epigenetic in nature rather than the more transient heat shock protein expression. Significant proteins are being analysed for identity and function.

Wood decay diseases in grapevine nursery: focus on scion cuttings and rootstocks. V. VIGUÈS[1], O. YOBREGAT[1], B. MILLE[1], F. BÉRUD[2], V. AYME-SEVENIER[3], C. BAPTISTE[4] and P. LARIGNON[4]. *[1]Institut Français de la Vigne et du Vin, Pôle Sud-Ouest, V'Innopôle 81310 Lisle sur Tarn, France. [2]Chambre d'agriculture du Vaucluse, 2260, route du Grès, 84100 Orange, France. [3]SPBPVV, 384, route de Caderousse, 84100 Orange, France. [4]Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France. E-mail: virginie.vigues@vignevin.com*

The aim of this study was to evaluate the impact of variety, sanitary state of mother vine fields and the distance from the mother vine crown along the cane on the rate of contamination by fungi associated with wood decay diseases on scion cuttings, rootstocks and grafted vines. To study the impact of varieties, woody tissue of 1580 scion cuttings of five varieties (Duras, Sauvignon Blanc, Syrah, Mourvedre, Gamay) and 1580 rootstock cuttings (3309 Couderc, 110 Richter, Gravesac, Fercal) was analyzed using a microbiological method. To study the impact of distance from the mother vine crown along the cane, 3 repetitions of 100 scions and 3 repetitions of 100 rootstocks from each zone were analyzed. Three zones on the canes were defined: the base (near the mother vine crown), the middle and the end of the cane. To study the impact of sanitary state, three Sauvignon Blanc, three Mourvedre and six 110R mother fields were chosen. The choice was based on the increasing ages of the vines, mortality of the mother vines (dead plants, missing plants) and decay diseases (foliar symptoms for the grapevines). For each mother field, 80 grafted buds and 80 rootstocks were analyzed. Finally, these studies showed no impact either of varieties, or of sampling areas or of sanitary state on the contamination rate by fungi associated with wood decay diseases in woody tissues of scion and rootstock cuttings. In total, 0.13% of scion cuttings and 0.06% of rootstocks were contaminated by esca fungi (*Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*) and 0.3% of scion cuttings and 2% of rootstocks, by fungi associated with Botryosphaeria dieback.

Analysis of a Spanish grapevine nursery indicates the prevalence of *Cylindrocarpon* sp. as the most frequently isolated fungal pathogen in both grafted plants and nursery soils. M.A. OLEGO, V.M. GARCÍA-MARTÍNEZ, J.J.R. COQUE and E. GARZÓN. *Instituto de Investigación de la Viña y el Vino (IIVV). Universidad de León. Campus de Vegazana s/n. 24071-León, Spain. E-mail: egarzon@unileon.es*

The occurrence of fungal grapevine trunk pathogens associated with grapevine nurseries has been previously reported from studies conducted in several countries. Here we describe a study conducted in a Spanish commercial nursery from the Comunidad Foral de Navarra (Spain). A total of 193 grafted plants were analyzed from years 2008 to 2010. Each plant was analyzed by monitoring the presence of fungal pathogens at five different points: roots, basal rootstock (roots insertion point), distal rootstock (5 cm below the graft), graft and

scion. A total of 86 plants (44.6%) were infected by at least one fungal pathogen. *Cylindrocarpon* sp. was the more frequently isolated pathogen (39.4%). Other species detected were *Phaeomoniella chlamydospora* (infecting 3.6% of the plants), *Diplodia seriata* (2.6%), *Phaeoacremonium aleophilum* (1.5%) and *Eutypa lata* (1.0%). These results indicate that many plants are contaminated in the grapevine nurseries and that in this case, *Cylindrocarpon* sp. was the prevalent pathogen, being specially detected in both roots and basal rootstock. However *Cylindrocarpon* sp. was also frequently detected in grafts (6.2%) and scions (7.8%). An analysis of the nursery soils (35 samples) was performed in parallel indicating that *Cylindrocarpon* sp. was present in the 74.3% of the soils analyzed. This data suggests that the control of the *Cylindrocarpon* sp. populations in soils could be a suitable strategy to reduce the rate of *Cylindrocarpon* sp. infection in nursery vines.

An innovative nephelometric *in vitro* method to evaluate the effect of different doses of a biological sterilizer treatment used in nurseries to control *Diplodia seriata* and *Neofusiccocum parvum*. CALMES BENOÎT[1], ZEKRI OLIVIER[2]. *[1]IRHS FungiSem, 2 bd Lavoisier, 49045 Angers cedex, France. [2]MERCIER Frères S.A.R.L, 16, Rue de la Chaignée, 85770, Vix, France.*

Treatments against fungal growth are usually evaluated first through fungicide sensitivity screenings using suitable growth media in Petri dishes. Nephelometry is a direct method of measuring light scattered by particles in suspension. Since the scattered light intensity is directly proportional to the suspended particle concentration, nephelometry could be used for recording microbial growth in liquid medium through turbidity measurements, especially for studying filamentous fungi. The effect of a biological sterilizer used at different concentrations on the growth of *Diplodia seriata, Neofusiccocum parvum* and *Trichoderma atroviridae* was assessed by using this nephelometric method. *Diplodia seriata* is often found in grapevine propagation material, while *N. parvum* is a virulent species within the grapevine trunk disease complex and *Trichoderma atroviridae* (I-1237) is used as a biological control agent during the production process in nursery. The biological sterilizer was used at concentrations of 6, 25, 50 and 100%. We observed inhibition of fungal growth between 90 and 100% for the 3 fungi at the 2 highest product concentrations, while we observed an inhibition of fungal growth between 80 and 95% for the two pathogens at the concentration of 25% when *Trichoderma atroviridae* growth inhibition was just 50%. The results obtained suggest first than the biological sterilizer could be used for growth reduction of some pathogens and second that nephelometry could be a good alternative method for treatment assessment.

Evaluation of factors that influence the viability of hot-water treated planting material in Spanish nurseries. D. GRAMAJE[1,2] and J. ARMENGOL[1]. *[1]Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. [2]Department of Crop Protection, Institute of Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain. E-mail: dgramaje@ias.csic.es*

Hot water treatment (HWT) at 53ºC for 30 min has been shown to be an effective control method for blackfoot and Petri disease pathogens in Spanish grapevine nurseries. However, there is still confusion in nurseries about the efficacy and safety of HWT. The anecdotal reports of unacceptably high rates of mortality when HWT is applied by nurseries to commercial batches of cuttings prior to callusing and to young rooted vines ready for dispatch has resulted in a reluctance by Spanish nurseries to use HWT. Several critical factors, including plant hydration and cold storage, have emerged in the production of quality grapevine planting material. In order to determine the most reliable HWT protocols, the effects of HWT at 53°C for 30 min., hydration (post-hydration HWT or no post-hydration HWT) and cold storage (0, 1, 2 and 4 weeks) on sprouting and shoot length and weight in dormant grafted plants (Tempranillo cultivar grafted onto 110 Richter rootstock) were evaluated. Eight bundles of 10 cuttings were treated for each factorial combination, and eight additional bundles of 10 untreated cuttings were prepared as controls (no-HWT). Dormant grafted plants were immediately planted in two field sites. At the end of the growing season, cutting sprouting and shoot length and weight were evaluated. Little variability was found in the percentages of sprouting among treatments. A significant reduction in shoot length and weight data was observed for all treatments with respect to the control. There was a significant reduction in values for all variables studied in non-hydrated cuttings stored during 4 weeks in cold room immediately after HWT. This finding indicates that extended cold storage times could be detrimental to planting material, especially when plants have not been previously hydrated following HWT.

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Hot water treatments used to manage infections caused by fungal trunk pathogens in the grapevine propagation process in Peru. J. MUNIVE[1], D. TAMAYO[1], C. CASTILLA[1,2] and L.A. ÁLVAREZ[2]. *[1]Vivero Los Viñedos SAC. Panamericana Norte km 508,5 Virú, La Libertad - Peru. [2]Departamento de Sanidad Vegetal, Universidad Nacional*

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Lasiodiplodia theobromae and *Phaeoacremonium parasiticum* are the main pathogens associated with infections of dormant cuttings in the grapevine propagation process in Peru. Hot water treatment (HWT) was used to manage natural infections by these pathogens. *In vitro* assays using mycelia and conidia of these pathogens determined a treatment of 53°C for 30 min as the minimum temperature/time combination required to reduce *P. parasiticum* infections, and 52°C for 30 min to control infections caused by *L. theobromae*. The response of rootstocks to HWT was quite variable; Dog Ridge, Harmony, MGT 101-14 and R-110 tolerated temperatures up to 53°C for 30 min, while Freedom and Salt Creek were less tolerant. The response of scions was more homogeneous. In controlled assays HWT was highly effective against infections caused by *L. theobromae,* and reduced infections caused by *P. parasiticum* in dormant cuttings. In addition, the HWT demonstrated a positive effect on the sanitation of planting material by controlling other fungi and arthropods that may be present on woody material. HWT represent an effective way of eliminating infections in the grapevine wood in Peru, thus preventing the development of fungal trunk pathogens in young vines.

Research on the incidence of pathogens leading to Esca and other trunk diseases in grapevine propagation material in Germany. A. KORTEKAMP, J. KÖCKERLING and J. EDER. *Department of Phytomedicine, State Service Center Rheinpfalz for Research, Teaching and Consulting in Viticulture, Horticulture and Rural Development, Breitenweg 71, D-67435 Neustadt/Weinstrasse, Germany. E-mail: andreas.kortekamp@dlr.rlp.de*

As in other vine growing countries, Esca in German vineyards is caused by a complex of fungal pathogens. Even though *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum*, and *Fomitiporia mediterranea* are the most prevalent pathogens in affected trunks, *Botryosphaeria obtusa*, *Ilyonectria* spp., and *Trichoderma* species were the dominant pathogens found in propagation material and one year old shoots. A survey of a 20-year old rootstock mother block of five different varieties over three growing seasons (2009 to 2011) revealed that nearly all rootstock mother plants exhibited Esca symptoms such as wood degradation. One year old woody cuttings from these mother plants showed dark brown or black streaks in the vascular tissue, as well as tyloses and gums in the xylem, mostly located in the basal part of the shoot. The main fungi isolated from the one year old cuttings were saprophytic species such as *Alternaria* sp., *Aspergillus* sp., *Chaetomium globosum*, *Cladosporium* sp., *Gliocladium* sp., *Graphium* sp., *Phomopsis viticola* and *Sclerotinia* sp. However, *Ilyonectria* spp. and *B. obtusa* were also frequently detected. Cuttings made from apparently healthy shoots taken from mother vines with Esca symptoms in the trunk performed poorly in propagation compared to cuttings taken from healthy mother vines. Only 23% of the grafted plants taken from symptomatic mother vines could be grown successfully in the nursery compared to 65% in the control group even though all shoots with wood symptoms had been discarded before propagation. Analysis of rooted plants ready for planting from a private nursery showed that about 30% of these plants were infected with *Pa. chlamydospora* and/or *Botryosphaeria*. In a second experiment, ten different rootstock-scion combinations were investigated. The rate of infestation with *B. obtusa* varied depending on the combination and reached up to 50% (Riesling). For infection experiments, cuttings of several grapevine cultivars each bearing one node were inoculated with *Pa. chlamydospora*, *Pm. aleophilum*, and *B. obtusa*. These cuttings showed a reduced production of green shoots compared to the untreated control, especially in the case for Riesling which seems to be more susceptible to Esca pathogens compared to other cultivars.

DISEASE MANAGEMENT

Optimising pruning wound protection to manage eutypa dieback. M.R. SOSNOWSKI[1], M.R. AYRES[1], T.J. WICKS[1] and E.S. SCOTT[2]. [1]*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia.* [2]*School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond SA 5064 Australia. E-mail: mark.sosnowski@sa.gov.au*

The fungus *Eutypa lata* infects grapevines through pruning wounds, colonising wood of cordons and trunks and causing eutypa dieback. Wound treatments reduce infection by *E. lata*, and benomyl and carbendazim, which were the most effective fungicide treatments, are no longer available for use on grapes in Australia. To provide data to assist registration in Australia, five alternative fungicides, three natural products and two surfactants were selected for field evaluation, based on results from previous research. Treatments at different concentrations were applied by hand and wounds inoculated with ascospores of *E. lata*. Twelve months later, treated canes were removed and assessed by isolation on PDA. Results indicated that three of the fungicides significantly reduced infection by *E. lata* and efficacy increased with dose rate; tebuconazole (59-88% control), fluazinam (21–58%) and pyrimethanil (33–53%). There was no benefit in adding surfactants Pentrabark or Du-Wett and natural products did not reduce infection significantly. The fungicides were also evaluated in the greenhouse using a

detached cane assay where treatments were assessed 4 weeks after application and results suggest it may offer a rapid alternative method of evaluation. Commercial sprayers (air-blast, air-shear, fan and recycle) were evaluated with the aim of improving the efficiency of applying pruning wound treatments. Using tebuconazole, some sprayers achieved control of eutypa dieback similar to hand-application, providing deposition was maximised by focussing spray at the pruning wound zone using water volume of 600 L ha^{-1}. Results will be discussed in relation to optimising management of eutypa dieback.

Effects of cultural practices on grapevine trunk diseases: results of a long-term experiment. V. DUMOT[1], G. SNAKKERS[1], P. LARIGNON[2], P. LECOMTE[3], P. RETAUD[4], S. DAVID[5], E. MENARD[1] and L. LURTON[1]. *[1]Bureau National Interprofessionnel du Cognac, 23, Allées Bernard Guionnet - BP 18 - 16100 Cognac Cedex, France. [2]IFV, Domaine de Donadille, 30230 Rodilhan, France. [3]INRA, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), 33140 Villenave d'Ornon, France. [4]Chambre Régionale d'Agriculture de Poitou-Charentes, 3, boulevard Vladimir, 17100 Saintes, France. [5]Lycée Georges Desclaude, rue Georges Desclaude, 17100 Saintes, France. E-mail: vdumot@bnic.fr*

A collaborative study was carried out in the Cognac area (France) to evaluate the effects of cultural practices on Eutypa dieback and esca. A vineyard of 2 hectares of cv. Ugni blanc was planted in 1991 and three factors were assessed: training system, fertilization, and rootstock. Two training systems were compared: guyot system and bi-lateral cordon. Three levels of fertilization were applied: no fertilizer, 50N.0P.100K each year, and 100N.0P.100K each year plus basic dressing. Four rootstocks were compared: 41B, Fercal, RSB and Rupestris du Lot. The experimental design comprised 336 plots of ten plants. Each plot was a combination of the three factors. Foliar symptoms and mortality were recorded every year from the date of planting until 2010. The variables used as a result were the proportion of plants that showed one symptom at least one year, and the percentage of dead plants. Results showed significant effects on foliar symptoms and on mortality, but not always in the same way. The training system had a strong effect, especially on Eutypa dieback symptoms, likely linked to the number and the size of the pruning wounds. The guyot system had less foliar symptoms but more dead plants. The higher the fertilization, the more severe the symptoms and the mortality were. Rootstock 41B had less symptoms of Eutypa dieback, but more symptoms of esca. The three other rootstocks had similar results. The mortality was highest for Rupestris du Lot. This study provides information that can assist winegrowers with managing trunk diseases.

Control of grapevine trunk diseases in California. W.D. GUBLER and F. PEDUTO. *Department of Plant Pathology, University of California Davis, One Shields Avenue, Davis, CA 95616, USA. E-mail: wdgubler@ucdavis.edu*

Control of grapevine trunk diseases has been illusive for many years with attempts to find chemical barriers or cultural methods that would stop or reduce spore germination and fungal penetration into the pruning wound . In recent years we have been able to register myclobutanil and thiophanate methyl as a tank mix that can be applied by tractor as an over the vine spray on fresh pruning wounds with multiple applications if necessary. While this treatment is effective in stopping infection, it lasts only 2–3 weeks while pruning wounds remain susceptible for 6–8 weeks during the winter. Due to increased susceptibility of pruning wounds during winter months and decreased susceptibility during late winter and early spring months, we developed the practice of double pruning in order to allow growers with large acreage to be able to wait until late winter or early spring to make the final pruning. This practice of double pruning allows approximately 90–95% disease control simply due to rapid wound healing in late winter and early spring. Followed by a fungicide (myclobutanil and thiophanate methyl) application, this practice should give nearly 100% disease control under California conditions. Further to this new control strategy, we have developed and new product (Vitiseal) that when used alone gave 100% disease control in field and laboratory studies. Vitiseal is an organic treatment when used alone but when applied with fungicide, the fungicide has a longer decay rate thus giving an extra measure of control for a longer duration. In the last year we have tested 2 isolates of *Trichoderma viride* that have shown excellent control of canker diseases when applied in late winter or early spring. One of these isolates was recovered from grapevine pruning wounds 3–4 weeks after pruning and has been documented to be one of the first natural colonizers of pruning wounds. This fungus is highly aggressive and colonizes pruning wounds rapidly. One isolate is being developed into a biocontrol product.

***Trichoderma atroviride* SC1 is a good wound colonizer and can protect grapevine from infections of *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora* in nurseries and vineyards.** D. PRODORUTTI[1], A. PELLEGRINI[1], A. COLOMBINI[1], B. CHARLOT[2], and I. PERTOT[1]. *[1]Fondazione Edmund Mach, via E. Mach 1, S. Michele all'Adige 38010, Italy. [2]AGR Sarl, 39 rue de Wissous, 91320 Wissous, France. E-mail: daniele.prodorutti@iasma.it*

Phaeoacremonium aleophilum (Pal) and *Phaeomoniella chlamydospora* (Pch) are two of the most important species associated with Esca disease in grapevines. They are frequently isolated from vines showing symptoms

of Esca disease in different countries. Plant colonization by these two microorganisms takes place via wounds. Therefore wound protection during all stages of grapevine propagation and in established vineyards is of extreme importance to prevent infection by these Esca-associated microorganisms. However, products containing either chemicals or biocontrol agents have given inconsistent results so far. The main reason for failure is most likely the short duration of protection. The strain *T. atroviride* SC1 was isolated from decayed wood, it has an outstanding capacity of colonizing wounds and it is a strong producer of hydrolytic enzymes. We evaluated its capability of colonizing wounds and efficacy of preventing Pal and Pch infections either under natural infections and artificial inoculation in established vines. *T. atroviride* SC1 was able to colonize wounds for several months after application and to protect plants along the growing season. When applied in the nursery process it successfully colonized almost all parts of the plants (rootstocks and scions) and prevented infections by Pal and Pch during grafting. The level of colonization was stable during the entire process giving a percentage of colonization of plants close to 100% at the time of commercialization. The trials were carried out for 3 years in several sites in Italy and France, indicating a good adaptability to different environmental conditions and consistency of results. *T. atroviride* SC1 may represent an interesting strain for further developments in the protection of grapevine wounds from Esca-associated microorganisms.

Methodological approach for an effective and reliable field assessment of biological control agents against grapevine trunk diseases. E. PAJOT, E. MOUNIER, C. BRIER, F. CORTES and A. COUTANT. *Agrauxine, 2 Rue Henri Becquerel, 49070 Beaucouze, France. E-mail: emmanuel.pajot@agrauxine.fr*

Esquive® WP is a biological control product containing the fungus *Trichoderma atroviride* strain I-1237. The product was registered in France for the prevention of grapevine pruning wound infection by *Eutypa lata*, the causal agent of Eutypa dieback. Since 2009 Esquive® has been applied to pruning wounds in order to investigate its effect on the expression of dieback symptoms, and on other grapevine trunk diseases such as Esca and Black Dead Arm (BDA). The experimental protocol was improved over two seasons (2010 and 2011), and with the support of statistical analyses (Principal Components Analyses) it was shown that specific experimental plan parameters were important to assess the efficacy of biological control agents (BCA) on trunk disease expression under natural field conditions. Observations and statistical analysis of the 2011 data, supported by results from previous years, allowed us to come to the following conclusion. For a BCA like *Trichoderma* to express significant efficacy, in particular towards complex grapevine trunk diseases, three main experimental factors are required; i) trials spanning multiple years and repetition of treatment; ii) the level of symptom expression during the year; iii) the sample size (number of plants) assessed. For example, on the bases of our observations we can conclude that when you note a sample with a number of plants by modality up to 500, associated with a level of trunk disease expression up to 5% in the control, you can make significant difference between the control and the BCA modality concerning the symptom disease expression. So, under natural conditions, to efficiently assess a new BCA product on grapevine trunk diseases, these parameters should be used.

Biocontrol of grapevine trunk pathogens with vine-specific antagonistic endophytic fungi. V. GONZÁLEZ, M. L. TELLO and P. ANDRÉS. *Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Agroalimentario (IMIDRA), Finca "El Encín". Apdo. 127, Ctra. N II, Km 38,20, 28800 Alcalá de Henares, Madrid, Spain. E-mail: vicente.gonzalez.garcia@madrid.org*

One of the future challenges related with grapevine disease management will be the implementation of new control strategies, especially after the banning of certain chemical fungicides, traditionally employed for the control and suppression of several important diseases like Esca, Petri or BDA. The present study explores the potential employment of several grapevine fungal endophytes belonging to genera previously reported as antagonists in other plant hosts, to prevent and control the development of grapevine trunk pathogens. Thus, seven endophytic isolates of the genera *Epicoccum* (4) and *Aureobasidium* (3) were tested to control the development of four grapevine pathogens (*Cadophora luteo-olivacea*, *Cylindrocarpon macrodidymum*, *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*) in two experiments. Direct antagonism tests were performed by dual culture of pathogens and endophytes. Potted grapevine seedlings were inoculated with both pathogens and endophytic strains at both simultaneously or by inoculating with the pathogen some days after the endophytic antagonist. In addition, a formulation of different fungal isolates as air-dried hydrogels (sodium alginate beads) was assessed. The capability and degree of control showed by the endophytic strains assayed in both direct confrontation and *in planta* tests are discussed, as well as the suitability of hydrogels to be employed as effective carriers for biocontrol agents.

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Use of a green-grafting technique for plant sanitation in the control of grapevine wood pathogens. P. LARIGNON[1], C. BAPTISTE[1], J. F. MALLET[2], J.P. GRANIER[2] and P. BLOY[2]. *[1]Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France. [2]Institut Français de la Vigne et du Vin, Pôle National Matériel Végétal, Domaine de l'Espiguette, 30230 Le Grau du Roi, France. E-mail: philippe.larignon@vignevin.com*

Green-grafting combines the simplicity of *in vivo* techniques with the benefits of working on young plant tissue. It enables fast production of grafted plants. Plant material is grown in a greenhouse, so they are not subject to contamination observed in the vineyard. This technique provides plants devoid of fungi involved in grapevine wood diseases (*Botryosphaeria* spp., *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., *Ilyonectria* spp., *Cadophora* spp., *Phomopsis* spp.). Material was collected from rootstock mother plants of different varieties (140 Ruggieri, 5BB, Rupestris du Lot, SO4, Riparia Gloire) and scion mother plants (Cabernet-Sauvignon) cultivated in greenhouse. One hundred and twenty four green grafts were produced, maintained for 2-3 weeks in climatic room (temperature = 25°C, relative humidity ~ 100%), then transferred to one-litre pots containing a standard commercial potting mix for 2 to 8 months in a greenhouse. Isolations made from different parts of green grafts (scion, graft union, rootstock) showed that the material from this form of production is free from pathogens, except for the agent of black foot disease (*Ilyonectria liriodendri*), isolated from the base of plants. Other fungi were isolated from the green grafts, including *Chaetomium globosum*, *Fusarium proliferatum*, *Trichoderma* spp., *Alternaria* spp., *Penicillium* spp. The two first fungi were isolated from different parts of the green graft at the same percentage suggesting they colonized the whole plant, while *Trichoderma* sp. was isolated from zones closed to the wounds, showing its incapacity to colonize woody tissues. To our knowledge, this is the first report of the production of plants in the nursery without the fungi involved in grapevine wood diseases (Petri disease, *Botryosphaeria* dieback, esca disease, *Phomopsis* decline).

***In vitro* evaluation of fungicides to control diatrypaceous fungi associated with grapevine trunk disease.** D. GRAMAJE[1,2], M.R. AYRES[3], F.P. TROUILLAS[4] and M.R. SOSNOWSKI[3]. *[1]Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. [2]Department of Crop Protection, Institute of Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain. [3]South Australian Research and Development Institute, Adelaide, SA 5001, Australia. [4]Department of Plant Pathology, University of California, Davis, CA 95616, USA. E-mail: mark.sosnowski@sa.gov.au*

A number of Diatrypaceous fungi have been isolated from the wood of cankered grapevines and shown to be pathogenic when inoculated in stems of potted vines. Until now management strategies have focused on the development of procedures and products to prevent or reduce *Eutypa lata* infection through pruning wounds. In a study conducted in Australia, six fungicides were evaluated *in vitro* for their efficacy in reducing mycelial growth of two isolates each of *Eutypa lata*, *Cryptovalsa ampelina*, *Diatrypella vulgaris*, *Eutypa leptoplaca* and *Eutypella citricola*, and one isolate of *Eutypella microtheca*. The fungicides carbendazim, fluazinam, tebuconazole, and prothioconazole + tebuconazole, incorporated in PDA at 1 or 10 ppm, were effective at reducing or inhibiting mycelial growth of all Diatrypaceae spp. tested. Pyraclostrobin reduced mycelial growth of most of the fungal species tested by at least 50%. Pyrimethanil was least effective at reducing mycelial growth of these pathogens. Five of the six fungicides evaluated in this *in vitro* study have demonstrated efficacy against Diatrypaceae spp. but require further evaluation in the field. This study is the first approach for fungicide evaluation against mycelial growth of diatrypaceous fungi, other than *Ea. lata*. It will contribute to the development of integrated control strategies for grapevine trunk diseases.

Preliminary screening for the possible biological control activity of a *Chaetomium* sp. isolate on grapevine trunk disease agents. A. SPAGNOLO[1], M. MAGNIN-ROBERT[1], P. LARIGNON[2], C. CLÉMENT[1] and F. FONTAINE[1]. *[1]Université de Reims Champagne Ardenne, UFR Sciences Exactes et Naturelles, Unité de Recherche Vignes et Vins de Champagne - EA 4707, Laboratoire de Stress Défenses et Reproduction des Plantes, Moulin de la Housse - Bâtiment 18, BP 1039, 51687 Reims Cedex 2, France. [2]Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, Domaine de Donadille, 30230 Rodilhan, France. E-mail: florence.fontaine@univ-reims.fr*

Esca disease, Eutypa dieback and Botryosphaeria dieback are three main fungal trunk diseases of grapevine, severely affecting vineyards worldwide. Control strategies for these diseases are mainly based on the application of chemicals, which have principal drawbacks of environmental pollution and ecological imbalances. As a matter of fact, research for biological control agents able to prevent or at least reduce the development of the diseases is nowadays of considerable interest. Species of the Ascomycete genus *Chaetomium* have shown to be active as biocontrol agents against several fungal plant pathogens. In this study, the potential biological control ability of a *Chaetomium* sp. isolate on the esca agents, *Phaeomoniella chlamydospora* (Pch) and *Phaeoacremonium aleophilum* (Pal), and on two Botryosphaeria dieback agents, *Neofusicoccum parvum* (Np) and *Diplodia seriata* (Ds), was eval-

uated by dual culture tests on agar plates. Two strains of each phytopathogenic species were tested. The *Chaetomium* sp. isolate used in this study (Cha1) belonged to a collection obtained mainly from asymptomatic trunk wood of 26-year-old standing vines cv. Chardonnay. These vines did not show any foliar symptoms related to esca disease and/or Botryosphaeria dieback for the last 10 years. Results showed the capability of Cha1 to overgrow colonies of the two Np and Ds strains and one strain of both Pch and Pal. The possible antagonistic effect of Cha1 against esca disease and Botryosphaeria dieback agents will be further evaluated through dual artificial infection on potted rooted cuttings.

Evaluation of *Trichoderma atroviride* as biocontrol agent against five Botryosphaeriaceae grapevine trunk pathogens. C. PINTOS, V. REDONDO, O. AGUÍN, M. CHAVES, C. RIAL and J.P. MANSILLA. *Estación Fitopatolóxica Do Areeiro, Deputación Pontevedra, Subida a la Robleda s/n. 36153 Pontevedra, Spain. E-mail: cristina.pintos@depo.es*

Species of the genus *Trichoderma* have been widely investigated as biocontrol agents and could become a potential alternative to routine chemical control. *Trichoderma* species have also been tested as biological agents to protect pruning wounds against grapevine trunk pathogens. *Trichoderma atroviride* (brand name TIFI: Giten Biological, Tarragona, Spain) was tested *in vitro* and in greenhouse trials against five Botryosphaeriaceae fungi: *Diplodia corticola*, *Neofusicoccum australe*, *N. luteum*, *N. mediterraneum* and *N. parvum*. *In vitro* antagonism of *T. atroviride* was evaluated in dual-cultures with the Botryosphaeriaceae species. The results indicated that the inhibition percentage of radial growth of pathogens ranged between 43.6 and 60.7%. Two additional biocontrol tests using detached grapevine canes in the laboratory and potted vines in greenhouse were performed. Mycelial plugs of Botryospaheriaceae species and *T. atroviride* were placed on the same inoculation sites following two procedures: *T. atroviride* was inoculated either before or after inoculations with the pathogens. Post-inoculation with *T. atroviride* reduced necrosis lengths of pathogens by 6.7–58.3% (in detached canes) and 22.1–69.2% (in potted vines). Pre-inoculation with *T. atroviride* had a stronger inhibition effect with reduction in necrosis lengths by 90.4–96.8% in detached canes and 65.7–91.9% in potted vines. In addition, reisolation of the pathogens decreased in both assays. Botryosphaeriaceae species were re-isolated on average from 56% when *T. atroviride* was inoculated after pathogens compared with 38% when the antagonist was inoculated prior to pathogens. *Trichoderma atroviride* was isolated in all cases 10 cm from the inoculation sites. Results suggest that *T. atroviride* could be used as a biocontrol agent against Botryosphaeriaceae pathogens on grapevine plants.

How to avoid Esca on grapevine? – From lab to field. A. KORTEKAMP and J. KÖCKERLING. *Department of Phytomedicine, State Service Center Rheinpfalz for Research, Teaching and Consulting in Viticulture, Horticulture and Rural Development, Breitenweg 71, D-67435 Neustadt/Weinstrasse, Germany. E-mail: andreas.kortekamp@dlr.rlp.de*

Esca is in Germany a serious disease of grapevine eventually leading to the death of the entire plant. The risk of an infection event and disease outbreak seems to depend on the pruning system, since the number of diseased plants in the vineyard increases with number of wounds. Unfortunately, there are no plant protectants available in Germany that can prevent infections of freshly created wounds. Thus, wounds created after pruning and sawing still remain untreated. In order to investigate the properties of plant protectants, sealing products, and biological products containing antagonistic microorganisms, an *in vitro*-test system based on sterilised wooden shoots was developed. For this purpose, internodes of the cultivar Müller-Thurgau were cut in half after sterilisation, treated with test products, placed into sterile agar in glass tubes, inoculated with *Phaeoacremonium aleophilum*, *Phaeomoniella chlamydospora*, and *Fomitiporia mediterranea*, and incubated at 21°C. This test system allowed a fast and easy evaluation of an antifungal efficacy of fungicides and other compounds against Esca pathogens within ten to twenty days post inoculation. Most active fungicidal agents were tebuconazole in combination with mancozeb and pyraclostrobin in combination with metiram. These fungicides were also used in the field to protect freshly created wounds that were inoculated with *P. aleophilum* and *P. chlamydospora* one day after fungicide application. Alternatively, some wounds were covered with sealing products, sprayed with a suspension of *Trichoderma harzianum* spores (Trichostar®) or treated otherwise to inhibit fungal infections. Fungicide mixtures, the antagonistic fungus, and the sealing products were able to reduce the infection by 60 to 70% in the case of *P. chlamydospora*.All fungicides and alternative treatments were less effective against *P. aleophilum*.

Field evaluation of fungicidal activity towards *Botryosphaeriaceae* canker and Excoriosis. C. REGO[1], T. NASCIMENTO[1], A. DIAS[2] *and* R. CORREIA[3]. *[1]Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal. [2]Sustinia, INOV-ISA, Tapada da Ajuda, 1349-017 Lisboa, Portugal. [3]Syngenta Crop Protection, Ribera de Loira 8-10 3ª planta, 28042 Madrid, Spain. E-mail: crego@isa.utl.pt*

On grapevine, Botryosphaeriaceae species and *Phomopsis viticola* are associated with wood necrosis but they are also able to infect both young and mature tissues as well as green shoots causing brown cankers or

dark elongated spots with cracks and purplish margins, respectively. A three-year trial (2008, 2009, 2010) was conducted in a 12 year-old vineyard of cv. Aragonês (= Tempranillo), in Alentejo to evaluate the effectiveness of five fungicides, azoxystrobin (Quadris®), difenoconazole (Score®), ciprodinil+fludioxonil (Switch®), copper oxychloride (Cuprocol®) and tebuconazole (Horizon®) against Bot Canker *(Botryosphaeriaceae* species) and Excoriosis (*Phomopsis viticola*). Three timings of application were carried out: after pruning, at growth stages C/D and after pruning + growth stages C/D. A total of 18 treatment combinations (three times of application x five fungicides and control) were applied on grapevines in a completely randomized design. Incidence and severity were evaluated during late spring. After pruning, copper oxychloride applications resulted in a significant reduction of disease incidence and severity as compared to the other fungicide treatments and control. At growth stages C/D, difenoconazole provided a significant reduction in incidence and severity of both diseases. These treatments provided a reduction in incidence and severity of diseases by 50% and 65%, respectively, compared to the untreated control. In conclusion, this combination of treatments seems to be a strategy to be taken into consideration for the control of Botryosphaeriaceae species and *P. viticola* under field conditions.

Selected foliar fertilization in a strategy for the reduction of esca foliar symptoms. F. CALZARANO[1], V. D'AGOSTINO[1], C. AMALFITANO[2], L. SEGHETTI[1] and S. DI MARCO[3]. *[1]Università degli Studi di Teramo, Dipartimento di Scienze degli Alimenti, Via C.R. Lerici, 1, 64023 Mosciano S.A. (TE), Italy. [2]Università di Napoli "Federico II", Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Via Università 100, 80055 Portici, Napoli, Italy. [3]CNR, IBIMET, Via Gobetti 101, 40129 Bologna, Italy. E-mail: fcalzarano@unite.it*

Results obtained in previous investigations have stimulated these trials that evaluate a commercial foliar fertilizer based on calcium and magnesium, plus adjuvants that facilitate their penetration, bioavailability and distribution in the plant. In 2010, nine treatments were performed between the phenological stages "third leaf unfolded" and "bunch closing". A consistent decrease in the expression of foliar esca symptoms was observed, and an increase of *trans*-resveratrol content in the leaves occurred after the first four treatments. In 2011, in order to investigate the most active components in the fertilizer, the treatments were repeated by separately applying calcium, magnesium or a tank mixture of both. The trial was extended to two other additional vineyards. The commercial fertilizer confirmed the decrease of foliar symptoms and the increase of *trans*-resveratrol in the leaves. The application of the mixture calcium + magnesium or calcium or magnesium separately also showed a reduction of symptoms, although to a lesser extent than that of the commercial preparation. This modularity of effects, would suggest an activity of calcium and magnesium in the manifestation of symptoms, even in the absence of adjuvants, and that the physiology of the plant may play a role in foliar expression. Numerous reports on the effect of calcium on various plant diseases are present in the literature. Less known is the activity of magnesium, which has shown contradictory effects when investigated in different pathosystems. Further studies on the mechanisms of action are in progress.

Protection of pruning wounds against the trunk diseases: results of a long-term experimentation. V. DUMOT[1], G. SNAKKERS[1], P. LARIGNON[2], P. LECOMTE[3], P. RETAUD[4], S. DAVID[5], E. MENARD[1] and L. LURTON[1]. *[1]Bureau National Interprofessionnel du Cognac, 23, Allées Bernard Guionnet - BP 18 - 16100 Cognac Cedex, France. [2]IFV, Domaine de Donadille, 30230 Rodilhan, France. [3]INRA, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), 33140 Villenave d'Ornon, France. [4]Chambre régionale d'agriculture de Poitou-Charentes, 3, boulevard Vladimir, 17100 Saintes, France. [5]Lycée Georges Desclaude, rue Georges Desclaude, 17100 Saintes, France. E-mail: vdumot@bnic.fr*

A collaborative study has been carried out in the Cognac area (France) to find solutions to protect plants from decline diseases Eutypa dieback and esca, by protecting the pruning wounds from infection. A vineyard of cv. Ugni blanc was planted in 1991 to compare the effects of chemical pruning wound protectants (ATEMICEP, composed of carbendazime and cyproconazole, or ESCUDO, composed of carbendazime and flusilazol), physical protection with a mastic (LAC BALSAM), foliar spraying of copper solutions at bud-break, and late pruning. The experimental design is composed of 122 plots of ten plants. Foliar symptoms of decline diseases and mortality have been recorded every year from the date of plantation until 2010. The variables used for assessment were the proportion of plants that showed one symptom in at least one year, and the percentage of dead plants. Results show global significant effects of the modalities, but few of them differ significantly from the control. Only the late pruning shows less symptoms of Eutypa dieback than the control, but it doesn't show less esca symptoms. Foliar symptoms of esca disease are fewer for both chemical protections. The ATEMICEP modality presents a low mortality, but not significantly different from the control. Physical protection and copper spraying have no efficacy, copper spraying even increases the Eutypa dieback symptoms. The products used in this trial for chemical protection are no longer allowed. Moreover, the time needed to apply them (around 20 hours per hectare) prevents this technique from being used by winegrowers. Only late pruning can be applied: it can be advised in the situations of high sensibility to Eutypa.

The fact that esca seems not to be dependent on the time of pruning suggests that Eutypa and esca diseases don't follow the same mechanism.

A possible positive interaction between tebuconazole and electrolysed water as a wood penetrating formulation in propagation material against *Phaeomoniella chlamydospora*. S. DI MARCO[1], D. BOSSIO[2], F. OSTI[1], M. BALEANI[2], G. BERTAZZA[1] and L. MUGNAI[2]. [1]*IBIMET-CNR,Via Gobetti 101, 40139 Bologna, Italy.* [2]*DiBA, Protezione delle piante, P.le Cascine 28, 50144 Firenze, Italy. E-mail: s.dimarco@ibimet.cnr.it*

Research on trunk diseases has shown that infections in the nursery are not only due to basal and graft union infections of the cuttings via the wounds but also to systemic infection from mother vines. To reduce cane infections the application of a highly penetrating fungicide, tebuconazole (Folicur®SE), provided in a special – devoted to wood treatments – formulation was tested at hydration. Cut ends of cuttings were sealed by wax, immersed in a fungicide suspension, and penetration into the wood of tebuconazole verified by HPLC analysis. To further improve both penetration into the wood and efficiency of the funcidide, tebuconazole (very active in reducing *Phaeomoniella chlamydospora* mycelium growth but not so efficient on spore germination) was also combined with electrolyzed water (EAW), very active in inhibiting spore germination, and previously studied for a possible use in hydration. The HPLC data showed that, even if systemic fungicide as triazoles can not be transported in the sap of cuttings as it happens in vines in the field, still the passive absorption of Folicur®SE in non active tissue was high enough to reach the active concentration, in all wood parts after soaking, and to mainatain it even after 3 months from treatment. Different trials were carried out in a commercial nursery on cuttings artificially or naturally infected with *P. chlamydospora*. The use of the Folicur®SE formulation without or, even more with EAW, provided a strong reduction of the infections in naturally and artificially infected cuttings.