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ABSTRACTS

Pathogen Identification and Detection

Occurrence of two new species of *Togninia* in California. A. Eskalen, S.N. Rooney-Latham and W.D. Gubler. *Department.of Plant Pathology, University of California, Davis, CA, USA.*
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Esca and Petri disease are two of the most destructive diseases of grapevines worldwide. In California, these diseases are caused by multiple species of *Togninia* (anamorph: *Phaeoacremonium*). The teleomorphs of *Phaeoacremonium aleophilum*, *P. mortoniae*, and *P. viticola* were recently confirmed as *Togninia minima*, *T. fraxinopennsylvanica*, and *T. viticola*, respectively. However, teleomorphs for other *Phaeoacremonium* species were unknown. In this study, grapevines showing esca symptoms were surveyed during summer and fall of 2004 and 2005. Samples were collected from native trees surrounding the vineyards, and from symptomatic grapevines. Perithecia of two new species of *Togninia* were found on the surfaces of old pruning wounds and in the cracks of cordons and trunks of the *Vitis vinifera* cv. Thompson seedless from Yolo County, Riesling from Mendocino Co. and Chardonnay from Sonoma Co. Morphologically these perithecia resemble other *Togninia* spp. and when plated onto PDA-tet medium, ascospores formed colonies similar to other *Phaeoacremonium* spp. Molecular data confirmed these perithecia to be two new species of *Togninia* on grapevine, *Togninia californica* nom. prov. and *Togninia davisiana* nom. prov. Perithecia of *Togninia davisiana* were also observed on dead vascular tissue of declining ash trees (*Fraxinus latifolia*) in Yolo and Sonoma counties.

Identification of a portion of the mating type (MAT-2) gene in *Togninia minima*. S. Rooney-Latham, A. Eskalen and W.D. Gubler. *Department of Plant Pathology, University of California, Davis, CA, USA.*
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The teleomorph of *Phaeoacremonium aleophilum* was recently shown to be *Togninia minima* (Diaporthales). *In vitro* mating studies have confirmed that this fungus sexually reproduces using a bi-allelic heterothallic mating system. *T. minima* perithecia have also been identified on standing grapevines in infected vineyards throughout California. Sexual reproduction among the higher fungi is controlled by a single regulatory mating type locus (MAT). The mating type locus exists in alternate forms called idiomorphs, each thought to encode proteins with alternate DNA-binding motifs. Degenerate primers previously designed for other heterothallic pyrenomycetes were used to amplify a portion of the MAT-2 mating type of California isolates of *Togninia minima*. Sequencing of this product, which included the HMG-box, allowed for the identification of more specific primers. The nucleotide sequence (~300bp) of this idiomorph showed homology to the HMG-box of other related ascomycetes, and included a putative 95 bp intron in the same position as these fungi. Identification of *T. minima* mating types using PCR is faster and cleaner than traditional pairing studies and would be very beneficial for population studies, especially in geographic regions where only the anamorph has been identified.

Botryosphaeria canker of grapevines in California; characterization and identification of the causal agents. J.R. Úrbez-Torres[1], G.M. Leavitt[2] and W.D. Gubler[1]. *[1]Department of Plant Pathology, University of California, Davis, CA 95616, USA. [2]University of California Cooperative Extension, 328 Madera Ave. Madera, CA 93637, USA.*
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Botryosphaeria canker was first reported in California in 1987. Since then, *Botryosphaeria rhodina* has been well established as the causal agent of the disease in the state, and is now considered an endemic pathogen in the arid climate grapevine-growing regions of southern California. Botryosphaeria canker affects spurs, cordons and trunks, causing dieback to the grapevine. The fungus enters primarily through fresh pruning wounds, eventually creating a wedge-shaped canker which is the typical symptom in the earliest stages of the disease.

Perennial cankers grow in the vine for several years causing death of the vine parts. Therefore, Botryosphaeria canker decreases vineyard longevity, reduces yields and increases production costs as a result of cultural and chemical preventive measures as well as removing diseased/dead wood from the vine after infection. *Botryosphaeria* species have recently been recognized as important grapevine pathogens in the main grape production areas worldwide, and have been associated with several vascular symptoms such as cankers, wood streaking, necrotic lesions, and trunk dieback. In order to know if more *Botryosphaeria* species were involved in grapevine dieback in California, as well as evaluate their incidence and importance in the state, field surveys were conducted in 2004 and 2005 throughout the main grape areas of California. Over 1,700 perennial cankers from spurs, cordons and/or trunks were collected from the predominant wine, table and raisin grape cultivars of California. Fungal isolation showed *Botryosphaeria* species as the most prevalent fungi associated with cankers in California. Based on morphological characters and partial sequence analysis of ITS and β-tubulin genes, nine *Botryosphaeria* species were identified from cankers of grapevines, including *B. australis*, *B. dothidea*, *B. lutea*, *B. obtusa*, *B. parva*, *B. rhodina*, *B. sarmentorum*, *B. stevensii* and *B. viticola*. Occurrence and distribution of *Botryosphaeria* species varied with location. In order to determine the importance of *Botryosphaeria* species in grapevine health in California, pathogenicity tests were conducted on mature vines. Dormant grapevines cv. Chardonnay were pruned to 4–5 buds. Fresh pruning wounds were inoculated using mycelium plugs of the nine *Botryosphaeria* species found in California. Internal lesions were measured 12 months after inoculation. Preliminary results verified the pathogenicity of the nine *Botryosphaeria* species, however, virulence varied among species. Isolates of *B. rhodina* were the most virulent based on extent of spread in the wood, while isolates of *B. obtusa* were the least virulent. More studies designed to characterize the virulence of *Botryosphaeria* species on grapevines in California are currently underway. The findings confirm that at least nine *Botryosphaeria* species are associated with Botryosphaeria canker of grapes in California.

Aspergillus vine canker of table grapes caused by *Aspergillus* Sect. *Nigri* in California. T.J. Michailides[1], W. Peacock[2], P. Christensen[1] and D.P. Morgan[1]. *[1]Department of Plant Pathology, University of California-Davis, Kearney Agricultural Center, Parlier, CA 93648, USA.[2]University of California, Cooperative Extension, Tulare County, Tulare 93274, USA.*
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A vine canker disease was first observed in the San Joaquin Valley, CA, in fall 1989 on vigorous 1-year-old cv. Redglobe vines. Since then, the same canker has been also observed on Crimson Seedless, Chardonnay, and Grenache vines in Tulare, Kern, Fresno, and Riverside (Coachella Valley, CA) counties. In affected vineyards, the disease resulted in the retraining of 2 to 6% of vines the following spring using a shoot originating from below the canker. All these infections started through wounds caused by removing lateral shoots or leaves when the vine was topped to form cordons, or possibly through growth cracks that occur on very rapidly growing 1-year-old shoots of vigorous cultivars. The first symptoms usually appear in August, as red pinhead size drops of sap on the surface of discolored tissue. By October to November, the canopies of vines that have been girdled by the canker prematurely display fall colors and become very distinct from healthy vines. The trunk is slightly swollen and spongy where the canker occurs. Internal canker tissue is discolored and dead. Black spores are abundant within the canker, on the surface of the canker, or both. Callous tissue is often associated with the canker as the vine attempts to repair the damage with new tissue. Canker length can range from 3.5 to 26.5 cm (average 7.0 cm) and can affect the shoot's cross section by partially to completely girdling the shoot. Isolations from cankers or from black sporulation inside the canker on acidified potato dextrose agar (APDA) consistently yielded *Aspergillus niger* v. Tiegh. Koch's postulates have been completed using isolates of *A. niger* (Michailides *et al.,* 2002, Plant Disease 86, 75). The objective of the 2001 and 2002 studies was to determine whether the three commonly isolated species of *Aspergillus* sect. *Nigri*, *A. niger, A. carbonarius,* and *A. japonicus*, could cause symptoms of Aspergillus vine canker. One isolate of each of these three species isolated from grapes was used in pathogenicity studies. Ten well-matured current-season canes of Redglobe grapes in an experimental vineyard at Kearney Agricultural Center were inoculated by inserting a 7-mm plug of mycelium from actively growing cultures on APDA into a cut made with a 7-mm cork borer. Ten canes were inoculated with a 7-mm plug of APDA and used as non-inoculated controls. To avoid dehydration of inoculum, inoculated sites were sealed with Parafilm. Inoculations of grapevines with only *A. niger* or *A. carbonarius* resulted in cankers of 32 and 20 mm, respectively, while canes inoculated with *A. japonicus* were not infected. These results were confirmed in three laboratory experiments in which consistently *A. niger* resulted in 25, 47, and 151 mm canker length, and *A. carbonarius* in 27, 26, and 33 mm canker length; while *A. japonicus* did not cause any canker. These results suggest that among the three species of *Aspergillus* in sect. *Nigri* isolated from grapevines, only *A. niger* and *A. carbonarius* can cause Aspergillus vine canker. *Aspergillus* species in the sect. *Nigri* have been previously reported to be among the pathogens involved in the bunch rot com-

plex. In addition, *A. carbonarius* was reported as the main source of ochratoxin A contamination in dried vine fruits and wines in Europe.

***Fomitiporia polymorpha* is a recently detected white rot basidiomycete on North American grapevine.** M. Fischer. *Staatliches Weinbauinstitut, Merzhauser Str. 119, D-79100 Freiburg, Germany.*
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Wood of grapevine is an important source of nutrients for a number of basidiomycetes. Pileate and resupinate fruit bodies of an unknown species of *Fomitiporia* were collected on a variety of deciduous and coniferous trees in the late 1980s in California. Microscopic and cultural characters together with molecular studies showed all these specimens to belong to a new species, designated *F. polymorpha* (Fischer and Binder, 2004). Recently, a vegetative mycelium derived from esca associated grapevine and provided by the group of Douglas Gubler (UC Davis) was assigned to *F. polymorpha* by an analysis of the nuclear encoded ITS region. Little is known about the life strategy of *F. polymorpha* in the field and to which extent vegetative and/or generative structures of the fungus occur on grapevine. The presentation provides a description of the species and refers to problems of its taxonomic classification and identification. The possible pathogenic significance of *F. polymorpha* is also discussed.

References

Fischer, M. and M. Binder, 2004. Species recognition, geographic distribution and host-pathogen-relationships: a case study in a group of lignicolous basidiomycetes, *Phellinus* s.l. *Mycologia* 96, 799–811.

A single method for the molecular detection of *Phaeomoniella chlamydospora* from mycelium, grapevine wood and soil samples. L. Gaforio, A. Gómez and M.L. Tello. *Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Finca "El Encín," Autovía A-2, km 38,200. Alcalá de Henares 28800, Madrid, Spain.*
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Petri disease, which causes decline and dieback of young grapevines, is a serious problem for grape growers and nurseries. Disease diagnosis implies the detection of the involved pathogens, especially *Phaeomoniella chlamydospora* which has been identified as a major casual agent. The molecular detection of this fungus is possible by species-specific amplifications of Internal Transcribed Spacer (ITS) fragments of ribosomal DNA. Because of the various dispersal modes of the fungus it is necessary to extract fungal DNA from a variety of samples, i.e. vine tissues, soil and fungal cultures. In this work we evaluated different molecular detection protocols to obtain a single accurate method to be used with all kind of samples, independently of their origin. We analyzed three groups of samples: (i) mycelium grown on two culture media: potato-dextrose agar (PDA) and potato-dextrose broth (PDB); (ii) soil prepared by either obtaining a soil suspension in water or grinding with a mixer mill and (iii) wood cores from symptomatic and asymptomatic vine tissues. A commercial kit and the buffer CTAB were used to ectract DNA. Total DNA was quantified by means of spectrophotometry and both DNA quantity and quality were compared to select the best extraction method. Sensitivity of the DNA extraction techniques and of the PCR reactions was tested. PDB proved to be more appropriate than PDA to culture *Pa. chlamydospora* for DNA extraction from mycelia. No amplification from the fungus grown on PDA was possible due to inhibition by the agar. Both extraction protocols (commercial kit and buffer CTAB) gave a good yield of DNA of a purity acceptable for PCR reactions. The detection level of the PCR was 1 pg of DNA template. The PCR efficiency of pathogen detection from soil was affected by the extraction method. With *Pa. chlamydospora*-DNA isolated from soil, only samples which had been ground into a fine powder gave good yields of DNA with both extraction protocols. However, only DNA isolated by CTAB led to PCR amplification. This method allowed the detection of *Pa. chlamydospora* at a concentration of 100 conidia per gram of dry soil and was validated by analysis of various artificially infected substrates and subsequent re-isolation of viable fungal propagules. When wood samples were analyzed, no positive PCR results were obtained when DNA was isolated with the commercial kit. *Pa. chlamydospora* was detected not only in necrotic tissues but also in some of the asymptomatic tissues. In conclusion, a buffer CTAB can be used as a single and adequate method for DNA extraction from grapevine wood, soil and mycelium with good efficiency and accuracy. This techique is both more economical and simpler than many other conventional protocols.

Characterization of *Cylindrocarpon* species, the cause of black foot disease of grapevines in California. E. Petit and W.D. Gubler. *Department of Plant Pathology, University of California, Davis, CA, USA.*
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We investigated phylogenetic divergence, morphological difference, and pathogenic variation among *Cylindrocarpon* isolates associated with black foot disease of grapevine (*Vitis* sp.) in California. To assess phylogenetic divergence, we sequenced the internal transcribed spacer (ITS) of the nuclear ribosomal DNA (rDNA), partial β-tubulin (BT) gene introns and exons, and the small subunit mitochondrial rDNA. Isolates associated with

black foot disease belonged to two paraphyletic species, *Cylindrocarpon destructans* and *C. macrodidymum*. The morphology of these isolates was in agreement with published descriptions of both species. We found that *C. macrodidymum* isolates were reliably distinguished from *C. destructans* isolates, in culture by a unique orange-dark brown colony color on 2% malt extract agar, and genetically by a species-specific 52 bp DNA insertion in the BT region. Selected isolates of each species inoculated onto grapevine rootstock 5C caused typical black foot disease symptoms. This is the first report of *C. macrodidymum* in California.

Characterization of *Eutypella vitis*, a potential pathogen of grapevines. S.A. Jordan and A.M.C. Schilder. *Department of Plant Pathology, Michigan State University, East Lansing, MI 48824, USA.*
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Eutypa dieback is a destructive disease of grapevines that causes major losses to the juice grape industry in Michigan, especially on *Vitis labrusca* 'Concord'. While *Eutypa lata* is considered to be the primary cause of Eutypa dieback, the potential pathogenicity of another fungus, *Eutypella vitis*, was examined. Isolates of *El. vitis* collected from mature 'Concord' grapevines expressing symptoms of Eutypa dieback were tested for pathogenicity on potted dormant cane cuttings. Each cane was inoculated 2 cm below the top node with a 3-mm mycelial plug. After three and six months, canes were rated for foliar symptom severity and the length of the tissue necrosis was measured. Sections of necrotic tissue were surface-sterilized and plated on PDA amended with ampicillin. While foliar symptoms caused by Michigan isolates of *El. vitis* were not as severe as those of virulent isolates of *E. lata*, several isolates of *El. vitis* had foliar severity values significantly greater than the agar-inoculated control and displayed symptoms characteristic of Eutypa dieback. Similar results were found for shoot death and tissue necrosis development, indicating a range of virulence for isolates of *El. vitis*. Several of the *El. vitis* isolates were recovered from the necrotic tissue. In addition, growth rate, cultural characteristics, appearance and size of ascospores and conidia, and the ability to produce phytotoxic secondary metabolites were investigated.

First observations of esca disease in the Trentino area, northern Italy: monitoring of spores, evolution of symptoms and evaluation of incidence. L. Michelon, C. Pellegrini and I. Pertot. *SafeCrop Centre, Istituto Agrario di S.Michele all'Adige, Via Mach 1, S .Michele all'Adige TN, 38010, Italy.*
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Esca disease is a serious disease of young and old vines in several grape cultivation areas in Italy. The most important causal agents are *Phaeomoniella chlamydospora (Pch)*, *Phaeoacremonium aleophilum* (*Pal*) and *Fomitiporia mediterranea (Fmed),* but other fungi, like *Botryosphaeria* spp. or *Eutypa lata*, are also often associated with the disease. In the Trentino area (northern Italy) the incidence of esca disease is low, but in recent years symptomatic plants have become more common, especially on some cultivars such as Sauvignon blanc, Cabernet Sauvignon and Traminer. The Trentino area was selected because it could represent a first stage of the epidemic and give important elements for understanding its development. The aims of this study, part of a national research project on esca, coordinated by the University of Firenze, are: to study the development of symptoms in vineyards and to estimate disease incidence in the Trentino area, to verify the presence of airborne spores of *Pch*, *Pal* and *Fomed* in Trentino's infected vineyards, and to determine when and under what conditions they are released. Airborne spore monitoring was carried out weekly in two infected vineyards using two methods: by glass microscope slides and by continuous volumetric spore traps (Burkard Spore Traps). From April 2005 to March 2006, no spores of the three main fungi involved in the disease were captured except on one day only. During summer 2005, in the two monitored vineyards, esca symptoms were assessed weekly on the vines and weather conditions were recorded. Symptom appearance was in line with the indications of the bibliography. In the first part of the season diseased leaves showed the classic "tiger-stripe" pattern; subsequently the chronic symptoms became acute. In both vineyards no clear correlation was seen between weather conditions and symptom expression. At the end of summer disease incidence was evaluated in five of the main vine growing areas of the Trentino area. One hundred vineyards with different cultivars and vine ages were randomly selected and 200 vines were assessed. In 2005 the average incidence of esca in the area was 1.3%. The cultivars with the highest esca incidence were Nosiola, Sauvignon blanc, Traminer, Cabernet Sauvignon and Marzemino; these cultivars are already known to be more sensitive to esca.

Localization and quantification of fungi in esca diseased grapevine trunks. H. Richter, K. Gindro, R. Pezet and O. Viret. *Agroscope Switzerland Changins, Department of Mycology, Nyon 1260, Switzerland.*
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Esca is one of the oldest grapevine disease, where multiple fungal species interact, obstruct the vascular system until the plant dries out. The slow development of esca until the sudden apoplectic decline is meanwhile a serious vinification problem as the plants contribute grapes of lower quality. Localization and quantification

of fungi was performed on some 40 plants showing the sudden apoplectic decline. "Direct"-PCR (Gindro *et al*., 2005, Vitis 44[3],139–142) was performed on crushed mycelia, without any isolation step of DNA, using fungal universal ITS primers. Over 50 different species were identified aligning ITS sequences and using morphological criteria. Interesting to note is that the fungal diversity and the quantitative location are centered to the grafting point and not to the head of the trunk. Fungi were grouped as follows. Esca: *Phaeomoniella chlamydospora, Fomitiporia mediterranea, Phaeoacremonium angustius* and *P. aleophilum*. Wood disease: *Eutypa lata, Botryosphaeria obtusa, Phomopsis viticola* and *Cylindrocarpon destructans*. Saprophypes or pathogens: *Cladosporium herbarum, Bionectria ochroleuca, Acremonium alternatum* and *Alternaria alternata*. Others: *Alternaria, Fusarium, Penicillium, Aspergillus, Botrytis, Mucor, Phoma*. The aim of this work is to use molecular marker to detect and differentiate the 8 to 10 most important fungi for an early detection on nursery material and in a non destructive plant system.

Identification and characterization of Cylindrocarpon species, the cause of black foot disease of grapevine in Chile. J. Auger, M. Esterio and I. Pérez. *Universidad de Chile, Facultad de Ciencias Agronómicas - Departamento de Sanidad Vegetal, Casilla 1004 - Santiago, Chile.*
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Black foot disease caused by Cylindrocarpon species is a serious problem of grapevine in many countries. In Chile black foot affects young table and wine grape planting throughout the country. Grapevines from one to five years-old showed reduced vigor with smaller-sized trunks, shortened internodes, uneven wood maturity, sparse foliage, and small leaves with interveinal chlorosis and necrosis. In cross section, the base of the trunk appeared necrotic and xylem vessels were plugged with black inclusions and tyloses. Severe root symptoms showed black sunken necrotic lesions, included a reduction in root biomass and root hairs, and were frequently associated with nematode and root weevil larvae. Further root abnormalities included the formation of secondary root crowns with roots growing parallel to the soil surface. Since infected grapevines gradually decline and eventually die, infection by this pathogen is causing a significant reduction in yield. This prompted an investigation into local Cylindrocarpon populations occurring on grapevines in Chile, using multigene phylogeny, morphological characteristics and pathogenicity. Isolates were collected from grapevines with black foot symptoms, also considering California isolates. To assess phylogenetic divergence, the isolates were analyzed using sequence data from the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA, the β-tubulin gene region and the small sub-unit mitochondrial rDNA. Based on DNA divergence, this study detected *Cylindrocarpon destructans* and *C. macrodidymun*. Selected isolates of each species inoculated onto one-year-old Red Globe plant cultivars caused typical black foot disease symptoms.

Morphology, phylogeny and pathogenicity of Diatrypaceous fungi associated with *Vitis vinifera* decline in California. F. Trouillas and W.D. Gubler. *Department of Plant Pathology, University of California, Davis, CA, USA.*
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Grapevine cordon and trunk canker diseases constitute the primary causes of plant mortality and economic losses to the California grape industry. For many years it was thought that the decline and dieback observed in the California grape-growing regions was due primarily to *Eutypa lata* (Pers.: Fr.) L. R. Tul. & C. Tul. (Syn: *E. armeniacae* Hansford & Carter) (Diatrypaceae), the causal agent of Eutypa Dieback. However, recent surveys in northern California vineyards have revealed the occurrence of additional species of Diatrypaceae in the wood of symptomatic grapevines, perhaps indicating new threats to California's grape industry. Diatrypaceous fungi were also commonly found on the dead or declining wood of the native vegetation surrounding vineyards. A few species were isolated from twig cankers of California bay laurel, California buckeye and Fremont cottonwood, raising questions regarding their role and potential to cause disease in natural ecosystems. Morphological examination together with phylogenetic analyses allowed the identification and separation of nine species of Diatrypaceae from grapevine, seven of which constitute first reports for California. These include the previously reported *E. lata* and *E. leptoplaca*, as well as *Diatrype* sp., *Cryptovalsa ampelina, Diatrypella* sp., *Diatrype stigma*, *Diatrype whitemanensis*, *Cryptosphaeria pullmanensis* and *Eutypella* sp. Preliminary results of pathogenicity tests on grape cuttings indicate that Diatrypaceous fungi are capable of colonizing grapevine wood. However, the ability of these fungi to cause disease remains to be determined.

Initial investigation of grapevine trunk health in Marlborough, New Zealand. D.C. Mundy[1] and M.A. Manning[2]. *[1]HortResearch Marlborough, Marlborough Wine Research Centre, PO Box 845, Blenheim, New Zealand. [2]HortResearch Auckland, Mt Albert Research Centre, Private Bag 92169, Auckland, New Zealand.*
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The Marlborough vineyard area has expanded very rapidly over the past decade. Currently less than ten percent of vines are more than 15 years old. Hence

the long term sustainability of wine grape production in the region will be dependent on the longevity of the vines that are currently planted. Our initial investigations of vines in Marlborough were focused on the older vines in the region. The aim was to identify fungi associated with vine decay, establish a range of sites to monitor disease progress and calculate the possible cost to the industry that trunk fungi might cause. A survey of 3772 vines in a 24-year-old Sauvignon Blanc vineyard assessed the incidence of dead or poorly growing vines. Some 0.9% of vines had already had to be replaced and a further 5.9% were either dead or in poor health. In a ten-year-old Riesling vineyard 8.3% of the 1870 vines observed were dead, and 2.7% appeared to be in poor health. Using a figure of 5% loss of production, due to dead or poor-health vines for an industry grower production model, a 14.1% reduction in vineyard profitability was calculated using the industry farm model. During the removal and replanting of vines in a Cabernet Sauvignon vineyard 100 vines were inspected for decay in July 2005. Many of the vines had no outward signs of disease but when the vine heads were cut open they showed substantial decay. Isolation of decayed tissue was made onto Potato Dextrose Agar plates. *Botryosphaeria obtusa* was the most common fungal species isolated from the decaying tissue. Further investigation is required before the exact impact of the different grapevine wood fungi can be determined but the possible implications to the wine industry are large as shown in the calculations using industry data. With additional study other areas of interest such as the quality of fruit produced from infected vines could also be determined. Only once the fungi that are responsible have been identified and the potential cost of damage calculated can cost-effective management tools be evaluated.

Characterization of *Cylindrocarpon* isolates from grapevines in Spain. S. Alaniz, M. León, A. Vicent, J. García-Jiménez, P. Abad and J. Armengol. *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.*
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Black-foot caused by *Cylindrocarpon* species is an important disease of grapevines occurring in all the major viticulture regions throughout the world (Halleen *et al*., 2006a). In Spain, surveys carried out in recent years in grapevine nurseries and young vineyards have confirmed the importance of *Cylindrocarpon* spp. affecting this crop. This pathogen was found in grapevine nurseries very early in the planting material production process, in grapevine plants ready to be planted, and in young vineyards showing decline symptoms. In all cases, *Cylindrocarpon* spp. was isolated mostly from the rootstocks, preferably from the basal end (Aroca *et al*., 2006; Giménez-Jaime *et al*., 2006). In Spain no studies have been carried out to determine which *Cylindrocarpon* species are found associated with this disease. In this work the phenotypical and genetic characters of 82 isolates, from different locations in Spain, were studied. The strains were cultured on spezieller nährstoffarmer agar (SNA) and potato dextrose agar (PDA). Colony characters such as texture, color and the nature of the growing margin were observed. The sporulation, size and shape of conidia and chlamydospores were measured. To determine the effect of temperature on colony growth the strains were incubated at 5, 10, 15, 20, 25, 30 and 35°C. Partial sequence of the β tubulin (BT1) gene region of the genome was amplified using primers BT1a/BT1b. A unique and conserved 52-bp insertion in the BT1 sequence, which is a specific marker for *Cylindrocarpon macrodydimum,* was found in 56 of the isolates. The rest of the isolates (26) were identified as *C. liriodendri* (Petit and Gubler, 2005; Halleen *et al*., 2006b). Phenotypical data were subjected to multivariate factorial analysis. According to this, the isolates were clearly separated into two groups which were in agreement with the BT species identification. *C. macrodydimum* isolates were differentiated from *C. liriodendri* by producing fewer conidia, having longer and wider macroconidia with lower growth rates at 5 and 10°C. Selected isolates of each species inoculated onto rooted cuttings of grapevine rootstock cv. 110 R caused typical black-foot disease symptoms. Both *Cylindrocarpon* species are present in all grapevine growing regions in Spain.

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References

Aroca A., F. García-Figueres, L. Bracamonte, J. Luque and R. Raposo, 2006. A survey of trunk disease within rootstocks of grapevines in Spain. *European Journal of Plant Pathology* 115, 195–202.

Giménez-Jaime A., A. Aroca, R. Raposo, J. García-Jiménez and J. Armengol, 2006. Occurrence of fungal pathogens associated with grapevine nurseries and the decline of young vines in Spain. *Journal of Phytopathology* 154, 598–602.

Halleen F., P.H. Fourie and P.W. Crous, 2006a. A review of Black foot disease of grapevine. *Phytopathologia Mediterranea* 45, 55–67.

Halleen F., H.J. Schroers, J.Z. Groenewald, C. Rego, H. Oliveira and P.W. Crous, 2006b. *Neonectria liriodendri* sp. nov., the main causal agent of black foot disease of grapevine. *Studies in Mycology* 55, 227–234.

Petit E. and W.D. Gubler, 2005. Characterization of *Cylindrocarpon* species, the cause of black foot disease of grapevine in California. *Plant Disease* 89, 1051–1059.

Genetic diversity among isolates of *Phaeomoniella chlamydospora* on grapevines. L. Mostert[1,2], E.C.A. Abeln[2], F. Halleen[3] and P.W. Crous[2]. [1]*Centraalbureau voor Schimmelcultures, P.O. Box 85167, 3508 AD Utrecht, The Netherlands.* [2]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa (current address).* [3]*ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa.*
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Phaeomoniella chlamydospora is one of the main causal agents of Petri disease and esca of grapevines. Although it is known to have a coelomycete synanamorph, no teleomorph has thus far been reported, and its disease cycle remains largely unknown. Mostert *et al*. (2006) compared the genetic diversity of *P. chlamydospora* isolates from different grapevine-growing countries using amplified fragment length polymorphisms (AFLPs). Sixty-three isolates from South Africa, and 25 from grapevine regions in Australia, France, Iran, Italy, New Zealand, Slovenia and the USA were studied. Two primer combinations were used producing 138 scorable markers, of which 33% were polymorphic. Unweighted paired group method of arithmetic averages (UPGMA) analysis showed a high similarity (≥94.5%) among the different isolates. The overall low level of genetic variation confirmed asexual reproduction to be dominant in the field. Different genotypes were found among isolates of *P. chlamydospora* within the same grapevine, suggesting multiple infections from different inoculum sources. Isolates from different production areas and countries had a high percentage of similarity and clustered together, indicating the absence of a genotype-geographic structure. The presence of the same genotype in different vineyards and production areas suggests that long range dispersal through aerial inoculum or infected plant material plays an important role in genotype distribution.

References

Mostert L, E.C.A. Abeln, F. Halleen and P.W. Crous, 2006. Genetic diversity among isolates of *Phaeomoniella chlamydospora* on grapevines. *Australasian Journal of Plant Pathology* 35, 453–460.

***Cylindrocarpon liriodendri*, the main causal agent of black foot disease of grapevines.** F. Halleen[1,6], H-J. Schroers[2], J.Z. Groenewald[3], C. Rego[4], H. Oliveira[5] and P.W. Crous[3,6]. [1]*ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa.* [2]*Agricultural Institute of Slovenia, Hacquetova 17, p.p. 2553, 1001 Ljubljana, Slovenia.* [3]*Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD, Utrecht, The Netherlands.* [4]*Laboratório de Patologia Vegetal "Veríssimo de Almeida," Tapada da Ajuda, 1349-017 Lisboa, Portugal.* [5]*Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal.* [6]*Department of Plant Pathology, University of Stellenbosch, P. Bag X1, Matieland 7602, South Africa.*
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Black foot disease of grapevines (*Vitis* spp.) is a serious decline and dieback disease in most areas where grapevines are grown. Mainly two species of *Cylindrocarpon*, *C. destructans* and *C. macrodidymum*, are associated with this disease. Recent studies have revealed significant molecular variation within the former, but only slight molecular variation within the latter, indicating that *C. destructans* presents a complex of several species. The present study elucidates the taxonomic status of *C. destructans*-like isolates associated with black foot disease of grapevines. Grapevine isolates were studied morphologically, subjected to DNA analyses of their ITS and partial β-tubulin genes, and were mated in all combinations *in vitro*. *Cylindrocarpon destructans* strains isolated from grapevines in Europe and South Africa appeared morphologically and genetically identical, and had identical ITS and partial β-tubulin gene sequences. Phylogenetic analyses placed these strains in a clade closely related, but clearly distinct from other clades with *C. destructans*-like anamorphs obtained from various herbaceous or woody hosts. Only the ex-type strain of *Cylindrocarpon liriodendri* had identical sequences to strains isolated from grapevines, and could also not be distinguished based on morphological characters. The grapevine isolates were therefore reidentified as *Cylindrocarpon liriodendri*. *Cylindrocarpon liriodendri* formed perithecia in heterothallic conditions and the holomorph of this species were described as *Neonectria liriodendri*. *Neonectria liriodendri* is genetically distinct from the ex-type strain of *Neonectria radicicola*, which originated from *Cyclamen* in Sweden. Both ex-type strains also differ from at least two other clades comprising additional *C. destructans*-like strains. Many of these strains originated from *Panax* sp., which is the host of the type of *C. destructans*. Our phylogenetic analyses indicate that *C. destructans* is not the anamorph of *N. radicicola* and that *N. liriodendri*, *N. radicicola* and several *C. destructans*-like taxa may have evolved independently within the same phylogenetic species complex.

Semiselective media for isolation of *Phaeomoniella chlamydospora* from vine and soil samples. L. Gaforio and M.L. Tello. *Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Finca "El Encía," Autovía A-2, km. 38,200, Alcalá de Henares 28800, Madrid, Spain.*
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The isolation of *Pa. chlamydospora,* causal agent of Petri disease, from grapevine tissues and from infect-

ed soil has its special difficulties. This pathogen grows slowly on traditional media and is easily overgrown by saprophytic fungi and bacteria. A semiselective medium for *Pa. chlamydospora* has to support its growth but at the same time must suppress or reduce the growth of most of the other epiphytic microflora. In this study, four chemicals, folpet, captan, benomyl, and bengal rose (BR) were tested at different concentrations, together with the antibiotic streptomycin. Mycelial growth, sporulation and spore germination rate were evaluated for three isolates of *Pa. chlamydospora* and seven common accompanying fungal genera: *Alternaria*, *Fusarium*, *Epiccoccum*, *Rhizopus*, *Coniothyrium*, *Botryosphaeria* and *Cylindrocarpon*. Data on mycelial growth were subjected to GLM repeat measures ANOVA while sporulation and spore germination rates were analyzed using GLM. Results showed no inhibition of mycelial growth of *Pa. chlamydospora* by folpet when tested without streptomycin but an inhibition of 30% when this antibiotic was added to the medium. Bengal rose, with and without streptomycin, reduced mycelial growth by 40% and 60% respectively, while benomyl and captan completely inhibited mycelial growth. The highest sporulation rates were obtained with media supplemented with high concentrations of folpet and bengal rose and their combinations with streptomycin. Benomyl, folpet and BR resulted in the best conidial germination rates, these last two also when combined with streptomycin. The semiselective media PDA+folpet 10 ppm + streptomycin 1 g l^{-1} (medium 1) and PDA+BR 0.15 g l^{-1} + streptomycin 1 g l^{-1} (medium 2), which permitted acceptable spore germination and mycelial growth of *Pa. chlamydospora,* were selected to assay the other fungi. Medium 1 had optimal control of *Conyothirum* and *Cylindrocarpon* while medium 2 partially suppressed *Fusarium* and *Alternaria*. Both media moderately inhibited *Epiccocum* and *Botryosphaeria*. Spore germination rates in medium 1 were lower than in medium 2 with all genera except *Alternaria*, for which the medium 2 spore germination rate was higher. When medium 1 and medium 2 were tested with woody samples of naturally infected grapevines the *Pa. chlamydospora* isolation rate increased by 50% and 40% respectively compared with isolation on PDA alone. With artificially inoculated vineyard soils, the increase in isolation was circa 60% with both media. The contamination rate from wood samples decreased from 61% with PDA to 13% with medium 1 and to 19% with medium 2. The semiselective medium PDA+folpet (10 ppm)+streptomycin 1 g l^{-1} could facilitate and improve the *Pa. chlamydospora* isolation process from wood and soil samples. When samples are heavily contaminated with *Alternaria*, it may be necessary to use the semiselective medium PDA+BR(0.15 g l^{-1})+streptomycin 1 g l^{-1}.

Identification of fungal pathogens associated with grapevine cankers in the main grape-growing areas of the United States and Mexico. J.R. Úrbez-Torres[1], G.M. Leavitt[2], J.C. Guerrero[3], J. Guevara[4], K. Striegler[5], A. Allen[5] and W.D. Gubler[1]. *[1]Department of Plant Pathology, University of California, Davis, CA 95616, USA. [2]University of California Cooperative Extension, 328 Madera Ave. Madera, CA 93637, USA. [3]Departamento de Agricultura, Universidad de Sonora, Hermosillo 83000, Sonora, Mexico. [4]Campo Experimental Costa de Ensenada (INIFAP), Ensenada 22800, Baja California, Mexico. [5]Institute of Continental Climate Viticulture & Enology, University of Missouri-Columbia, Columbia, MO 65211, USA.*
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The grapevine industry is one of the most important agricultural sectors in the United States and northwest Mexico and it currently comprises over 435,000 hectares. Fungal trunk diseases have become a growing threat to grapevines worldwide causing millions of dollars in losses each year. Grapevine cankers are one of the main causes of grapevine dieback not only in the United States and northwest Mexico but in all countries where grapes are grown. In order to determine which fungal pathogens are associated with these cankers, field surveys were conducted in the main grape-growing areas of the United States, including California, Oregon, Arizona, Missouri, Arkansas, New York, Pennsylvania, Maryland and Virginia; and in Mexico, including Baja California and Sonora. A total of 233 vineyards were surveyed and over 2,300 samples of cankered spurs, cordons and trunks were collected from grapevines showing dieback symptoms. Fungal species isolated from cankers were first identified by their morphological characteristics. Morphological identification was confirmed by analysis of the internal transcribed spacer region (ITS1-5.8S-ITS2), a partial sequence of the β-tubulin (BT2) gene, and the translation elongation factor 1-α (EF1-α). Morphological identification along with DNA analysis showed *Botryosphaeria* species to be the most prevalent fungi associated with cankers in all the grapevine production areas surveyed. Nine *Botryosphaeria* species (*B. australis*, *B. dothidea*, *B. lutea*, *B. obtusa*, *B. parva*, *B. rhodina*, *B. sarmentorum*, *B. stevensii* and *B. viticola*.) were identified as associated with cankers in California, two (*B. obtusa* and *B. stevensii*) in Oregon, four (*B. dothidea*, *B. obtusa*, *B. parva* and *B. rhodina*) in Arizona, Missouri and Arkansas, and three (*B. dothidea*, *B. obtusa* and *B. parva*) in New York, Pennsylvania, Maryland and Virginia. Two *Botryosphaeria* species were associated with cankers in Mexico, including *B. rhodina* in Sonora, and *B. obtusa* and *B. rhodina* in the Ensenada wine grape production area of Baja California. No other fungal pathogen was isolated from cankers in northwest Mexico. The second most prevalent fungus

was *Eutypa lata*, which was isolated from cankers in all areas except southern California, Arizona and northwest Mexico. *Phomopsis viticola* was occasionally isolated from cankers in California and Missouri. Other sporadically isolated fungi from cankers in California and Oregon were *Clonostachys* sp., *Truncatella angustata*, *Alternaria alternata*, *Pestalotia* sp., and the teleomorphs of *Phomopsis* spp., *Diaporthe helianthi* and *Diaporthe phaseolorum*. This study is the first report of *Botryosphaeria* species associated with grapevine cankers in the main grape-growing regions of the United States. The findings also confirmed *Botryosphaeria* species as the main fungal pathogens associated with cankers in northwest Mexico, and both *Botryosphaeria* species and *Eutypa lata* in the United States.

Basidiomycetes associated with heart rot of grapevines in Australia. J. Edwards, J. Cunnington, S. Salib and I.G. Pascoe. *Victorian Department of Primary Industries – Knoxfield Centre, 621 Burwood Hwy, Knoxfield, Victoria 3180, Australia.*
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Fomitiporia mediterranea is responsible for the white heart rot component of the esca disease complex of *Vitis* in southern Europe. In Australia, similar heart rots have been observed in grapevines but associated fungal fruiting bodies have proved difficult to find. In order to determine the affinities of Australian species, we sequenced a portion of the nuclear rDNA large subunit from forty isolates: 37 from grapevine, one from *Eucalyptus* sp., one from *Dodonea viscosa* (native hop-bush), and one from a fence post made of *Eucalyptus* wood. The sequences were compared with other species of Hymenochaetaceae. The Australian isolates were identified as species of *Fomitiporia* or *Inonotus s. str.* A single isolate was probably *Fuscoporia contigua*. There were two species of *Fomitiporia*: *F. australiensis* (from grapevine, *Dodonea* and the *Eucalyptus* fence post) and an undescribed species (from grapevine and the *Eucalyptus* sp.). Three species of *Inonotus s. str.* were found. We have not been able to identify any of these three species. One of these taxa was encountered much more frequently than the other two. Given that both *Fomitiporia* species were also isolated from native Australian hosts, it seems likely that the species encountered are native to Australia.

The effect of water borne microorganisms and hot water treatment on grapevine propagation and development. H. Waite[1] and M. Cole [2]. *[1]Northern Melbourne Institute of TAFE, Corner Cooper St & Dalton Rd, Epping, Victoria, 3076 Australia. [2]Agpath Pty Ltd, 105 Gunn Rd, Vervale, Victoria, 3814 Australia.*
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There is a perception that hot water treatment (HWT) sterilises the surface and the wood of cuttings. However many microorganisms associated with grapevines and contaminated water are quite robust and are dispersed by HWT. Isolation of water borne microorganisms from poorly performing vines that show browning of the vascular tissue suggested that these microorganisms were opportunistic pathogens in the vines, gaining entry to vine tissue through wound contact with contaminated water during propagation. The effects of 2 bacterial isolates, *Rahnella aquatilis* and *Enterobacter intermedius*, and a yeast isolate, *Trichosporon pullulans*, and HWT on graft healing and root and shoot development of Shiraz PT23 grafted to 1103 Paulsen (+/-HWT) were assessed by inoculating the bases of grafted cuttings and the graft union at the time of grafting. The vines were incubated and then grown in a commercial nursery. Root number, percentage graft healing and shoot length were assessed at leaf fall. HWT did not affect graft healing or root numbers. Scion shoot length was apparently promoted by HWT. None of the microorganisms had any effect on root number, but *R. aquatilis* and *E. intermedius* significantly reduced graft healing. Shoot length was suppressed by all 3 microorganisms, but the correlation between graft healing and shoot length was weak, indicating that the effect of the organisms on shoot growth was independent of their effects on graft healing. Microbial contamination of graft wounds during propagation is likely to be a contributing factor in the poor performance of grapevine planting material. It is strongly recommended that the practice of using untreated water, and of soaking cuttings and precut buds, be stopped.

Host Pathogen Interactions

Characterization of the population structure of *Eutypa lata* and identification of associated fungi in grapevine wood cankers. P.E. Rolshausen[1], N.E. Mahoney[2], R.J. Molyneux[2] and W.D. Gubler[1]. *[1]Department of Plant Pathology, University of California, Davis, CA, USA [2]USDA/ARS, Albany, CA, USA.*
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Eutypa lata is the causal agent of branch dieback of trees and is responsible for shortening the lifespan of vineyards. The fungus invades open wound vessels by means of ascospores released after rain, and slowly kills the wood over the years. Variability in the susceptibility of grape cultivars to the disease was reported. Grapevines can suffer multiple infections of *E. lata* each year. Moreover, pruning wounds are also the point of entry of many other wood decay fungi. Recent reports have indicated the presence of a complex of fungi co-inhabiting grapevine wood cankers. However, the structure of these fungal communities has not been studied extensively and

their economic impact has never been assessed. The objective of our study was first to characterize the fungal community found in association with *E. lata* in cankers of symptomatic grapevines. The second objective was to establish the population diversity and structure of *E. lata* by sorting the number of isolates recovered from individual cankers of susceptible and tolerant grapes. Finally, the secondary metabolite profile of these isolates was characterized. The sampling was done in 2 consecutive years from 2 vineyards in the Napa valley, CA. Samples were collected from 20 grapevines of the cultivars Merlot and Cabernet Sauvignon known to be tolerant and susceptible to *E. lata* respectively. The spur positions showing Eutypa dieback foliar symptoms were flagged in the spring. A 1-cm-thick slice of the cordon with discolored wood was cut off at that spur position the following dormant season. Three wood zones consisting of the living sapwood, the dead sapwood and the marginal wood of canker lesions were separated from each other. Wood chips from each zone were surface-sterilized and plated on potato dextrose agar. Fungi recovered were sub-cultured and identified morphologically or by BLAST search of the ITS rDNA region in the GenBank database. The total number of *E. lata* isolates was determined by Vegetative Compatibility Groups (VCGs) and each VCG was confirmed as *E. lata* based on its PCR-RFLP patterns. Secondary metabolite production was determined for 6 VCGs isolated from each cv. in the first field, where a total of 3, 2 and 1 VCGs were found in 3 individual cankers. All 12 VCGs were grown in a liquid culture of wood cane extracts, and secondary metabolites were extracted after 1 month of growth and were identified and quantified by high liquid performance chromatography. Our results indicated that *E. lata* was recovered successfully from cankers and canker margins, but also from apparently healthy wood. *E. lata* in the cankers was commonly found in association with *Phomopsis viticola, Phaeoacremonium aleophilum* and *Botryosphaeria* spp. A greater number of *E. lata* VCGs was found in the susceptible cv. Cabernet Sauvignon than in the more tolerant Merlot in the two fields sampled. We recovered a total of 47 and 35 *E. lata* isolates from Cabernet Sauvignon and 30 isolates from Merlot from the two vineyards. In Merlot, the same VCG was often identified from the inside to the margin of the canker, while Cabernet Sauvignon presented different VCGs from these two zones. The analysis of secondary metabolites produced by the 12 VCGs indicated a different qualitative profile between VCGs from Merlot and those from Cabernet Sauvignon. Eutypine was always produced by at least one of the VCGs recovered from each sample of Merlot. Our results suggest that the host could play a role in the selection pressure on *E. lata*, i.e. the most virulent isolates might only be able to infect tolerant cvs., while susceptible cvs. would most likely be infected by a broader pool of isolates.

Toxins and secondary metabolites from the fungus *Phomopsis* spp., a pathogen responsible of vine excoriosis. E. Abou-Mansour[1], M-L. Goddard[1], N. Mottier[1], D. Christen[2] and R. Tabacchi[1]. [1]*University of Neuchâtel, Institute of Chemistry, Laboratory of Analytical Chemistry, Rue Emile Argand 11, CH-2009 Neuchâtel, Switzerland.* [2]*Phytopathology Group, Institute of Plant Sciences, Swiss Federal Institute of Technology, 8092 Zurich, Switzerland.*
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Nine strains of the fungus *Phomopsis* spp. were collected from a grapevine field in Ticino, Switzerland and analysed for their secondary metabolites. Five new metabolites , four furanones and cytosporone F, together with three known compounds phomopsolide B, and two xanthones were isolated from three strains identified as *Phomopsis* sp., *Phomopsis viticola* Sacc., and the *Phomopsis viticola* complex. The structures of the compounds isolated were determined by spectroscopic methods, mainly by intensive use of 1D and 2D NMR experiments and by mass spectrometric measurements. Biological assays against *Vitis vinifera* leaves and grape callus were evaluated and the antibacterial activity of the new isolated compounds was assessed. Phomopsolide B showed the greatest activity on grape leaves and against callus assay. The metabolism of phomopsolide by grape leaves was also investigated and the detoxified compound identified. Furthermore, an LC-(API)-MS method was developed and used for the detection of identified metabolites when *Phomopsis* sp. was grown on pruned grapevine wood.

Influence of the cultivar and the fungal isolate on the spread of *Eutypa lata* within grapevines. M. Sosnowski[1,2], R. Lardner[1,3], T. Wicks[2] and E. Scott[1,3]. [1]*Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond SA 5064, Australia.* [2]*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia.* [3]*School of Agriculture, Food and Wine, University of Adelaide, Glen Osmond, SA 5064, Australia.*
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Eutypa dieback, caused by the fungus *Eutypa lata*, is one of the most important trunk diseases of grapevine worldwide, causing yield decline and eventually killing the vines. Symptoms include external wood cankers and wedge-shaped staining of internal woody tissue, stunted shoots with chlorotic leaves, often cupped and with tattered margins. Although the expression of foliar symptoms of eutypa dieback is influenced by the grapevine cultivar and the isolate of *E. lata*, the effect of the cultivar and the isolate on the spread of mycelium or staining in the grapevine wood has not been documented. Rooted grapevine cuttings were inoculated with *E.*

lata mycelia in two shadehouse experiments. In Experiment 1, cuttings of Grenache were inoculated separately with 28 isolates, collected from various regions of southern Australia. In Experiment 2, cuttings of Grenache, Cabernet Sauvignon and Merlot were inoculated with 12 isolates. The severity of foliar symptoms (percentage affected) was assessed during the following two spring seasons (8 and 20 months after inoculation). Twenty-four months after inoculation, he bark was removed and the extent of stained wood tissue measured above and below the inoculation point. To determine the extent of colonisation by *E. lata*, the stems were surface sterilized and sections cut at 5 or 10-mm intervals above and below the inoculation site to a distance of approximately 100 mm beyond any staining, then plated onto potato dextrose agar and assessed for the presence of *E. lata*. In a third experiment, fresh wounds on 1-year-old canes of mature vines (Cabernet Sauvignon, Gamay, Grenache, Merlot, Pinot Noir, Riesling, Semillon and Shiraz) in a vineyard in the Barossa Valley, South Australia, were inoculated with ascospores of *E. lata*. Vines were assessed for foliar symptoms in the spring of each year following inoculation. Inoculated spurs were removed 3 or 4 years after inoculation and dissected to assess the extent of staining. The extent of staining beyond the inoculation point was measured and the presence of *E. lata* was confirmed using an *E. lata*-specific DNA probe. In Experiment 1, 24 isolates induced foliar symptoms, with severity ranging from 2 to 36%. Over 24 months up to 230 mm of grapevine stem was colonised by *E. lata*. There were significant differences among isolates for both symptom severity and the extent of colonisation by *E. lata*, although there was no correlation between these factors. In Experiment 2, seven isolates induced foliar symptoms on Grenache, and two isolates on Cabernet Sauvignon and Merlot. Only one isolate induced foliar symptoms on all three cultivars. The mean severity of foliar symptoms was significantly greater in Grenache than in Cabernet Sauvignon or Merlot. There was significantly more staining in Grenache and differences were apparent in the extent of colonisation by the isolates. The greatest mean distance colonised by *E. lata* ahead of stained tissue was 46 mm in Cabernet Sauvignon, 35 mm in Merlot and 34 mm in Grenache. The greatest distance from which *E. lata* was isolated ahead of staining was 75 mm, in a Grenache vine. The average growth rate of *E. lata* in the wood of Cabernet Sauvignon, Merlot and Grenache was 15, 13 and 18 mm year^{-1} respectively. In the third experiment, foliar symptoms were not observed on any vines inoculated with *E. lata* 3 or 4 years previously. The extent of staining attributed to *E. lata* differed significantly among cultivars, with means ranging from 10 mm year^{-1} (Merlot, Gamay, Grenache and Semillon) to 18 mm year^{-1} (Cabernet Sauvignon and Shiraz). There was considerable variation in the amount of staining within cultivars and, in some cases, staining attributed to *E. lata* extended up to 50 mm year^{-1} in Cabernet Sauvignon and Shiraz vines. This study showed that the virulence of *E. lata* varies depending on the isolate and the grapevine cultivar. Vines differed in their resistance to the spread of *E. lata*, and the severity of foliar symptoms was not related to the amount of colonisation of woody tissue. The growth rate of *E. lata* was greater in young cuttings compared to mature vines, with maximums of 115 and 50 mm year^{-1}, respectively. The staining of wood typically associated with eutypa dieback did not always correlate with the presence of the fungus, and *E. lata* grew up to 75 mm in advance of staining. This suggests that wood staining may, in part, be caused by other wood-inhabiting fungi, or by the wound response and that control of infected vines requires removing at least 10 cm of wood beyond the stained tissue. The absence of foliar symptoms in the field 4 years after inoculation, despite observing symptoms in the first season in rooted cuttings, may be due to differences in inoculation method, environmental conditions or vine physiology. Information from this study will help to optimise strategies for removing infected tissue from vines, thus maintaining productivity of grapevines with eutypa dieback and reducing the economic impact of the disease.

Characterization and early detection of grapevine (*Vitis vinifera*) stress responses to esca disease by *in situ* chlorophyll fluorescence and comparison with drought stress. D. Christen[1], S. Schönmann[1], M. Jermini[2], R.J. Strasser[3], G. Défago[1] and I. Wagschal. [4]. *[1]Phytopathology Group, Institute of Plant Sciences, Swiss Federal Institute of Technology, CH-8092 Zürich, Switzerland. [2] Agroscope, Swiss Federal Research Station for Plant Production of Changins, Centre of Cadenazzo, CH-6594 Contone, Switzerland. [3] Bioenergetics Laboratory, University of Geneva, CH-1254 Jussy-Geneva, Switzerland. [4]Department of Phytopathology, Institute of Integrative Biology, Swiss Federal Institute of Technology, Zurich, Switzerland.*
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Esca disease, as well as other trunk diseases of grapevine, is an important wood disease that impedes the water transport in plants by clogging the xylem vessels. This type of effect is not detectable for years, due to the long latency time of the diseases. In a field experiment, the susceptibility of *Vitis vinifera* cv. Cabernet Sauvignon and Merlot to esca disease was evaluated by visual assessment of foliar symptoms and by necrosis and white rot indexes. C. Sauvignon was highly susceptible and Merlot was tolerant to esca. The characteristics of the fast chlorophyll *a* fluorescence transient were investigated in attached leaves by using the so-called JIP-test. The fluorescence transient was analyzed and plants without visible esca foliar symptoms were compared with

those showing symptoms. In C. Sauvignon, alteration of the photosynthetic apparatus could be detected two months before the appearance of foliar symptoms in autumn. To our knowledge, this is the first report of early detection of esca disease using a nondestructive method. For Merlot, only one JIP-test parameter was affected. However, when both cultivars were compared, the relationship of the performance index (defined by the density of reaction centers (RCs) and by the relative yields j_{Po} and y_o; $PI_{ABS} = [RC/ABS][j_{Po}/(1–j_{Po})][y_o/(1–y_o)]$) versus the calculated rate of the electron transfer (ET_0/ABS) permitted us to separate the highest performing cultivar Merlot from the susceptible C. Sauvignon. Also, the method used allowed us to detect modification of photosystem II (PS II) performance in greenhouse-grown Riesling×Sylvaner after a drought stress. Finally, the comparison of the fluorescence transients of esca-affected and of drought-stressed grapevines provided information on the two differentiated functional-behaviour patterns of the PS II for both stress types. These results suggest that esca infection cannot simply be interpreted as a water transport deficit through xylem dysfunction, but that other reaction mechanisms in the plants must be considered. The possibility of using fast chlorophyll *a* fluorescence monitoring as a wood decay early detection tool is discussed.

Recent developments of research on the factors influencing symptom appearance in esca diseased grapevines in Italy. L. Mugnai[1], F. Peduto[1], L. Calamai[2], G. Mattii[3], G. Marchi[1], G. Surico[1] and E. Zini[1]. *[1] Dipartimento Biotecnologie Agrarie - Sezione Patologia vegetale, P.le delle Cascine 28, 50144 Firenze, Italy. [2]Dipartimento di Scienza del Suolo e Nutrizione della Pianta, P.le delle Cascine 18, 50144 Firenze, Italy. [3] Dipartimento di Ortoflorofrutticoltura, Viale delle Idee 30, 50019 Sesto F.no, Italy.*
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Not many plant diseases attain the complexity of esca complex, and even now research is still adding more bits to the puzzle and more questions to be answered. Different pathogens are involved, and there are various infection sources not only for all these pathogens as a whole, but also for each single pathogen. In addition, the external factors influencing symptom development in affected vines are still poorly understood. Among many other lines of research, an interdisciplinary approach was used to analyse the physiological changes occurring in vines before and during symptom expression. The influence of the watering regime, changes induced in photosynthetic activity and gas exchange, and changes induced in the amino acid content and water-stress related parameters are all under study, and preliminary results are reported and discussed. Relating these different approaches to each other can help us to understand and monitor the role of single factors, as in the case of the role that phytotoxins have in the development of foliar symptoms.

Effect of esca and eutypa dieback toxins on the regulation of grapevine defence responses. Wagschal, D. Christen and G. Défago. *Department of Phytopathology, Institute of Integrative Biology, Swiss Federal Institute of Technology, Universitätsstr. 2, CH-8092 Zürich, Switzerland.*
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Eutypa dieback and esca disease are insidious grapevine trunk diseases leading to the decline of vineyards by congesting the xylem vessels, and eventually killing the vines. They are characterized by long latency times. Eutypa dieback is caused by *Eutypa lata*, whereas esca disease is caused by a complex of pathogens, which were shown to produce several toxic compounds (e.g. eutypine, 4-hydroxybenzaldehyde, 3-phenyllactic acid). No *Vitis* cultivar is resistant to trunk diseases, but Merlot is rather tolerant and is thought to possess a specific aldehyde reductase responsible for detoxification. Some *Trichoderma* strains are able to degrade these toxins, and could be used as biocontrol agents. It is therefore hypothesized that at low concentrations the toxins are able to stimulate plant defences and reduce disease. Induced resistance is associated with an increase in transcription of the genes possibly involved in resistance and the production of defence molecules such as stilbenes. We therefore examined the effect of the esca and eutypa dieback toxins on the elicitation of cell suspensions and leaves of grapevine. A time-course and dose-effect experiment was carried out on cell cultures of *Vitis vinifera* cv. Gamay to determine the cytotoxicity levels of the poisonous molecules. The kinetics of toxicity of all the tested molecules revealed that they act within a frame of few hours only. Moreover, the induction of some genes of interest was studied by Real-Time RT-PCR. *GST*, *GLU*, *PGIP*, *PIN*, and *LOX9* gene transcripts were shown to accumulate upon treatment with the toxic compounds, whereas other genes involved in defence, such as *PAL*, *STS*, and *CHIT,* were not affected. In order to test our theory of induced resistance, grapevine cell suspensions were put in a first contact with 2 μM of toxin. Sixteen hours later they received 5 μM, as well as cells which had not been pre-treated. At the level of the cell death rate, no significant difference could be observed. However, *GLU*, *PGIP*, and *LOX* transcripts accumulated significantly faster and to a higher extent in the pre-treated samples, while *GST* and *PIN* transcript abundances were increased to the same extent in both lots of samples. The same analyses were conducted on grapevine leaves, by absorption of the toxins through the petioles. Kinetics of the manifestation of the foliar symptoms, and

of the volume of solution uptake, were monitored. With all the toxins tested, there was a reddening of the surroundings of the main veins, followed by necrosis. It was also noticed that with the higher concentrations of toxins, there was a significantly higher uptake of solution by the detached leaf, suggesting increased transpiration. This would explain how a vine could switch suddenly from a latent form of the disease to an apoplecticform. These first analyses helped us to determine the toxin concentrations at which the plant is still able to initiate a defence reaction. Alarger screening for transcriptome reprogramming will be carried out in this way by EST microarray analysis in order to identify more candidate genes involved in the early perception of toxic compounds, signalling, cell defence, and detoxification. This knowledge will help speed up the selection of biocontrol agents for eutypa dieback and esca diseases, which at present is a very long process by conventional methods due to the several years of latency of these diseases.

Susceptibility of grapevine rootstocks to Cylindrocarpon disease. M.V. Jaspers[1], C.M. Bleach[1] and I.C. Harvey[2]. [1]*Lincoln University, PO Box 84 Lincoln University, Canterbury, New Zealand*. [2]*Plantwise Services, PO Box 181 Lincoln, Canterbury, New Zealand.*
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Cylindrocarpon black foot disease has been identified as a major cause of young vine death in South Africa, North and South America, Australia, New Zealand and several Mediterranean countries in the last 5–10 years. The purpose of the two (repeated) greenhouse studies was to investigate the susceptibility of the more commonly planted rootstocks in New Zealand. Rootstock plants were grown from the current season's cuttings and when 4–6 weeks old had their roots trimmed immediately prior to inoculation, either by soaking them in a spore suspension (10^6 ml^{-1}) or planting them in an infested medium. The young vines were grown in a greenhouse for 3–4 months and then assessed by scoring symptom expression, root growth and isolation of the pathogen from the roots and stem bases. The incidence of *Cylindrocarpon* spp. recovered from root and rootstock stem sections differed between trials. However, when rootstock varieties were ranked for resistance, there was general agreement about which were the most resistant in both trials. For root tissue, K 5BB, R140, and Schwarzman had the lowest pathogen incidence, but there was no strong correlation with root weights as expected. For stem tissue, Riparia Gloire, R140, 3309C and 420A had the lowest pathogen incidence, but these rootstocks had levels of stem blackening similar to those of the more severely linfected varieties. In the second trial, the rootstock stems of many varieties were seriously infected with *Botryosphaeria* spp., possibly affecting the incidence of the *Cylindrocarpon* spp. recovered, since the varieties with the highest incidence of *Botryosphaeria* spp. coincided with low *Cylindrocarpon* incidence. These results indicate that in greenhouse conditions all of the rootstock varieties commonly used in New Zealand vineyards are susceptible to *Cylindrocarpon* spp. in some degree. Field trials are currently being undertaken to verify these results under field conditions.

Epidemiology

Susceptibility of grapevine pruning wounds to trunk pathogen infection in South Africa. J.M. van Niekerk[1], F. Halleen[2] and P.H. Fourie[1]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa*. [2]*Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa*.
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Trunk diseases of grapevines cause decline and premature dieback of vineyards across the world. Several pathogens, which include *Eutypa lata*, *Phaeomoniella chlamydospora* and several *Phaeoacremonium*, *Botryosphaeria*, *Phomopsis* and basidiomycete spp., have been implicated in this complex of diseases. Wounds, especially pruning wounds, are the most important infection portals for these pathogens and pruning wound protection by means of chemical or biological control agents is recommended as preventative control measure. However, the period of pruning wound susceptibility has not been determined for most of the trunk disease pathogens. In order to address this issue, a trial was conducted in an 18-year-old Chenin Blanc vineyard in the Stellenbosch area of the Western Cape province of South Africa. Plants were pruned to 3 buds per cane at 2 stages (mid- [July] and late-winter [August]) during the dormant season of 2004. At each stage, 180 plants were pruned and the pruning wounds (20 wounds per treatment) spray-inoculated with 1 ml of a spore suspension of 1×10^4 spores ml^{-1} of *E. lata*, *Pa. chlamydospora*, *B. australis* and *P. viticola* directly after pruning, 1, 2, 3, 7, 10, 14, 17 and 21 days after pruning. As control treatments, pruning wounds were not inoculated but sprayed with 1 ml sterile water at the abovementioned times, or painted with a commercial non-fungicidal pruning wound sealant to limit further natural infection. Eight months after treatment, the incidence of these pathogens in the asymptomatic xylem and pith tissues (isolation zones) beneath the pruning wound scar was determined by means of isolations onto fungal growth media. Analysis of variance of the pathogen incidence data indicated significant pathogen × pruning

time × wound age at treatment × treatment interaction (P=0.0549). Xylem tissue generally yielded higher pathogen incidences than pith tissue, but susceptibility of both tissue types did not decline over the 3-week period, as was also obvious from trends depicted by quadratic regression lines for pathogen × pruning time × treatment combinations. Wounds made and inoculated in late winter generally yielded higher pathogen levels than mid-winter wounds. This effect was, however, not reflected by the water-treated and painted wounds, which generally yielded similar pathogen levels for both pruning times, with the exception of *Pa. chlamydospora*, which were more frequently isolated from painted wounds. The findings therefore illustrate that pruning wound protection agents should provide long-term protection of xylem and pith tissues against these pathogens, whether pruning was done during mid- or late winter.

Preliminary studies on the susceptibility of pruning wounds to contamination with fungi involved in grapevine decline diseases in Italy. S. Serra, M.A. Mannoni and V. Ligios. *Dipartimento di Protezione delle Piante - Università, Via De Nicola 9, 07100 Sassari, Italy.*
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Phaeomoniella chlamydospora (Pch) and *Phaeoacremonium aleophilum* (Pal) are associated with esca, a serious grapevine decline disease with a worldwide distribution. Another important grapevine decline disease is black dead arm, caused by *Botryosphaeria obtusa* (Bot). These fungi colonize the wood through wounds of any kind. The present study was undertaken to investigate the susceptibility of annual pruning wounds to infection. The experiment was carried out in a 15-year-old vineyard, cv Sauvignon, pergola-trained, located in the North of Sardinia, showing symptoms of both diseases. In 2005, one year-old canes were pruned during the dormancy period in January, February and March, leaving a 20–30 cm long spur on the vine. Each week from the pruning date to April–May the spurs, randomly distributed on 470 vines along two rows, were artificially inoculated with 40 μl of conidial suspension (Pch and Pal: 4000 conidia ml^{-1}; Bot: 2500 conidia ml^{-1}) on 20 wounds per fungus. An equal number of wounds was inoculated with sterile water. Four weeks after inoculation, spurs were collected and examined by cutting ten sections, 1 mm thick, from the top. Sections were placed on Petri dishes (five sections per dish) containing malt extract agar supplemented with tetracycline hydrochloride and streptomycin sulphate (50 mg l^{-1} of each) and incubated at 25°C for up to 20 days. Wounds on canes pruned in March were less susceptible to infection by Pch than those pruned at other times.By contrast, spurs pruned in the same period and inoculated with Pal and Bot had higher contamination percentages. All pruning wounds were also susceptible to Pal and Bot in late spring, when the grapevine is in full vegetative growth. These data need to be confirmed. Multi-year inoculations are planned to study this important way in which Pal, Bot, and Pch are spread.

Spore dispersal patterns of grapevine trunk pathogens in South Africa. J.M. van Niekerk[1], F. Halleen[2] and P.H. Fourie[1]. *[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602. [2]Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599.*
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Eutypa lata (Munkvold and Marois, 1995), *Phaeomoniella chlamydospora* (Mugnai *et al*., 1999), several species of *Phaeoacremonium* (Mostert *et al*., 2006a,b) and of *Phomopsis* (van Niekerk *et al*., 2005), and species of Botryosphaeriaceae (including *Botryosphaeria* and aggregate genera *Lasiodiplodia* and *Neofusicoccum*) (van Niekerk *et al*., 2004; Crous *et al*., 2006; van Niekerk *et al*., 2006) have been identified as trunk pathogens of grapevines and are known to infect wounds, especially pruning wounds. Airborne inocula should therefore be expected to play an important role in the epidemiology of these pathogens. Little is known about the spore dispersal of these pathogens as influenced by diverse climatic factors. For 14 weeks during each of the 2004 and 2005 pruning seasons (June to mid-September), multiple spore trapping was done with a Quest volumetric spore trap in an 18+-year-old Chenin Blanc vineyard in the Stellenbosch region, a winter-rainfall region in the Western Cape province of South Africa. An automatic weather station recorded temperature, rain, relative humidity (RH), wind direction and wind speed in the vineyard row adjacent to the spore trap. The spore trap disc was covered with clear adhesive plastic and sprayed with petroleum jelly. At regular intervals, the adhesive plastic segments, each of which represented a 6-hour period, were removed from the discs. Spores trapped during these periods were suspended in 10 ml sterile water and spread-inoculated onto five 65-mm Petri dishes containing water agar. After 36 hours of incubation at 22°C, all germinating spores observed at 50× magnification were transferred singly to 65-mm Petri dishes containing potato dextrose agar. These pure cultures were morphologically identified to at least genus level. In both seasons, spores of *E. lata*, *P. viticola* and Botryosphaeriaceae spp. were trapped throughout the trapping periods, but no spores of *Pa. chlamydospora* and *Phaeoacremonium* spp. were trapped. Spore events were mostly preceded by at least 0.03 mm rain, but spores of Botryosphaeriaceae spp. were also trapped when rain had

not been recorded. Correlation and step-wise regression analyses were done to determine the effect of selected climatic variables (in 6-hour increments) on spore events. Botryosphaeriaceae spp. spore counts correlated positively (P<0.1) with minimum temperature recorded in the 48 hours preceding the spore event, amount of rainfall, maximum RH, and mean wind speed during the spore event. The variables minimum and maximum temperature recorded in the 48 hours preceding the spore event, and maximum RH recorded during the spore event correlated positively (P<0.1) with the spore counts of *E. lata* and *P. viticola*. Step-wise regression analyses yielded prediction models for the spore dispersal for Botryosphaeriaceae spp. using the variables minimum temperature in the 48 hours preceding the spore event, minimum and maximum RH and rainfall during the event (R^2=0.40): of *E. lata,* using maximum temperature and rainfall duration in the 48 hours preceding the spore event (R^2=0.44); and for *P. viticola*, using maximum temperature and mean RH in the preceding 48 hours and maximum RH during the spore event (R^2=0.41).

References

Crous P.W., B. Slippers, M.J. Wingfield, J. Rheeder, W.F.O. Marasas, A.J.L. Phillips, A. Alves, T. Burgess, P. Barber and J.Z. Groenewald, 2006. Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* 55, 235–253.

Mostert L., F. Halleen, P. Fourie and P.W. Crous, 2006a. A review of *Phaeoacremonium* species involved in Petri disease and esca of grapevines. *Phytopathologia Mediterranea* 45, S12–S29.

Mostert L., J.Z. Groenewald, R.C. Summerbell, W. Gams and P.W. Crous, 2006b. Taxonomy and pathology of *Togninia* (*Diaporthales*) and its *Phaeoacremonium* anamorphs. *Studies in Mycology* 54, 1–115.

Mugnai, L., A. Graniti and G. Surico, 1999. Esca (black measles) and brown wood-streaking: two old and elusive diseases of grapevines. *Plant Disease* 83, 404–416.

Munkvold G.P. and J.J. Marois, 1995. Factors associated with variation in susceptibility of grapevine pruning wounds to infection by *Eutypa lata*. *Phytopathology* 85, 249–256.

van Niekerk J.M., P.W. Crous, J.Z. Groenewald, P.H. Fourie and F. Halleen, 2004. DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. *Mycologia* 96, 781–798.

van Niekerk J.M., J.Z. Groenewald, D.F. Farr, P.H. Fourie, F. Halleen and P.W. Crous, 2005. Reassessment of *Phomopsis* species on grapevines. *Australasian Plant Pathology* 34, 27–39.

van Niekerk, J.M., P.H. Fourie, F. Halleen and P.W. Crous, 2006. *Botryosphaeria* spp. as grapevine trunk disease pathogens. *Phytopathologia Mediterranea* 45, S43–S54.

Fruit symptoms of esca (black measles) induced by injecting culture filtrates and spore suspensions of the causal pathogen. T.S. THIND and W.D. GUBLER. *Department of Plant Pathology, University of California, Davis, USA.*
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Symptoms of black measles were induced both under laboratory and field conditions through injections of culture filtrates and spore suspensions of *Phaeoacremonium aleophilum* (Pa) and *Phaeomoniella chlamydospora* (Pc). Spore suspensions (10^5 spores ml^{-1}) of both pathogen species were prepared in distilled sterilized water from10-day-old cultures grown on PDA-Tet medium, while culture filtrates were prepared by growing the cultures on MA broth for one month and filtering through three layers of Whatman filter no. 1 to remove spores and mycelia. Laboratory experiments. Berry clusters of Thompson Seedless at two growth stages, pea-size, and well-developed firm, were surface-disinfected with a 10% bleach solution. Half of the bunches were injured by pricking the berry surface with a fine syringe needle producing a wound of approx. 0.5 mm. Inoculum or culture filtrate drops (20 μl each) of Pa or Pc were placed on the injured and the intact berry surfaces and incubated by placing the berries in small plastic boxes lined with moist paper towels at 25°C. Water inoculation served as controls. In another experiment, inoculum or culture filtrate drops were placed at the stalk end of the berries after gently removing the stalks while keeping the individual berries in an upright position by placing them on plastic blocks. Observations were recorded regularly for up to 10 days on symptom appearance, which took the form of a reddish-brown pigmentation. Reddish brown lesions were observed when spore suspensions or culture filtrates were inoculated on the injured berries, but the lesions remained restricted and did not extend beyond 2–3 mm. Young hard berries were more susceptible than mature berries. The reddish (in Pa) or dark brown (in Pc) pigmentation spread downwards (3–5 mm). Field experiments. Spore suspensions or culture filtrates of Pa or Pc were injected with a syringe into Thomson Seedless berries (approx. 100 μl per berry) at Tulare in the third week of July. Three bunches per plant were injected and three plants were used per treatment. In another experiment at Madera, culture filtrates of Pa and Pc were injected into cordons/twigs by making a slanting hole in the cordon with a battery operated drill near the base of the spur position and inserting a disposable plastic syringe containing 50 ml of culture filtrate or water deep into the hole. Symptom appearance on the berries was recorded twice at bi-weekly intervals. Typical black measle symptoms developed when berries in the vineyards were syringe-inoculated with spore suspension or culture filtrates. The pattern

of symptom development near the injection points indicated production of metabolites responsible for measle development on the berry surface. Symptom development was earlier with Pc spore suspension or filtrate inoculation. Symptom development was more rapid and pronounced with culture filtrate injections than with spore suspensions and was more severe with Pc filtrate. Injections of culture filtrates in the cordons also produced typical measle symptoms on the berries. No symptoms appeared with the water injections. The study indicated that the development of measle symptoms on berries may not be due to the direct infection of the berries by the pathogen spores but to the production of metabolites by the pathogen in the host, which metabolites are translocated to the berries in the early development stages.

Photosynthesis alteration before foliar esca expression. F. Fontaine[1], M. Boulay[2], N. Vaillant-Gaveau[1] and C. Clément[1]. *[1]Université des Sciences de Reims, Laboratoire Stress, Défense et Reproduction des Plantes, Bât. 18, BP 1039, 51687 Reims cedex 2, France.[2]Moët et Chandon, 6 rue Croix de Bussy, 51200 Epernay, France.*
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Esca is a destructive disease affecting vineyards all over the world. Several fungi and external factors are found associated with the disease but the main physiological causes are not fully understood. Esca is characterized by wood decay symptoms associated with two types of symptoms ranging from the rapid dehydration and death of the annual cane (apoplectic form), to the progressive loss of pigments from the leaves (chronic form). Previous results have shown that after foliar symptom expression, carbon metabolism is drastically affected, and the alteration of photosynthetic apparatus induces a reduction in the carbohydrate reserves of the canes. The aim of this work was to determine if the photosynthetic apparatus is affected before foliar symptom expression in its apoplectic and chronic forms. Gas exchange was measured from the beginning of June to September, 2005, with foliar symptom expression occurring in the middle of July. The experiment was carried out on 19-year-old plants of Chardonnay in a Champagne vineyard. Preliminary results showed that in the chronic form, the photosynthetic apparatus was affected until 15 days before foliar symptom expression, and that the alteration became worse thereafter. In the apoplectic form, photosynthesis could be seriously damaged one month before rapid leaf dehydration and defoliation. Thus, some plants presented an early alteration of their photosynthetic apparatus, before the leaf symptoms of esca were expressed. These preliminary results still need to be confirmed.

Apple as a possible alternative host for grapevine pathogens in California. L. Gallegos, S. Rooney-Latham and W.D. Gubler. *[1]University of California-Davis, Department of Plant Pathology, Davis 95616, USA.*
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In a study on canker-causing fungi in an apple orchard in Mendocino County, California, three important grapevine pathogens were isolated from vascular tissue of apple trees (*Malus domestica)*: *Eutypa lata,* the causal agent of Eutypa dieback in grapevine, *Phaeoacremonium angustius* and *Togninia fraxinopennsylvanica* (anamorph *Phaeoacremonium mortoniae*), fungi associated with vine decline and esca in grapevine. None of these pathogens have previously been reported on apple in California. Pathogenicity studies done previously in which several possible alternative hosts were inoculated with the esca pathogens, showed that apple was equally susceptible to infection by the *Phaeoacremonium* sp. as was grapevine. Furthermore, perithecia formed readily on artificially inoculated apple wood. However, natural infection of *Phaeoacremonium* sp. on apple has not been reported before. Cankers on apple branches were examined for fruiting bodies and perithecia were found. Ascospores from the perithecia were cultured and subsequent DNA sequencing confirmed the identification of the perithecia as *Togninia fraxinopennsylvanica*. Naturally occurring perithecia of *Phaeoacremonium sp*. have only recently been found on grapevine, and finding them on apple was unexpected but not surprising in the light of the study mentioned above. The apple trees from which these pathogens were isolated were situated in an area where grapevines are extensively grown. In addition to the grapevine pathogens, *Neofabraea malicorticis*, the causal agent of anthracnose canker on apple was also found. In a separate study, a *Neofabraea* sp. was isolated from several grapevine spurs in Sonoma County, suggesting a possible association between these canker-causing fungi. Pathogenicity studies are currently under way to determine which of the fungi examined above are responsible for canker formation on apple branches, as well as to identify the symptoms produced by the *Neofabraea* sp. on grapevine. The findings suggest that apple 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.

Genetic variation of *Fomitiporella* sp. associated with grapevine Chlorotic leafroll symptoms. J. Auger, M. Esterio and N. Aguilera. *Universidad de Chile, Facultad de Ciencias Agronómicas - Departamento de Sanidad Vegetal, Santiago Chile, Casilla 1004 - Santiago, Chile.*
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The genetic variability of *Fomitiporella* sp. was ex-

plored through the PCR-RAPD technique using 44 isolates recovered from grapevine plants with chlorotic leafroll symptoms from 5 locations in the Chilean Central Valley (V, VI, VII Regions and the Metropolitan Area). The use of 11 universal primers of the OPA series (Operon Technologies) amplified 146 polymorphic bands. A similarity dendrogram of the various isolates was constructed by means of the NTsys 2.0 program. In addition, the study included isolates from the Hymenomycetes: *Fomitiporia mediterranea, Phellinus igniarus* and *Phellinus* sp. The results suggest that *Fomitiporella* sp. isolates clustered depending on the location. As regards the isolates of the other Basidiomycetes analyzed and associated with wood damage in grapevines, there was a differentiation between the *Fomitiporella* sp. isolates and the *Fomitiporia mediterranea, Phellinus igniarus* and *Phellinus* sp. isolates, with three different clusters being differentiated. Since there were no isolate clusters in plants recovered with chlorotic leafroll symptoms in the field, these results suggest that clonal propagation of the fungus from plant to plant does not occur, and that field infection is through basidiospores.

Reference

Auger J., N. Aguilera and M. Esterio, 2005. Identification of basidiomycete species associated with wood decay symptoms of grapevine chlorotic leaf roll in Chile. *Phytopatologia Mediterranea* 44, 86 (abstract).

Cortesi P., M. Fischer and M. Milgroom, 2000. Identification and spread of *Fomitiporia punctata* associated with wood decay of grapevine showing symptoms of esca disease. *Phytopatology* 90, 967–972.

Fischer M., 2001. Diversity of basidiomycetous fungi associated with esca diseased vines. *Phytopatologia Mediterranea* 40, Supplement, S480 (abstract).

Pollastro S., A. Abbatecola, C. Dongiovanni and F. Faretra, 2000. Usage of molecular marker (PCR-RAPD) for studing genetic variability in *Phellinus (Fomitiporia)* sp. *Phytopatologia Mediterranea* 40, 362–368.

Economic loss in California's table grape vineyards due to measles. S.J. Vasquez[1], W.D. Gubler[2] and G.M. Leavitt[3]. *[1]University of California Cooperative Extension, Fresno County, CA 93702, USA.[2]University of California, UC Davis, Davis, CA 95616, USA.[3]University of California Cooperative Extension, Madera County, CA 93637, USA.*
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Measles has long plagued California grape growers with its cryptic expression of symptoms its and its lack of identifiable causal organism(s). Table grape growers experience the greatest losses because of the demand for unblemished fruit. Typical fruit symptoms include black speckling which gave measles its name. The black spots appear shortly after fruit set and continue through harvest, often coalescing, and turning the berries completely black. Fruit that is severely affected often cracks and may rot or dry into raisins. Grapes that fully mature, but then suddenly display measles symptoms near harvest, often have an acrid taste, even when only a few berries are affected. Chlorosis and necrosis are typical foliar symptoms, and, much like fruit symptoms, appear throughout the growing season at varying degrees of severity. Fresh-market grape production exceeds $1 billion a year with over 16 cultivars being exported to more than a dozen countries. Producing grapes for the fresh market is an intensely laborious production system with growers managing vineyards year-round at a cost exceeding $17,000 per hectare. Although all cultivars grown for the fresh market are susceptible to measles, 'Flame Seedless' and 'Thompson Seedless' are the most commonly affected. Some 'Thompson Seedless' vineyards in the San Joaquin Valley were surveyed to determine the economic impact of measles on grape production for the fresh market. Four 'Thompson Seedless' vineyards for the fresh market were surveyed for disease incidence and severity during the 1997–98 growing seasons. The vineyards were similar in characteristics including age (24–30 years old); they were grown on a standard T-trellis, and furrow irrigated. Disease severity scores for the fruit ranged from 0 (= no symptoms) to 5 (= 100 percent fruit symptoms) and were documented on 0.40 ha. Disease severity at harvest ranged from 35 to 54 percent for Madera and Kern, respectively. In 1998, disease severity at harvest ranged from 33 percent for Tulare-2, and 56 percent for Tulare-1. The high disease severity in fruit resulted in this report of yield and economic loss to California's fresh market grape industry.

Susceptibility of some grapevine varieties and rootstocks commonly used in Spain to decline-associated fungi. J. Luque[1], S. Martos[1], E. Torres[2] and F. Garcia[2]. *[1]Department Protecció Vegetal, IRTA - Centre de Cabrils; Ctra. de Cabrils s/n, E-08348 Cabrils, Spain. [2]Laboratori de Sanitat Vegetal; Via Circulació Nord, Tram VI, Carrer 3, Zona Franca, E-08040 Barcelona, Spain.*
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Surveys conducted in the last decade in Spain have detected most of the fungi already known to be associated with grapevine declines from other wine producing countries. In order to evaluate the susceptibility that some varieties and rootstocks common in Spain exhibit to these fungi, pathogenicity tests were carried out with different pathogen-variety combinations. The plant material used in the trials included three red cultivars (*Cabernet Sauvignon*, *Grenache* and *Tempranillo*), three white cultivars (*Macabeu*, *Parellada* and *Xarello*), and four rootstocks (*110R*, *140Ru*, *41B* and

SO4). The fungal pathogens tested were four *Botryosphaeria* spp. (*B. dothidea*, *B. lutea*, *B. obtusa*, *B. parva*), *Eutypa lata* and *Phaeomoniella chlamydospora*. Artificial inoculations were conducted on groups of 10 one-year old potted plants. Wounds were made on the bark surface of either canes or stems and inoculated with a mycelial plug of each fungus (on the canes, of the varieties; on the stems, of the rootstocks). Control plants of each variety and rootstock were inoculated in a similar way with sterile PDA plugs. The plants were maintained in a greenhouse with regular watering. The length of the vascular necroses was measured five months after the inoculations and this parameter was used in the statistical analysis as an index of host susceptibility.

Effects of esca disease on leaf gas exchanges of cv. Alvarinho in a vineyard of the Portuguese Vinho Verde Region. M.L. Felgueiras[1]; G. Chicau[2]; J.M. Moutinho-Pereira[3] and A.C.P. Dias[1]. *[1]Biology Department, Minho University - Campus de Gualtar 4710-057 Braga - Portugal. [2]Ministry of Agriculture, D.R.A.E.D.M. - Rua da Restauração No. 336, 4050-501 Porto - Portugal. [3]Trás-os-Montes e Alto Douro University, Apartado 1013, 5000-911 Vila Real - Portugal.*
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Esca is a complex disease that is usually not noticed until the grapevines begin to show external symptoms. However, grapevines can be infected for long periods of time while only rarely manifesting the typical leaf symptoms. Moreover, little is known about the consequences of esca on vineyard productivity. In Portugal, in the Vinho Verde region, Alvarinho sub-region, most vineyards are infected with esca. In this work we evaluated the impact of esca on some physiological parameters of grapevines cv.× Alvarinho. Plants showing the foliar symptoms of esca were selected. Ten plants were studied; two types of cordons were selected: infected cordons containing leaves with and without visible symptoms of esca, and asymptomatic cordons. Gas exchange was measured by the IRGA system on three different leaf materials of each grapevine: leaves from the infected cordon with (FD) and without (FApD) visible symptoms; and leaves (FB) from the asymptomatic cordon. Parameters studied were: net photosynthesis *(A)*; transpiration (*E*); stomatal conductance (*g*); and intercellular concentration of CO_2 (*Ci*). The leaves from the symptomatic cordon (FApB, FD), had significantly lower *A* values than those from the asymptomatic cordon (FB). This was particularly noticed in the leaves with external symptoms (FD), where *A* was extremely low. *E* and g_s were also severely affected in leaves from the symptomatic cordon, but the values were not significantly different between FApB and FD. C_i was significant increased in leaves from the symptomatic cordon (FApB, FD). These results indicated that esca reduced both the functional leaf area and the assimilation rate of the grapevines, and that all leaves from a symptomatic cordon were affected, regardless of whether they themselves showed symptoms or not.

Effect of inoculation with *Phaeomoniella chlamydospora* on mortality, graft strength and polyphenol content of young grapevines. A.B. Graham, L.D. Melton and B.G. Smith. *[1]University of Auckland, Department of Chemistry, Auckland, New Zealand.*
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The effect of inoculation with *Phaeomoniella chlamydospora* on the mortality, graft strength and polyphenol content of young grapevines was investigated in field trials and by measurement of the phenolic content using HPLC in grapevines subjected to both grafting and fungal inoculation. The grapevine cultivars used were Pinot Noir cl. 777 and Sauvignon Blanc cl. 1 UCD, grafted onto 101-14 Mgt rootstock. During graft development the total polyphenol and stilbene oligomer content in the xylem of the rootstock increased, while the resveratrol content decreased. Pinot Noir inoculated in the graft with *P. chlamydospora* had a greater total polyphenol and antifungal ε-viniferin content than Sauvignon Blanc and the uninoculated controls. The mortality of Pinot Noir after 10 weeks was less than that of Sauvignon Blanc vines. However, after 8 months, the surviving Pinot Noir vines had more brown discoloration of the xylem and graft region and a higher incidence of weak grafts than Sauvignon Blanc. Inoculation with large doses (10^6 cfu ml^{-1}) of *P. chlamydospora* directly onto the graft caused over 90% of vines to die or be rejected during grading. However, at lower doses up to 70% of the Sauvignon Blanc vines reached acceptable quality, compared with only 17% of Pinot Noir. In the second year of growth, at least 95% of the Sauvignon Blanc vines given low-to-moderate doses of *P. chlamydospora* retained their quality. Accumulation of polyphenols below the graft in response to fungal infection may have exacerbated thickening of the wound layer inside the graft, which may have interrupted the formation of xylem bridges across the graft in Pinot Noir vines more than in Sauvignon Blanc. Although *P. chlamydospora* can cause a gradual decline in the health of older grapevines in commercial vineyards, the field trials confirmed that this fungus poses a significant problem to viticulture nurseries as a cause of premature vine death and loss of vine quality.

Effects of hoja de malvón disease on the sensory properties and composition of Malbec wine from Mendoza, Argentina. F. Casassa[1], S. Sari[1], S. Avagnina[1], V. Longone[2], C. Césari[2], G. Escoriaza[2], C. Catania[1]

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Hoja de malvón is a wood grapevine disease that decreases vineyard productivity and longevity in Argentina. This study was conducted to evaluate the influence of this disease on wine quality and organoleptic attributes. Lots of 120 kg of grapes cv. Malbec harvested from vines with different degrees of disease symptoms from Luján de Cuyo, Mendoza, were crushed and wine was made in 100 l stainless steel tanks. Grapes were grouped in three categories according to plant symptoms (%): C1, no symptoms; C2, 10–50% symptoms; and C3, 50–100% symptoms. No differences among wines were found in alcohol, total acidity and pH. Volatile acidity and Color Index (IC: 420+520+620 nm) were higher in wines from C3, while Total Phenolics Index was higher in C2 wines. Wines from grapevines with no symptoms presented the greatest hue value (420/520 nm). During sensory analysis, C2 and C3 wines were perceived as having greater color intensity, violet hue and spicy notes than C1, while the latter was perceived as the fruitiest. No differences among wines were perceived in global aromatic intensity, concentration, astringency, bitterness or acidity in the mouth. According to the Kramer test, wine made from C2 grapes was preferred by the judges. This experiment was repeated during 2006 and wines are currently being made.

Presence of double-stranded RNA molecules in Cylindrocarpon liriodendri isolates infecting grapevine. T. Nascimento[1], F. Cardoso[2], C. Rego[1] and H. Oliveira[3]. [1]*Laboratório de Patologia Vegetal "Veríssimo de Almeida", Tapada da Ajuda, 1349-017 Lisboa, Portugal*. [2]*INETI-DB-UTPAM, Estrada do Poço do Lumiar, 22,1649-038 Lisboa, Portugal*. [3]*Instituto Superior de Agronomia, Departamento de Protecção das Plantas e de Fitoecologia, Tapada da Ajuda, 1349-017 Lisboa, Portugal*.
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In some fungi, double-stranded RNA molecules (dsRNAs) are responsible for morphological and physiological variation, which includes virulence. Results of pathogenicity tests carried out with a collection of *Cylindrocarpon liriodendri* isolates (previously identified as *C. destructans*) revealed that all caused typical disease symptoms of black foot disease in grapevine plants. Differences in morphological and virulence characteristics were detected, although no clear association was found between these parameters and age, geographic origin or even DNA data of isolates. To date, dsRNAs are reported to be responsible for up regulation of *C. destructans* virulence associated with ginseng root rot. The aim of the present study was to screen *C. liriodendri* isolates obtained in Portugal from grapevine for the occurrence of dsRNAs and to carry out their preliminary characterization. The presence of dsRNA was detected in 27% of the strains screened and nine of these strains were selected for dsRNA analyses. It was detected the presence of three different dsRNAs bands, one of about 1 kb (one isolate), another of 2 kb (four isolates) and a common band to all the isolates with approximately 15 kb. The 1 kb and 2 kb dsRNAs are similar to the smallest dsRNAs (6.0, 5.0, 2.5 and 1.5 kb) detected in *C. destructans* isolates from ginseng. The 15 kb dsRNA differs from these results but its nature was confirmed through cellulose (CF-11) column chromatography. Moreover, positive results for all the nine isolates were obtained by DAS-ELISA using dsRNA antibodies. The behaviour of dsRNA-cured strains is being carried out and compared with wild strains.

Stilbenic polyphenols from esca diseased wood and their role as ROI scavengers. C. Amalfitano[1], D. Agrelli[1], L. Mugnai[2], A. Evidente[1] and G. Surico[2]. [1]*Dipartimento di Scienze del Suolo della Pianta e dell'Ambiente, Università di Napoli Federico II, Via delle Scienze 100, Portici (NA) Italy*. [2]*Dipartimento di Biotecnologie Agrarie-Patologia Vegetale, Università degli Studi, P.le delle Cascine 28, 50144 Firenze, Italy*.
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In a previous work it was demonstrated that polyphenolic stress metabolites, such as resveratrol (3,5,4'-trihydroxystilbene) and many of its oligomers, generally accumulated in the brown red wood of esca-diseased grapevines (*Vitis vinifera* cv. Sangiovese). The concentration of some of these compounds was higher than in asymptomatic wood, that of others was lower and still others were altogether absent from asymptomatic wood. In this paper we discuss the identification of some other resveratrol oligomers. Moreover the role of polyphenolic stress metabolites as a scavenger of ROI was evaluated *in vitro* by the Fenton reaction (Fe^{II}-H_2O_2). Such polyphenols showed a different rate of conversion and they gave oxidised dark products suggesting a significant radical condensation between themselves. The rate of conversion improved synergistically when polyphenols were in the mixture. The data suggested that the polyphenolic stress metabolites act as ROI scavengers. Their ability to condensate may result in the formation of a chemical barrier against pathogen progression in the host.

Physiological effects of esca-related fungi on *in vitro* plants of grapevine cultivar Baga. J. Sofia[1], M.T. Gonçalves[2], C. Rego[3] and H. Oliveira[4]. [1]*DRABL, CEVDão, Quinta da Cale, 3520-090 Nelas, Portugal.* [2]*Dep. Botânica, FCTUC, Universidade de Coimbra, 3000 Coimbra, Portugal.* [3]*Laboratório de Patologia Vegetal "Veríssimo de Almeida", 1349-017 Lisboa, Portugal.* [4]*Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal.*
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The objective of this experiment was to evaluate and compare the effects of the three fungi more frequently isolated from Esca affected grapevines in the Dão wine region. *In vitro* plants of Portuguese grapevine cv. Baga were subjected to four inoculation treatments with isolates of: *Phaeomoniella chlamydospora* (*Pch*), *Phaeoacremonium aleophilum* (*Pal*), *Fomitiporia mediterranea* (*Fmed*) or left uninoculated (control). For inoculation, the third leaf from the apex of each plantlet was cut off and a mycelial plug rubbed against the fresh wound surface; on control plants a malt agar plug was used. Nine plants per treatment were prepared. After a 3-month growth period, the response of plants to infection was evaluated through the following parameters: disease symptom severity (according to a symptom severity rating scale), shoot biomass (ou stem and leaf biomass), water content, lipid peroxidation (determined by malondialdehyde - MDA - content in leafs), and chlorophyll content and fluorescence. Plants inoculated with *Pch* and *Pal* developed severe symptoms when compared with plants inoculated with *Fmed* or noninoculated controls. The water content of stems and leaves did also diminish. Although the chlorophyll content was not statistically affected by any treatment, the Fv/Fm value for plants inoculated with *Pch* was significantly diminished indicating photoinhibition and a stress situation. Also, plants inoculated with *Pch* have shown a significant increase of lipid peroxidation indicated by the increase of MDA production. Regarding the evaluated parameters, plants inoculated with *Fmed* were not significantly affected by any treatment, whereas those inoculated with Pal revealed an intermediate behavior. According to our results *Pa. chlamydospora* was the most virulent of the three fungi studied.

Spatial variability of soil salinity and grapevine dieback in Chardonnay and Shiraz. A. Somers[2], R. Thompson[3], Y. Qiu[1], S. Savocchia[1] and D. Lamb[4].[1]*National Wine and Grape Industry Centre, School of Wine and Food Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia.* [2]*NSW Dept. Primary Industries, Tocal Agricultural College, Paterson, NSW 2421, Australia.* [3]*175 Lawes Street. East Maitland, NSW 2323, Australia.* [4]*School of Biological, Biomedical & Molecular Sciences, University of New England, NSW 2351, Australia.*
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Stress due to biotic or abiotic factors often plays a crucial role in the manifestation of disease symptoms. An endophytic pathogen in healthy tissue can induce symptoms of the disease only when its host is physiologically weakened by stress. Soil salinity is a major factor limiting vineyard performance and it could also be a contributing factor in the dieback of grapevines. A survey of soil salinity and grapevine dieback was conducted in a vineyard planted with non-grafted Chardonnay and Shiraz in the Hunter Valley, New South Wales, Australia. We hypothesized that spatial soil variability might influence vine health and predispose them to trunk disease pathogens. The objectives were to assess the spatial variability of the soil, as well as vine health and grapevine dieback based on spatial correlations. Individual vines were scored according to the extent of dieback in the trunk and arms. A range of fungi were isolated from vines showing dieback symptoms. Of the 119 wood samples collected, the genera isolated were *Botryosphaeria*, *Epicoccum*, *Pestalotia*, *Alternaria*, *Greeneria*, *Phaeomoniella*, *Phaeoacremonium*, *Aureobasidium*, *Phellinus* and *Phomopsis*. The dominant genera isolated were *Alternaria* (43%), *Epicoccum* (41%), *Botryosphaeria* (36%) and *Phaeoacremonium* (14%) with others being isolated infrequently (1–10%). Soil electrical conductivity (EC) was assessed using EM38**.** Lateral variability of soil salinity in the vineyard was mapped using an EM38 electromagnetic sensor. Grapevine dieback was not strongly associated with soil salinity levels.

Epidemiology

Spatial analysis of eutypa dieback in Michigan 'Concord' vineyards using Spatial Analysis by Distance Indices (SADIE). S.A. Jordan, A. Jarosz and A.M.C. Schilder. *Department of Plant Pathology, Michigan State University, East Lansing, MI 48824, USA.*
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Eutypa dieback is a major limiting factor to the longevity of *Vitis labrusca* 'Concord' vineyards in Michigan. Previous studies analyzing the spatial pattern of infected vines have helped shape current management strategies as well as improve our understanding of how an infection can spread within and among vineyards. Recently, an innovative technique for evaluating spatial pattern, spatial analysis by distance indices (SADIE), has been adapted for use in several epidemiological plant disease studies. SADIE was developed to quantify the patterns of biological organisms for either mapped reference units or individuals by measur-

ing the degree of nonrandomness among measured counts. The aim of this study was to evaluate SADIE for use in studying the spatial distribution of infected 'Concord' grapevines in Michigan. Three vineyards from southwest Michigan (Vineyard A, n=1833; Vineyard B, n=1649; Vineyard C, n=1638) were scouted and mapped for disease incidence over 4 years (2003–2006). The ability of SADIE to detect aggregation of diseased vines is compared with more traditional methods such as spatial autocorrelation and other geostatistical approaches.

A natural history of *Fomitiporia mediterranea* carpophores in the Adriatic coastal environment.
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The role of *Fomitiporia mediterranea* in the development of the esca disease is a widely discussed subject. The biology, morphology and ecology of some *Hymenochaetales* which are more strictly involved or suspected to be involved in diseases such as grapevine esca are described in detail. Some notes are also given on the length of the latent phase, the age of carpophore production, the stages of carpophore formation, the durability of carpophores and of the natural decline of these fungi. In the course of a detailed survey in the Adriatic coastal area, and viewing the epidemiological problem of esca-disease as being the result of a possible transfer from heterologous host-plants to *Vitis vinifera*, a wide range of facultative bridge-plants has been observed with a peculiar statistical analysis in the Gargano area where the author recently performed a detailed mycological survey. Various spp. of cultivated and natural-standing trees were found to host living or dead agents of white-decays, which are considered quite similar to *Fom*. For this reason, various species of *Phellinus* and *Fomitiporia* are considered, due to the very slight dissimilarities between genera. Notes on ascomycetal or basidiomycetal associations or exclusions were recorded as a local statistic. It is well known that some imperfect fungi (such as *Pal, Pch*, etc.) are almost constantly active as rot agents. While mainly examining the samples of *Fomitiporia* and *Phellinus* from various hosts or matrices, a comparatively uncommon fungal community consisting of mycoparasites, mycophilous, and exclusive competitors for the substratum was also recorded. Notes on the vectors that could be implicated in the dispersal of fungi, especially during the late colonization phase of the whole etiological complex, are given so that the triggering mechanism in the wood-decay processes and the biocontrol of the connected pathologies can be better understood in relation to the local environment.

Foliar symptoms of eutypa dieback are consistent over years and vineyards.
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As a part of a medium-term research program, field trials on chemical and biological control of eutypa dieback were set up in farmer's vineyards in Rioja Alavesa. A split-plot design, with foliar sprays applied to main plots and pruning wound treatments applied to sub-plots was used. No significant differences in foliar symptoms among treatments were detected in the first two years. This gave us the occasion to study the factors affecting the symptom expression, in order to optimize experimental design. Results from two vineyards (Lapuebla y Barriobusto) in 2004 and 2005 are presented. Both were over 20 year old, planted with Tempranillo variety on 41B rootstocks and trained in free vase. In each vineyard, four blocks, ten plants long and eight rows wide, were marked. Each arm of each plant was scored for foliar symptoms of eutypa dieback, in an arbitrary 0 (no symptoms) to 5 (dead arm) scale, at Baggiolini's phenological stage H. The data so obtained were subject to variance analysis, in a factorial Vineyard×Year model. Within-vineyard variation was added to the model in the form of nested factors Block (Vineyard) and Plant (Block). The necessary replication to calculate the experimental error was given by the between-arm variation within plants. A square root transform of the symptom score was necessary to normalize the error distribution. The main factors Year and Vineyard accounted for most of the variability (MS>50). More interesting, they were independent from each other (no significant interaction). Next level of variation is given by the Block factor. There are significant differences between blocks, and blocks behave differently between years (10>MS>5). This probably reflects edaphic variation within the vineyards, although there might also be a variation between scoring persons (different persons scored different blocks), which should be incorporated to the model. Variation between plants is small (MS<1), but still significant, as compared to the experimental error based on between-arm variation. In contrast to the erratic expression of foliar esca symptoms (data not shown), the ones of eutypa dieback seem consistent over years and vineyards. This would mean that results from a few experimental vineyards could be applied to a wide viticultural area. Research effort could be concentrated on deeper epidemiological, etiological and control studies on a few sites, rather than more superficial studies over many sites. Sound maps of disease incidence within the vineyards could be constructed using the results from individual plants. Experimental blocks should carefully be

laid out, to account for most of the edaphic variation. It would be worth to score individual arms within plants, as this further reduces variability. This would be most important in vase-trained plants, were arms are lost to the disease from one year to the next.

Climatic factors influence seasonal variation in the foliar symptoms of eutypa dieback. M. Sosnowski[1,2], D. Shtienberg[3], M. Creaser[1], T. Wicks[2], R. Lardner[1,4] and E. Scott[1,4]. [1]*Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond SA 5064, Australia.* [2]*South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia.* [3]*Department of Plant Pathology, ARO, the Volcani Center, Bet Dagan 50250, Israel.* [4]*School of Agriculture, Food and Wine, The University of Adelaide, Glen Osmond, SA 5064, Australia.*
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Eutypa dieback, caused by the fungus *Eutypa lata*, contributes to the decline of vineyards by reducing growth and yield. Foliar symptoms, comprising stunted shoots with chlorotic leaves, often cupped and with tattered margins, are thought to be caused by toxins produced by the fungus. Foliar symptoms progress over many years, eventually killing the infected vine. However, recent reports suggest that the intensity of foliar symptoms varies from year to year. Because foliar symptoms are related to yield loss, it is important to establish the underlying causes of seasonal variation in symptom expression so that strategies may be developed to minimise losses due to the disease. Here, we examined relationships between weather parameters and the intensity of foliar symptoms of eutypa dieback. Five vineyards (cv. Shiraz) in the Coonawarra and Eden Valley regions of South Australia were assessed each spring for foliar symptoms of eutypa dieback between 1999 and 2004. Symptoms were assessed using a scale where shoots were rated from 0 (no symptoms) to 4 (severe symptoms) and the percentage of the canopy affected was estimated. Weather data were collected from automatic weather stations located within 5 km of each trial site. Coonawarra and Eden Valley are approximately 350 km apart and have mean annual rainfalls of 650 mm and 500 mm, respectively. For statistical analysis, three measures of disease intensity were applied: (i) disease incidence (DI), which is the percentage of all vines assessed that have a symptom rating ≥ 1; (ii) disease prevalence (DP), which is the mean percentage of canopy expressing symptoms in all vines; and (iii) severe disease incidence (SDI), which is the percentage of symptomatic vines that have a rating ≥ 3. Attempts were made to elucidate coincidence between weather parameters (e.g. minimum or maximum temperatures, accumulated temperatures [degree days], rain quantity, or the number of rain events) and eutypa dieback intensity measures. Incidence and severity of foliar symptoms varied from year to year for all vineyards; however, differences between regions were apparent. A number of possible relationships between climate and symptom expression were examined. An increase in DI was associated with the number of rain events occurring around the time of mechanical pruning in the previous winter. Conversely, a decrease in DI was positively related to spring temperature; the higher the temperature, the fewer vines expressed foliar symptoms (i.e. the more vines "escaped" disease symptoms this year when they had had symptoms in the previous year). A conceptual model was developed from these data to predict DI. It involves knowledge of DI in the previous year (DI_{i-1}), prediction of new infections (NI_{i-1}) by the rainfall events of the previous winter and the "escape" from disease symptoms (E_i) by spring temperature; $DI_i=DI_{i-1}+NI_{i-1}-E_i$. When this model was applied to the climatic data for the two locations, a similar trend was observed between the predicted and the observed incidence. DP and SDI were also related to weather variables. For each vineyard, DP decreased by 0.06% with each degree-day increase in the spring. Analysis also revealed that the greater the October rainfall, the lower the SDI; however, it also showed that, in the Eden Valley vineyards, SDI was reduced when October rainfall was very low. This study indicates that the variation in foliar symptoms of eutypa dieback from year to year may be caused by climatic factors rather than vineyard management practices such as pruning. The different trends observed between the regions may be due to climatic variation. An increase in DI between years appeared to be due to an increase in rainfall in the previous winter. This may be linked to the influence of rainfall on airborne inoculum; however, symptom expression has not been recorded within 15 months of infection in the field. Decreases in DI and DP were related to increased temperature in spring. Vines grow more vigorously in warmer conditions, and this could reduce the effect of fungal toxins on the foliage. Alternatively, the production of toxins by, or growth of, *E. lata* may decline in warmer conditions. It is also possible that a combination of these reasons may explain this relationship. The most interesting relationship here involved the effect of October rain on severe disease incidence of eutypa dieback. A complex interaction between host and pathogen may exist which leads to a reduction in disease incidence when moisture availability is high, possibly through the dilution of toxic compounds, and then a reduction of disease incidence during periods of very low moisture availability, which may be due to reduced production of toxins or lack of mycelial growth due to water stress on the fungus or to limited water movement for transport of toxins through the vine. It is important to note that the DI model must be validated using a different set of data before conclusions can be made about its accuracy. A number of relationships have

been observed, and a number of theories have been proposed to explain them. However, these relationships may be coincidental; and further investigation is under way to verify the relationships.

Notes on the relationship of manifest esca disease to vineyard slope. V. Robotic and R. Bosancic. *NA-VIP-Fruskogorac, 21131 Petrovaradin, Karlovacki put 1, Serbia.*
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Esca was first reported in northern Serbia, province Vojvodina in the viticultural area of Fruska Gora in 2000, and it is already a devastating disease in some other regions of Serbia as well and indeed in grape-growing regions all over the world. It has been established that *Phaeomoniella chlamydospora* Crous & W.Gams and *Phaeoacremonium aleophilum* W.Gams *et al*. are involved in the disease. Esca incidence in the vineyard may be influenced by the wide range of environmental factors such as rainfall, air temperature, vineyard slope, soil type, etc. In this paper we briefly report on how vineyard slope affects the incidence of diseased vines, as measured by counting all plants that showed esca symptoms during a three-year observation period. Field surveys were carried out yearly from 2002 to 2004 in one experimental vineyard in Fruska Gora. The vineyard was established in 1988 with the domestic white vine berry cultivar Zupljanka, area 1 ha, and containing 12 columns of which 8 were inspected each year. The average slope of the experimental plots was l5%. The highest and steepest part of the vineyard was denominated, A, the middle (and more level) part, B, and the lowest part, C. The incidence of esca was recorded on all vines in 8 columns (extending over all three altitudes) in September each year starting in 2002. Each vine was inspected for external symptoms and scord according to the following scale: 1. asymptomatic, healthy plants, 2. plants with chronic leaf symptoms, 3. withering of some shoots and clusters, 4. complete wilting of the crown, apoplexy, and 5. plants which did not revegetate after winter. With the symbol "0" we marked plants already missing before 2002. The total number of vines inspected was 2356 each year. Esca incidence increased in all investigated plots during the inspection period.In part A of the vineyard, the number of asymptomatic vines decreased from 81.90% (619) in 2002 to 81.00% (608) in 2003 and to 75.80% (606) in 2004; in part B, the number of healthy plants was 79.60% (637), 81.00% (612) and 74.30% (594) in 2002, 2003 and 2004 respectively; ands in part C it was 80.60% (645) in 2002, 77.60% (621) in 2003, and 76.50% (578) in 2004. Multivariable variance analysis (MANOVA) detected significant differences in asymptomatic vines between vineyard parts A, B and C (P=0.002) in the three years of the study. When comparing vineyard parts A, B and C in 2002–2004 by Roy's test, it was found that discrimination between vineyard parts was greatest in 2004 (P=0.000); in 2003 it was (P= 0.009) and in 2002 (P=0.112). In the graphic ellipse survey it is possible to find the mutual locations of esca symptoms in 3 different locations on vineyard slope. Part A in the graphic survey was separated from parts B and C in having healthier vines in 2002 than in 2004.The situation was similar when the number of healthy vines was compared between parts A; B and C in 2002, 2003, and 2004. Esca incidence was lower where the vineyard slope was steeper (part A) and the higher were the slope was lower and more level (parts B and C).

Disease Management

Hot water treatment of grapevine rootstock cuttings grown in a cool climate. A. Graham. *Corbans Viticulture Ltd, Auckland, New Zealand.*
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The standard hot water treatment (HWT) protocol for grapevine cuttings (50°C for 30 min) was modified to reduce nursery losses of grapevines grown in the cooler climate of New Zealand. Initial trials using 101-14 and 5C UCD rootstock cuttings taken from mother vines from four different viticulture regions showed that between 60% and 95% of the grafted vines died within six weeks when rootstock cuttings were subjected to HWT at 50°C for 30 min. In a second trial, 101-14 rootstock cuttings were subjected to temperatures ranging from 45–50°C or to cold water for 30 min prior to grafting with Sauvignon Blanc. HWT of rootstock at 45°C and 47°C for 30 min delayed budburst, but graft and root callusing in the hothouse was more uniform. After 6 weeks of propagation, including 3 weeks in the nursery field, there was less than 10% mortality in vines treated at 45°C and 47°C for 30 min compared with 60% mortality with HWT at 50°C. The incidence of fungi in vines treated at 45°C and 47°C, including known pathogens and endophytes in grafted rootstock was 11% and 3% respectively 3 weeks after treatment, as compared with 15% in the untreated controls. Six months after HWT at 47°C for 30 min, most of the xylem tissue samples taken from treated vines contained other endophytic fungi, while less than 5% contained *Phaeomoniella chlamydospora,* the causal agent of young vine decline or Petri disease, compared with an incidence of up to 40% in untreated vines. Therefore, HWT at 47°C reduced the incidence of *P. chlamydospora* in the rootstock to an extent comparable to that reported by other research groups in vines treated at 50°C for 30 min. The graft strength, stem diameter and root growth of both treated and untreated vines will be tested after leaf fall. These results will be included in this presentation.

Influence of *Glomus intraradices* on black foot disease caused by *Cylindrocarpon macrodidymum* on *Vitis rupestris* under controlled conditions. E. PETIT and W.D. GUBLER. *Department of Plant Pathology, University of California, Davis, CA, USA.*
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We examined the influence of *Glomus intraradices* (INVAM CA 501), an arbuscular-mycorrhizal fungus, on black foot disease caused by the fungus *Cylindrocarpon macrodidymum* on *Vitis rupestris* cv. St. George under controlled conditions. Both mycorrhizal and non-mycorrhizal grapevines were inoculated with the pathogen. Eight months after inoculation with the pathogen, we evaluated disease severity, vine growth, and mycorrhizal colonization. Mycorrhizal plants developed significantly fewer leaf and root symptoms than non-mycorrhizal plants (P=0.04 and P<0.0001, respectively). Of the grapevines inoculated with the pathogen, only the non-mycorrhizal grapevines had significantly less dry root and leaf weights compared to the non-inoculated control (P=0.0021 and P=0.0017 respectively). Mycorrhizal colonization was high (48.3% for the non-infected control and 54.5% for plants infected with *C. macrodidymum*) and was not significantly affected by inoculation with *C. macrodidymum* (P=0.2256). Thus, *V. rupestris* plants pre-inoculated with *G. intraradices* were less susceptible to black foot disease than non-mycorrhizal plants. Results from this study suggest that pre-plant applications of *G. intraradices* may help prevent black foot disease in the nursery and in the vineyard.

Potential use of chitosan in the control of grapevine trunk diseases. T. NASCIMENTO, C. REGO and H. OLIVEIRA. *Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.*
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Due to its fungistatic or fungicidal potential, chitosan, a high molecular-weight polymer that is non-toxic and biodegradable, has become an alternative to conventional fungicides. In addition, chitosan is reported to elicit defense mechanisms in plant tissues. In this study, we explored the *in vitro* fungicidal effect of chitosan on some of the most important grapevine wood fungi, such as *Botryosphaeria* sp. (dieback and cane blight), *Phomopsis* sp. (Phomopsis cane and leaf spot), *Eutypa* sp. (eutypa dieback), *Neonectria liriodendri* (black foot disease), *Phaeomoniella chlamydospora* (Petri disease and esca) and *Fomitiporia* sp. (esca). Inhibition of mycelial growth was evaluated at five decreasing concentrations 50, 25, 5, 2.5 and 0.5 mg a.i. l^{-1} of chitosan. Chitosan was effective in reducing mycelial growth of all the fungi. The lowest EC_{50} values were obtained with *Phaeomoniella chlamydospora, Fomitiporia* sp and *Botryosphaeria* sp., and the highest with *Neon. liriodendri*. All these were inferior to the recommended field rate (8.3 mg a.i. l^{-1}) with exception of the value obtained with *Neon. liriodendri*. Greenhouse experiments were carried out to evaluate the efficacy of foliar sprays of chitosan on potted grapevine plants (cultivar Castelão) growing in a substrate infested with *Pa. chlamydospora* or *Neon. liriodendri*. The effect of chitosan against *Neon. liriodendri* was similar to that achieved with some selected fungicides (carbendazim+flusilazole, cyprodinil+fludioxonil and tebuconazole). Chitosan significantly improved plant growth (plant height and number of roots) and decreased disease incidence compared with untreated plants. As regards *Pa. chlamydospora,* chitosan only reduced the disease incidence caused by this fungus.

Identifying effective management strategies for esca and Petri disease. A. ESKALEN, S. ROONEY-LATHAM and W.D. GUBLER. *Department of Plant Pathology, University of California, Davis, CA, USA.*
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Phaeomoniella chlamydospora causes Petri disease, and it also induces symptoms of esca, along with numerous species of *Togninia (Phaeoacremonium).* It has been shown that all of these pathogens can be transmitted from infected mother vines via contamination of the external bark or internal vascular tissue. It has also been demonstrated that pruning wounds are susceptible to infection by conidia of both *Pm. aleophilum* and *Pa. chlamydospora*. Several fungicides were tested on naturally infested dormant propagation materials. Prior to grafting, the dormant rootstocks and scions were soaked in a suspension of fungicide, *Trichoderma harzanium* or tap water (control). Treated cuttings were then grafted (Freedom×Crimson and Couderc 3309×Thompson seedless). One month after grafting, twenty-five grafted cuttings were visually evaluated for percentages of callusing and rooting. Cuttings were then planted in the field for one year after which they were uprooted and isolations were made. Vines treated with Ziram, Thiram, Topsin M, Lime sulfur, and hot water (30 min at 50°C) showed significant reductions in both *Pa. chlamydospora* and *Pm. aleophilum* after one year. The effectiveness of boric acid, Garrison, Cabrio and Topsin M to protect pruning wounds on grapevine wood blocks maintained in 228 ml French square bottles was assessed *in vitro*. Sterile, one-year-old, dormant grape canes of the cultivar Cabernet Sauvignon were used for wood block material. Wood blocks were dipped in each fungicide and placed aseptically on Van Teegham cells in French square bottles containing agar and one of the pathogens. The isolation techniques performed yielded 100% recovery of *Pm. aleophilum* and *Pa. chlamydospora* from the control wood blocks. Topsin M, Cabrio, and Cabrio in combination with Garrison showed the best control of both fungi on the

wood blocks. In addition to the lab tests, field trials were conducted to examine the potential use of these fungicides in commercial crop protection. Five fungicides including Topsin M, Garrison, Biopaste (5% Boric acid mixed with the tree wound dressing Treekote; Walter E. Clark & Son, Orange, CN, USA), Prevam and Cabrio were tested in two locations in California. Dormant grapevines of the cultivars Zinfandel and Chardonnay were pruned to 2-bud spurs in December 2004 at each site. One day after applying fungicides to fresh pruning wounds, spore suspensions of each fungus were used to artificially inoculate the grapevines. Non-inoculated grapevines (negative control) were used to determine the amount of natural infection in each field. Spurs were collected in the fall of 2005 and pathogens were re-isolated from wood onto PDA amended with tetracycline (0.1%). Topsin M, Biopaste, Garrison and Cabrio were the best wound protectants for both fungi. Control of *Pm. aleophilum* was 96, 92, 46 and 65 respectively for each fungicide, while control of *Pa. chlamydospora* was 91, 91, 59 and 85% respectively. This research illustrates the significance of using fungicides as wound protectants during dormancy and the propagation process.

A hot-water treatment to control *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* in Spanish grapevine nurseries. J. Armengol[1], D. Gramaje[1], D. Salazar[2], I. López-Cortés[2], A. Giménez-Jaime[1], A. Crespo[1], E. H. Albaráñez[1] and J. García-Jiménez[1], *[1]Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain; [2]Departamento de Producción Vegetal, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.*
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Hot-water treatment (HWT) has been reported as a promising method to control *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* in grapevine propagating material. To date, most HWT protocols have focused on treatments at 50°C for 30 min (Crous *et al*., 2001; Edwards *et al*., 2003; Fourie and Halleen, 2004; Waite and May, 2005). The purpose of this research was to evaluate HWT of dormant rootstocks and grafted cuttings using a broader range of temperatures. Rootstocks (41-B, 161-49C, 110-R, 140-Ru and 1103-P) and grafted cuttings (Tempranillo/110-R; Merlot/110-R; Bobal/1103-P and Tempranillo/161-49C) were immersed in a temperature-controlled water bath at 50, 51, 52, 53 or 54°C for three lengths of time: 30, 45 and 60 min. Four groups of 10 cuttings were treated at each temperature and time with their respective controls and planted in a completely randomized design in a field where grapevine had never been grown. Cultural practices followed common nursery guidelines. At the end of the growing season, the sprouting of cuttings and the length and weight of the shoots were evaluated. In a second experiment, the effect of HWT on *P. chlamydospora* and *P. aleophilum* was studied. Healthy cuttings of 100-R rootstock were vacuum-inoculated with conidial suspensions (10^6 conidia ml^{-1}) of one isolate of each of the pathogens (Rooney and Gubler, 2001). The cuttings were subjected to the treatments indicated above. Four groups of 10 cuttings were treated for each temperature, time and isolate, with their respective controls. Isolations were made immediately after treatment from sections (10 cm long) that were cut from the basal end of the cuttings. The cuttings were planted and evaluated as described above. Results demonstrated that rootstocks and grafted cuttings can tolerate temperatures from 50°C to 53°C without significant reduction in sprouting or growth. *P. chlamydospora* was more sensitive than *P. aleophilum* to the experimental temperatures for all lengths of time. Satisfactory control percentages for both pathogens were obtained at temperatures over 51°C. These findings are promising in order to develop an effective HWT to control Petri disease in grapevine propagation material in Spanish grapevine nurseries.

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References

Crous P.W., L. Swart and S. Coertze, 2001. The effect of hot-water treatment on fungal occurring in apparently healthy grapevine cuttings. *Phytopathologia Mediterranea* 40, S464–S466.

Edwards J., I.G. Pascoe, S. Salib and N. Laukart, 2004. Hot water treatment of grapevine cuttings reduces incidence of *Phaeomoniella chlamydospora* in young vines. *Phytopathologia Mediterranea* 43, 158-159.

Fourie P.H. and F. Halleen, 2004. Proactive control of Petri disease of grapevine through treatment of propagation material. *Plant Disease* 88, 1241–1245.

Rooney S.N and W.D. Gubler, 2001. Effect of hot water treatments on eradication of *Phaeomoniella chlamydospora* and *Phaeoacremonium inflatipes* from dormant grapevine wood. *Phytopathologia Mediterranea* 40, S467–S472.

Waite H. and P. May, 2005. The effects of hot water treatment, hydration and order of nursery operations on cuttings of *Vitis vinifera* cultivars. *Phytopathologia Mediterranea* 44, 144–152.

Effect of methyl jasmonate and salicylic acid on in vitro plants of Cabernet Sauvignon in the presence of Inocutis jamaicensis. C. Césari[1], M. Ponce[2], G. Escoriaza[1], V. Longone[1] and M. Gatica[1]. *[1]Laboratorio de Fitopatología, EEA Mendoza, Instituto Nacional de Tecnología Agropecuaria, San Martín 3853, 5507, Luján de Cuyo, Mendoza, Argentina. [2]Laboratorio de Fisiología Vegetal, Facultad de Ciencias Agrarias, Uni-*

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Hoja de malvón is one of the most important grapevine diseases in Argentina; however, no effective control method of this disease is yet available. *Inocutis jamaicensis* is one of the important pathogens associated with the disease. Methyl jasmonate (MeJ) and salicylic acid (SA) are molecules known to stimulate plant defenses. Therefore, experiments applying hydro alcoholic solutions of MeJ and SA at different concentrations (0.1, 1 and 10 ppm) with or without the presence of *I. jamaicensis* were conducted. Plantlets of the cv. Sauvignon were grown *in vitro* in a Galzy grape micropropagation medium under controlled conditions of light and temperature. Sterile water was applied to plantlets used as controls. The number of leaves, foliar area; leaf, root and stem dry weight, chlorophyll, polyphenol and anthocyanin concentrations were recorded. The presence of *I. jamaicensis* reduced the number of leaves and chlorophyll concentration in all plants treated with MeJ, SA, and water. Although the application of MeJ increased the phenolic phytoalexines, this was not enough to prevent infection with *I. jamaicensis*. In the presence of *I. jamaicensis*, applications of MeJ at all concentrations increased the phenolic concentration, while in the absence of the fungus, phenolic concentration only increased when 10 ppm of MeJ was applied. SA had no effect on the phenol concentration. In another experiment, the effect of MeJ at 0.1 ppm in the presence of *I. jamaicensis* was compared with applications of water and a hydroalcoholic solution to plants used as controls. MeJ at this concentration reduced the percentage of dead leaves and increased the number of green leaves whereas the control plants showed symptoms of *I. jamaicensis* (a lower number of leaves, and a greater proportion of green leaves with red edges). These results are a first contribution to our knowledge of the relation between *Vitis* sp. and wood fungus related to plant defense induction.

Effect of boron fertilizer applications on grapevine pruning wounds on bud break, boron levels in leaf tissue and vine yield. R.J. Smith[1]. *[1]UC Cooperative Extension, 133 Aviation Blvd., Suite 109, Santa Rosa, CA 95403, USA.*
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North Coast wine grape growers commonly apply a wound protectant at the time of pruning to reduce the incidence of Eutypa dieback and other fungal canker diseases. An increasing number of growers apply boron-based products to pruning wounds to prevent fungal infections. Previous research has shown that 5% boric acid (17.5% a.i. boron) mixed with a commercial paste and applied to pruning wounds can control eutypa dieback in grapevines (Rolshausen and Gubler, 2005). Many growers utilize boron fertilizer as a convenient replacement for boric acid and make solutions of fertilizer to either spray directly onto the vines or mix with paste and daub onto pruning wounds. Various concentrations of B are applied in this manner. Boron fertilizer directed to pruning wounds has not been evaluated for phytotoxicity, thus a study was conducted to monitor the effects of commonly used rates of boron fertilizer applications made to pruning wounds on bud break, vine tissue B concentrations and crop yield. Trials were established January 2005 in two blocks of Chardonnay vines in the Alexander Valley U.S. Viticultural Area in Sonoma County, California. Two rates of a liquid boron fertilizer product (guaranteed analysis 10% B; contains 132 g B l^{-1}) were applied to pruning wounds in spray and paste applications. Sprayed treatments were applied at 30 gallons per acre and paste treatments applied in a commercial paste (Doc Farwell's Seal & Heal). Treatments were as follows: 5% fertilizer solution (0.5% B; 6600 mg kg^{-1}), 50% fertilizer solution (5% B; 66000 mg kg^{-1}) and an untreated control. In addition, a 1% fertilizer solution (1% B; 1320 mg kg^{-1}) was applied only as a spray. Treatments were applied on the same day that the vines were pruned. Each trial was a randomized complete block design with 6 treatments and 4 replications. Shoot growth stage of basal, first and second node positions was determined using the modified Eichorn-Lorenz system on each spur on all vines to calculate percent bud break by node position (Coombe, 1995). In both trials, applied boron concentration affected bud break differently by node (P<0.001). Basal nodes had the <u>*greatest*</u> percent bud break with both the spray and paste applications of 66000 mg kg^{-1} B (P=0.001) whereas those treatments resulted in the <u>*lowest*</u> percent bud break in nodes 1 (P=0.0394) and 2 (P<0.001), the latter node being located immediately below the cut surface of the spur. Average percent bud break in node 2 was 56% and 58% in vines that received the spray and paste treatments of 66000 mg kg^{-1} respectively, but bud break in node 2 ranged from 95% to 99% with all other treatments, including the control. No symptoms of foliar boron phytotoxicity were seen in either trial at any time during the 2005 season. In Trial 1, the highest rate of sprayed boron fertilizer was the only treatment that resulted in significantly greater levels of B in leaf blades over all other treatments at bloom (P=0.0001) and veraison (P=0.0002) averaging 120 mg kg^{-1} and 146 mg kg^{-1} respectively. Yield was not affected by treatment in either trial. Average cluster number per vine was not significantly different by treatment within each trial. Although bud break of node 2 was reduced by the highest concentration of applied boron, that treatment did not result in less fruit because basal nodes had significantly higher bud break and clusters produced on basal shoots had a compensating effect on yield.

References

Coombe, B.G. 1995. Adoption of a system for identifying grapevine growth stages. *Australian Journal of Grape and Wine Research 1*, 104–110.

Rolshausen, PE, and Gubler, WD 2005, Use of boron for the control of Eutypa dieback of grapevines. *Plant Disease* 89, 734–738

Soil-borne grapevine diseases caused by fungi in Japan. H. Nasu and K. Inoue. [1]*Okayama Agricultural Experiment Station, Akaiwa, Okayama 709-0801, Japan. E-mail:* hideo_nasu@pref.okayama.jp

Three fungal species, *Rosellinia necatrix* (white root rot), *Armillaria tabescens* (armillaria root rot) and an unknown species have been the main causal agents of soil-borne diseases of grapevine in Okayama Prefecture, Japan. The ecology and the control of these diseases are presented here. White root rot: this disease is well-known as a soil-born disease all over the world. In screening tests of about 100 fungicides against this disease, the most effective were fluazinam (active ingredient 39.5%, trade name: Frownside SC) and fluazinam soil-drench at either 790 ppm or 395ppm. These treatments were very effective. Fluazinam was registered in Japan as a fungicide in June 1998. Nevertheless, it will be hard to find acceptance because of its high price and the unfamiliar nature of the soil-drench method. Armillaria root rot: this is a minor disease caused by *Armillaria tabescens* and recently reported for the first time in Japan. It occurred in some vineyards established on reclaimed land near mountains. Before the vineyards were established, the area was a peach orchard that was already infected with this fungus. The effectiveness of both fluizanam fungicides is being assessed against this disease. Unidentified pathogen (decay fungus?): this fungus has been observed for some time and now occurs in many vineyards. Symptoms include: tattering of the root epidermis, giving off a mushroom-like smell, and weakening of the growth. The mycelium does not however move into the xylem of the main roots. Since the fungus in question grows on the grape roots, we think that it is the causal organism but it has not yet been identified. The fungus is now being identified by PCR. The primary inoculum may come from bark compost applied to the fields.