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ABSTRACTS

***Fomitiporia mediterranea*, a new basidiomycete species associated with esca of grapevine in Europe: biology, ecology, and distribution.** M. FISCHER and H-H. KASSEMEYER. *Staatliches Weinbauinstitut, Merzhauser Str. 119, D-79100 Freiburg, Germany. E-mail:* michael.fischer@wbi.bwl.de

Fomitiporia mediterranea is described as a new wood-decaying basidiomycete species associated with esca of grapevine in European wine-growing countries. Characters of the fruit body are essentially identical with those of the closely related species, *Fomitiporia punctata*. *F. mediterranea* is distinct by the sequences of the ribosomal ITS1-5.8S-ITS2 region and by larger mycelial growth rates at temperatures between 15°C and 35°C. While *F. punctata* is confirmed as a homothallic species, *F. mediterranea* is shown to be outcrossing, exhibiting a unifactorial mating behaviour with a multiple allelism of the mating type factor, *A*; single spore isolates of *F. mediterranea* are intersterile in pairing tests with *F. punctata*. *F. mediterranea* is widespread all over southern Europe and is shown to exist in France, Italy, Greece, and Spain. In these regions, the species not only occurs on *Vitis vinifera*, but also on a number of other hardwood genera. In Central Europe, *F. mediterranea* seems restricted to *V. vinifera*. A one-year study shows that *F. mediterranea* seems to be the main causal agent for esca in Germany. It is associated with 63% of esca affected grape plants; age of plants showing symptoms of wood-decay is between 4 and 42 years. The species often occurs side-by-side with *Eutypa lata* (22%), less often with species belonging to *Phaeomoniella* or *Phaeoacremonium* (8%).

Teleomorph associations and genetic diversity occurring in *Phaeoacremonium*. L. MOSTERT[1], P.W.C. CROUS[2], J.Z. GROENEWALD[1], W. GAMS[2] and R. SUMMERBELL[2]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa.* [2]*Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, Netherlands. E-mail:* mostert@cbs.knaw.nl

Petri disease or black goo is a serious disease of young and old vines in most areas where grapevines are cultivated. The disease is typically associated with grapevines exhibiting a slow dieback as well as stunted growth. The dominant fungus associated with Petri disease is *Phaeomoniella chlamydospora* (Chaetothyriales). Several species of *Phaeoacremonium* (*Pm.*) are also associated with these disease symptoms, namely *Pm. aleophilum*, *Pm. angustius, Pm. inflatipes, Pm. mortoniae, Pm. parasiticum*, *Pm. rubrigenum* and *Pm. viticola*. Hausner *et al*., 1992 (*Canadian Journal of Botany* 70, 724–734) reported comparable Phialophora-like anamorphs for two *Togninia* species. To investigate possible teleomorph association, isolates of *Pm. aleophilum*, *Pm. parasiticum* and *Pm. angustius* were mated. Perithecia of the genus *Togninia* were observed between compatible strains of each of these species after 3–4 weeks for *Pm. aleophilum* and 12 weeks for *Pm. parasiticum* and *Pm. angustius*. The genetic diversity within these species was also determined by means of phylogenetic analyses of the nrDNA internal transcribed spacers (ITS1, 5.8S and ITS2) and the translation elongation factor 1 alpha (EF-1α). The generic placement of teleomorphs within *Togninia* (Calosphaeriales) was further confirmed via phylogenetic analyses of the mitochondrial small subunit. From these sequences, morphological and mating data, we conclude that *T. minima* is the teleomorph of *Pm. aleophilum*, and that it has a biallelic heterothallic mating system. An epitype and mating type tester strains are also designated for *T. minima*. We found that both

mating types of species can occur in the same vine and that recombination can commonly occur in the field, thus explaining the high level of genetic diversity observed in some species.

Further investigations into the first report of the teleomorph of *Phaeoacremonium aleophilum*. S. Rooney Latham[1], A. Eskalen[1], W.D. Gubler[1], T.C. Harrington[2] and D.L. McNew[2]. [1]*Department of Plant Pathology, University of California, One Shields Ave., Davis, CA 95616, USA.* [2]*Iowa State University, Department of Plant Pathology, 221 Bessey Hall, Ames, Iowa 50011. E-mail:* snrooney@ucdavis.edu

The hyphomycete genus *Phaeoacremonium* contains species associated with grapevine diseases throughout the world. Spores of *Phaeoacremonium* species have been trapped in infected vineyards, but neither asexual nor sexual fruiting structures have been identified in the field. Pairing studies were set up using *Phaeoacremonium* isolates from infected grapevines and type isolates obtained from Centraalbureau voor Schimmelcultures (CBS). The isolates from infected grapevines shared the most commonly isolated colony morphology on agar-based media, a buff to grey colour with a yellow-pigmented diffusion. These isolates were previously identified as *P. aleophilum* based on molecular data. After 35 days, perithecia were found forming on wood chips and agar of many pairings. Perithecia formed in a heterothallic-type mating system, with isolates representing one of two different mating types. Perithecia were dark, globose and formed solitarily or in small groups. At maturity, perithecia had long necks, which held sticky droplets of ascospores at their tips. Dissections of perithecia showed clavate to subclavate asci, each containing eight hyaline, allantoid to ellipsoid ascospores. F_1 progeny isolates were obtained and crossed back with the two mating types, confirming fertility and heterothallism. Molecular analyses of the ITS and small subunit regions, both of the nuclear ribosomal DNA, showed that perithecia and F_1 progeny isolates were genetically identical to *P. aleophilum*. However, when compared to other known sequences of ascomycetes, no similar matches were found. Further comparisons with herbaria specimens suggest a probable inclusion into a genus collected a century prior. However, because of the age of the specimens, we were unable to collect any useable nucleic acid for analysis.

Molecular and biochemical characterisation of *Eutypa lata* in Australia. R. Lardner, B. Stummer and E. Scott. *Cooperative Research for Viticulture, P.O. Box 154, Glen Osmond, SA 5064, Australia. Department of Applied and Molecular Ecology, University of Adelaide, PMB 1 Glen Osmond, SA 5064, Australia. E-mail:* richard.lardner@adelaide.edu.au

Eutypa dieback of grapevines, caused by *Eutypa lata*, is a major threat to the sustainability and productivity of vineyards worldwide. Foliar symptoms, caused by translocatable toxins produced by the fungus in the xylem of the wood, do not become visible until several years after infection. Vines may not display symptoms every season, or may display symptoms only at the start of a season. Little is known about the spread and distribution of the pathogen in infected vines. We aim to develop DNA probes in order to gather information about the location of the pathogen in infected vines, and to assess potential control agents. Two approaches have been used to develop DNA probes specific to *E. lata*. First, SCAR (sequence characterised amplified region) markers, derived from RAPD (randomly amplified polymorphic DNA) fragments, were generated in order to detect the pathogen using the polymerase chain reaction. Second, RFLP (restriction fragment length polymorphism) probes specific to *E. lata* were developed for use in Southern hybridisation experiments, such as slot blot assays. SCAR and RFLP markers were tested for their specificity towards 26 isolates of *E. lata* from Australia, and 12 other grapevine inhabiting fungi, including *Phellinus* sp., *Botrytosphaeria ribis*, *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, as well as grapevine DNA. Both types of marker were capable of detecting all isolates of *E. lata* but no other fungi. However, further analysis of these markers using 11 isolates of *E. lata*, which were also analysed for secondary metabolite production (Mahoney *et al*., 2003), resulted in the detection of only nine isolates. The two anomalous isolates originated from Valley oak in California (isolate E178) and from grapevine in New

Zealand (isolate SS1#1). Analysis of secondary metabolite production following growth of the same 11 isolates on a range of media showed that these two isolates had profiles significantly different from most other isolates (Mahoney *et al.,* 2003 [*Phytochemistry* 64, 475–484]). Isolate E178 has previously been subjected to ITS- and AFLP-based analysis by DeScenzo *et al.,* 1999 (*Phytopathology,* 89, 884–893), who suggested that this isolate, which was significantly different to the majority of other *E. lata* isolates analysed, might be re-classified as *E. armeniacae*. Similarly, recent research (Long *et al*., this issue) suggests that isolate SS1#1 is not *E. lata*, and, on the basis of ITS sequence data, it seems probable that this isolate belongs to the genus *Cryptovalsa* (P. Long, *pers. com.*). Both sets of markers were used to detect *E. lata* in infected grapevine wood. Use of SCAR markers in a PCR-based assay revealed that this approach was prone to inhibition by phenolic compounds, and false negative results were frequent. Various DNA extraction protocols were used to isolate DNA from grapevine material which was suitable for use in PCR, however, only extractions conducted using the Bio-101 soil DNA extraction kit (Bio-101, USA) resulted in the consistent amplification of *E. lata* DNA from infected wood. However, RFLP markers consistently detected *E. lata* DNA in infected wood using a slot blot assay. This approach will be used to gather information on the spread of the pathogen in grapevines and as a tool to assess the efficacy of potential control agents against *E. lata*.

Producing the sexual stage of *Eutypa lata in vivo* and characterizing parents and progeny. P.G. Long[1], C.L. Nicoll[1], L.K. Davis[1] and R.E. Bradshaw[2]. [1]*Institute of Natural Resources, Massey University, Palmerston North, New Zealand.* [2]*Institute of Molecular Biosciences, Massey University, Palmerston North, New Zealand. E-mail:* P.G.Long@massey.ac.nz

Eutypa lata is a major pathogen of grapevines worldwide. It infects the plant through pruning and other wounds and colonises stems and cordons. There is a delay of up to four years before the sexual stage is produced in natural conditions and studies on the genetics of the fungus have been hampered by the failure to produce the sexual stage in culture. We inoculated *E. lata* isolates onto potato dextrose agar (PDA), pieces of autoclaved, old grapevine cordons (GC) and autoclaved blackcurrant shoots (BL). Each of six single ascospore isolates from one field-collected perithecium was self- or cross-fertilised with a mixture of the other five isolates on each of these media. Incubation was for eight weeks at 15°C in the dark, followed by 1°C for four weeks. After fertilisation, they were returned to 1°C for 19 weeks and then incubated under a 12 h/12 h light/dark regime with temperatures of 15°C and 10°C respectively. Perithecia were produced in all isolate combinations on BL after a further 16 weeks. They were not produced on PDA and five perithecia only were found on one piece of GC. There was a minimum of 19 and a maximum of 38 ascospores per ascus with an average of 32 in all perithecia examined. Ascospores from self- and cross-fertilised treatments were viable, with germination of 93–100% on PDA. The fungus was shown to be homothallic and we are currently developing a suite of RAPD markers to characterize the parents and progeny to determine whether outcrossing took place in attempted cross-fertilisations.

ITS and β-tubulin phylogeny of *Cylindrocarpon* spp. associated with black foot disease of grapevine. F. Halleen[1], P.W. Crous[2] and J.Z. Groenewald[3]. [1]*ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa.* [2]*Centraalbureau voor Schimmelcultures, Uppsalalaan 8, CT Utrecht 3584, The Netherlands.* [3]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7600, South Africa. E-mail:* francois@nietvoor.agric.za

Over the last few years a drastic reduction has been observed in the survival rate of vine cuttings due to a decline disease present in nurseries, as well as in young vineyards in the Western Cape Province of South Africa. *Cylindrocarpon* species, which cause black foot disease of grapevines, have also been found to be associated with the decline of these vines. The present study was therefore undertaken to identify the *Cylindrocarpon* species isolated from local vines and to establish their association with black foot disease. The 28 *Cylindrocarpon* iso-

lates studied were obtained from various grapevine nursery plants, as well as plants displaying typical decline and black foot symptoms. The isolates were subjected to PCR analysis of the rRNA operon's internal transcribed spacers (ITS1, 5.8S and ITS2) and a portion of the beta-tubulin gene. Phylogenetic analysis using maximum parsimony of the two regions in separate and combined analyses clearly grouped the isolates into two well-supported main clades, each of which could further be divided into two distinct subclades. Whether these clades represent new species or variation within the C*ylindrocarpon* and *Neonectria* species remain to be established. The molecular delimitation of the two major clades was strongly supported by the cardinal temperature requirements for growth of these isolates. Isolates from the first major clade had an optimum growth rate at 25°C and did not grow at 30°C, whereas isolates from the second major clade had an optimum growth rate at 30°C.

Molecular systematics of *Phialophora*-like fungi from declining grapevines. L.A. Long, E.L. Stewart, N.G. Wenner and B.E. Overton. *The Pennsylvania State University, Department of Plant Pathology, Buckhout Laboratory, University Park, PA 16802, USA. E-mail:* els4@psu.edu

The status and causes of grapevine decline in the states of Pennsylvania and New York (USA) have been under investigation since March 2001. A survey has been conducted across these two states revealing the extent of vine decline in the region. From each site visited, wood tissue samples were taken from the trunk and roots of declining vines and plated onto 2% acidified malt agar. Fungi frequently identified from these symptomatic vines included *Phaeomoniella chlamydospora, Phaeoacremonium* species, *Phialophora* species and *Phialophora*-like isolates. The objectives of this research were to assess the genetic diversity of selected *Phialophora*-like isolates and to determine the role of these organisms in grapevine decline. Molecular sequence data from the internal transcribed spacer (ITS) region ITS1-5.8S-ITS2, the large exon of translation elongation factor 1α (tef), and the partial sequence of reverse polymerase subunit 2 (RPB2) gene regions have been analyzed using PAUP with *Phaeomoniella chlamydospora* as the outgroup. The *Phialophora*-like isolates clustered in two clades: a *Phialophora* clade, and a *Phaeoacremonium* clade. The *Phialophora*-like isolates clustering in the *Phaeoacremonium* clade do not match published sequences of *Phaeoacremonium*. Based on the sequence data, *Phialophora*-like isolates clustering in the *Phaeoacremonium* clade will be preferentially selected for pathogenicity testing.

Pathogenicity of a *Phialophora* species on grapevines in California. A. Eskalen and W.D. Gubler. *Department of Plant Pathology, University of California, One Shields Ave., Davis, CA 95616, USA. E-mail:* els4@psu.edu

A *Phialophora* species has been isolated from symptomatic grapevine wood including spurs, cordons and trunk from eight different grapevine production regions in California. Greenhouse experiments have demonstrated that this fungus is an effective pathogen of grape seedlings. One month-old seedlings (*Vitis vinifera* 'Carignane') were inoculated by root dip and *Phialophora* sp. was isolated from 65% of the seedlings. Symptoms included a significant reduction in the number of roots and overall stunting of plant height. Field inoculations have also demonstrated that *Phialophora* sp. is an aggressive pathogen of grapevines. Infection and vascular discoloration was found in spurs of 'Cabernet Sauvignon' and 'Thompson Seedless' vines inoculated with *Phialophora* sp. through pruning wounds. Vascular discolouration was measured ten months after inoculation by cutting the spurs longitudinally and evaluating the extent of vascular streaking. Average length of streaking in inoculated spurs was shown to be approximately 9.2 cm. Vascular streaking in control spurs was shown to be about 1.37 cm. Discoloured vascular tissues were cultured on potato dextrose agar amended with tetracycline and the pathogen was successfully recovered. The pathogen was not detected in control spurs. Internal transcribed spacers ITS1 and ITS2 were amplified and sequenced. ITS sequence data showed that this species of *Phialophora* is related to both *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora*.

The characterisation of *Eutypa* isolates associated with Eutypa dieback of grapevines in South Africa. S. Safodien[1], F. Halleen[1], P.W. Crous[2], A. Botha[3], J.Z. Groenewald[4], W.A. Smit[1] and L. Smit[1]. [1]*ARC Infruitec–Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa.* [2]*Centraalbureau voor Schimmelcultures, P.O. Box 85167, 3508 AD Utrecht, The Netherlands.* [3]*Department of Microbiology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa.* [4]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa. E-mail:* sieyaam@infruit.agric.za

Eutypa dieback of grapevines is a trunk disease that has a devastating impact on vineyards worldwide. Usually symptoms of the disease appear on vines two years after infection. Eutypa dieback can be costly, not only due to the magnitude of yield losses and vine destruction that may occur, but also as a result of the cost of replanting. The causal agent was first described as *Eutypa armeniacae*, the pathogen that causes dieback of apricots, but since 1987 this species has been considered a synonym of *Eutypa lata* (anamorph *Libertella blepharis*). However, the identification of *E. lata* can at best be only tentative since it is based solely on morphological characteristics. Also, if the *Eutypa* sp. responsible for Eutypa dieback is genetically divergent, the use of molecular tools would support identification based on biological characters (viz. morphological and cultural). Recently, it was proposed that at least two species that are capable of infecting vines are responsible for Eutypa dieback. Consequently, it was decided to investigate local *Eutypa* populations occuring on vines for the presence of different fungal species. Isolates were collected from vines and fruit trees with dieback symptoms, as well as from fruiting structures on infected wood. *Eutypa lata* isolates were also obtained from Australia and France. Molecular data derived from PCR amplification of the internal transcribed spacer (ITS) region of the ribosomal DNA operon and the β-tubulin gene region and their subsequent sequence data was compared to that of known species. Preliminary results from the ITS sequencing data grouped most of the isolates from South Africa and those obtained from Australia and France in a well-supported clade with a bootstrap support value of 87%, except for one isolate that was closely related to *E. leptoplaca*. This isolate also showed some sequence similarity to other species of *Eutypa*, viz., *E. consobrina*, *E. maura*, *E. astroidea*, *E. crustata* and *Eutypella prunastri*, part of the family Diatrypaceae. The grape isolates STE-U 5561 and 5562 and the fruit cultures formed a separate cluster showing sequence similarity to *Eutypella vitis*. Based on the fact that several of these morphologically similar isolates represent different species, the design of species–specific primers using the sequence data will be considered for pathogen identification in future.

Identification and partial characterisation of toxins from *Fomitiporia mediterranea* the rotting agent of esca disease. E. Abou-Mansour[1], L. Bovet[2], J. Polier [1], E. Martinoia[3] and R. Tabacchi[1]. [1]*Institute of Chemistry, University of Neuchâtel, Bellevaux 51, 2007 Neuchâtel, Switzerland.* [2]*Institute of Biology, University of Neuchâtel, Emile-Argand 11, 2007 Neuchâtel, Switzerland.* [3]*Labor für Molekulare Pflanzenphysiologie, Institut für Pflanzenbiologie, Universität Zürich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland. E-mail:* eliane.abou-mansour@unine.ch

The woody decay symptoms of esca have been associated with *Fomitiporia mediterranea* formely named *Fomitiporia punctata* (Wagner and Fischer, 2001 [*Mycological Research*. 105, 773–782]). Being a basidiomycete, it was stipulated that this fungus was responsible for rotting the wood based on laccases, phenyloxydases and peroxydases activities. The goal of this study was to determine whether *F. mediterranea,* is also able to produce toxins in addition to enzymatic activities. Two strains of *F. mediterranea* were cultured on solid and liquid phases in three different mediums under a dark-light cycle. The biological effects of *F. punctata* crude extracts were then analyzed on cell cultures of *Vitis vinifera* that were inoculated with liquid and mycelium crude extracts. Interestingly, the data showed that *F. mediterranea* secrete one or more compounds able to produce esca symptoms on grapevine leaf disc assay. Four-week-old *Arabidopsis thaliana* plants were also sprayed with the same extracts. Following the treatment, the plants stunted and showed a reduced growth, and the

leaves were paler and smaller, compared to the controls. *Arabidopsis thaliana* seedlings containing auxin-responsive construct DR5-GUS were not affected by the mycelium extracts, thereby suggesting that AIA is not involved in the phytotoxic effect. But the histochemical staining of the leaves with blue astra/saphranine suggested that lignin synthesis or degradation is involved. Confirming this hypothesis, RT-PCR analyses indicates that genes involved in the biosynthesis of lignin were differentially regulated after treatments with the fungus extracts. In order to identify the toxins, a high scale culture of *F. mediterranea* was performed. The ethyl acetate crude extract was subjected to biodirected fractionation. Several active compounds were isolated and are currently under identification. We believe that the activity of *F. mediterranea* is most probably the effect of the concomitance of several compounds from different chemical families.

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Production of stress metabolites in vine tissue cultures inoculated with *Phaeomoniella chlamydospora*. L. Mugnai[1], C. Amalfitano[2], A. Arrigo[2], M. Pasi[1], G. Surico[1] and A. Evidente[2]. *[1]Dipartimento di Biotecnologie Agrarie, Sez. Patologia vegetale, Università di Firenze, P.le delle Cascine 28, 50144 Firenze, Italy. [2]Dipartimento di Scienze del Suolo della Pianta e dell'Ambiente, Università di Napoli Federico II, Via Università 100, 80055 Portici, Italy. E-mail:* laura.mugnai@unifi.it

The accumulation of resveratrol, ε-viniferin and pterostilbene in vine callus inoculated or non-inoculated with *Phaeomoniella chlamydospora* (*Pch*) was measured using HPLC. Different tests were performed on callus from cultivars that had shown a varying susceptibility to esca in field surveys (Roussanne and Colorino, exhibiting a certain degree of resistance; Moscato, Pinot Bianco, Isabella, Riparia Gloire and Barbera 424, medium resistance; and Semillon, very susceptible). Pterostilbene was never detected under any of the conditions tested, ε-viniferin was absent or present in negligible amounts and all cultivars showed endogenous levels of resveratrol. Resveratrol presence was not closely correlated to esca susceptibility except for cv. Semillon (the most susceptible), which had the lowest endogenous resveratrol level. *Pch* inoculation caused an increase in resveratrol and ε-viniferin content. Such increase was faster and greater in resistant cultivars than in the susceptible cultivar. Two weeks after inoculation, resistant cultivars showed a resveratrol content 18–30 fold, and an ε-viniferin content 70–600 fold, than that recorded in the susceptible cultivar. In addition, *Pch*-inoculation was carried out on callus of three cultivars (Moscato, Isabella and Pinot Bianco) incubated in the dark or under light conditions. Nine days after infection, exposure to light was shown to highly influence the ratio between ε-viniferin and resveratrol content in infected callus cvs. Isabella and Pinot Bianco and the formation of other resveratrol derivatives in callus cv. Moscato. These results suggest that resveratrol and ε-viniferin play a role in the interaction between the fungus and its host.

Microscopic study of the trunk diseases symptoms in grapevine. P. Gomez[1], A. Baidez[1], M.D. Fuster[1], A. Ortuño[1], J.A. del Rio[1] and V. Frias[2]. *[1]Dpto. Biología Vegetal (Fisiología Vegetal), Facultad de Biología, Universidad de Murcia, Spain. [2]Agrométodos S.A. Pozuelo de Alarcón, Madrid, Spain. E-mail:* jadelrio@um.es

In the present communication, a morphological study of the xylem block associated to Petri disease is presented. Electron microscopy of stem and roots of young grapevines showed different forms (tyloses and aggregates) present in the lumen of the xylem, which are involved in the obstruction of water flow. The presence of fungal hyphae in the wall of the xylem is demonstrated. In the different tissues analysed, the presence of possible resistance forms of the microorganisms was detected. A tentative method for identifying fungi is described and a possible invasive pathway of fungi in young grapevines is proposed.

Secondary metabolites produced by southern Australian isolates of *Eutypa lata*. T.J. Wiechel[1], M.L. Creaser[2], F.M. Cole[1] and T.J. Wicks[2]. *Cooperative Research Centre for Viticulture, P.O. Box 154, Glen Osmond SA 5064, Australia. [1]Department of*

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Eutypa lata (Pers.:Fr.) Tul & C. Tul. is the causal agent of Eutypa dieback in grapevines. Eutypa dieback is a chronic disease that significantly reduces grapevine yield. *E. lata* infects through wounds and grows slowly in the vascular tissue producing foliar symptoms several years after infection. Stunted shoots with chlorotic leaves that may indicate *E. lata* infection are most easily seen in early spring. Studies have suggested that these foliar symptoms are a result of a phytotoxin, eutypine, produced by the fungus that inhabits the xylem. The aim of this study was to determine whether eutypine, a well-characterised phytotoxin, could be used as the basis of a rapid diagnosis system. The secondary metabolites of 19 isolates of *E. lata* growing on Pezet's medium were extracted using vacuum infiltration of ethyl acetate and separated by thin layer chromatography. After 14 days on Pezet's medium, numerous compounds were produced by a number of isolates. The metabolite Rf 0.34 was the most consistently produced compound. Only two isolates, L003 and M303, produced eutypine. After 42 days on Pezet's medium, eutypine was not produced by any of the isolates, but five other compounds were produced. After 56 days, five isolates produced a total of six metabolites with only L003 producing eutypine. After 70 days, seven isolates produced a total of four metabolites and again only L003 produced eutypine. This research demonstrates that more than one toxin may be involved in the production of foliar symptoms of Eutypa dieback.

Host-pathogen interaction of *Phaeomoniella chlamydospora*, causal organism of Petri disease, in grapevine tissue. E. Cottral†[1, 2, 5], I.G. Pascoe[1, 2], J. Edwards[1,2], G. Jaudzems[3] and P.A. Taylor[4]. [1]*Co-operative Research Centre for Viticulture, P.O. Box 154, Glen Osmond, SA, 5064, Australia.* [2]*Institute for Horticultural Development, Department of Primary Industries, Private Bag 15, Ferntree Gully Delivery Centre, Victoria, 3156, Australia.* [3]*School of Biological Sciences, Building 18, Monash University, Clayton, Victoria, 3800, Australia.* [4]*Crop Protection Approvals, Ltd, Level 1, 5, Everage Street, Moonee Ponds, Victoria, 3039, Australia.* [5]*Institute of Land and Food Resources, The University of Melbourne, Victoria, 3010, Australia. E-mail:* jacky.edwards@nre.vic.gov.au

Petri disease (previously known as Black goo decline) is caused by the fungus *Phaeomoniella chlamydospora* (Pch). Petri disease is a grapevine trunk disease that threatens the sustainability of young grapevines and has been diagnosed in Australia, New Zealand, South Africa, Europe and USA. Dark vascular streaks in the trunk that appear as black tarry goo in cross section are typical symptoms of vines infected with Pch. Although Pch is consistently isolated from vines infected with Petri disease, little is known about the infection path of the fungus. In this study, tissue-cultured grapevine plants (cultivar Chardonnay) were artificially inoculated with Pch. Infected tissue pieces were embedded in resin for examination by light and electron microscopy. Pch hyphae initially grow intercellularly, particularly between the parenchyma cells that lie adjacent to xylem vessels (paratracheal cells). The fungus then penetrates the paratracheal cell wall and grows intracellularly. The presence of the fungus inside these paratracheal cells is associated with the production of tyloses in the adjacent xylem vessel lumen. As infection progresses the fungus moves into the vessel lumen, possibly through vessel pits. The host reacts to the presence of the fungus by forming reaction zones around the hyphae. Staining with Toluidine Blue showed that these reaction zones are composed of polyphenolic compounds. The reaction zones were found to vary in electron density under the TEM. Many infected vascular bundles were occluded with brown phenolic compounds. Hyphae were not always associated with infected vascular bundles, suggesting the host response can occur ahead of the fungus.

Gene regulation in *Arabidopsis thaliana* after treatment with eutypine. L. Bovet[1], E. Abou-Mansour[2], S. Perrin-Chérioux[2], R. Tabacchi[2] and E. Martinoia[3]. [1]*Institute of Biology, University of Neuchâtel, Emile-Argan 11, 2007 Neuchâtel, Swit-*

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Eutypine, 4-hydroxy-3-(3-methyl-3-buten-1-ynyl) benzaldehyde, is a toxic compound secreted by *Eutypa lata* (Per: Fr.) Tul., the fungus responsible for a severe disease of grapevine and many other woody fruit plants such as apricot trees. Grapevine cells placed in the presence of eutypine produce a hydroxylated derivative of eutypine called eutypinol. This compound does not show any toxicity to the grapevine. We report here on biological assays carried out using eutypine, eutypinol and three other eutypine analogues, obtained in a synthetic way. Only eutypine showed a relevant activity on grapevine callus. Consequently we decided to analyse the biological effect of eutypine as well as gene regulation in the model plant *Arabidopsis thaliana*. To this end, we treated *Arabidopsis thaliana* with eutypine using two different methods, either by spraying the leaves or by measuring the growth of roots in agar plates containing the toxin. In both cases, eutypine was found to affect the plants by forming leaf necrosis or preventing root growth. After leaf treatments for 24 and 48h, eutypine catabolites were identified by HPLC coupled to an Ion Trap ESI/MS. DNA-microarray technology associated with RT-PCR analyses were performed to identify possible genes involved in eutypine detoxification in *Arabidopsis thaliana*. Interestingly, among several genes, some ABC transporters were identified suggesting that eutypine or degradation products might be transported within vacuoles or in the apoplastic compartment for complete cell detoxification. The data will be discussed in terms of identification of novel genes and proteins to help us develop new strategies for fighting eutypa dieback.

Resveratrol dimers in the brown-red wood of esca-diseased grapevines. C. Amalfitano[1], A. Arrigo[1], L. Mugnai[2], G. Surico[2] and A. Evidente[1]. *[1]Dipartimento di Scienze del Suolo della Pianta e dell'Ambiente, Università di Napoli Federico II, Via Università 100, 80055 Portici, Italy. [2]Dipartimento di Biotecnologie Agrarie, Sez. Patologia vegetale, Università di Firenze, P.le delle Cascine 28, 50144 Firenze, Italy. E-mail:* amalfita@unina.it

Resveratrol (3,5,4'-trihydroxy stilbene) and its biosynthetically related compounds, the viniferins (dimers or oligomers of resveratrol deriving from condensation by an oxidative dehydrogenation process), belong to a class of compounds described as stress metabolites produced by *Vitis vinifera* in response to fungal infection, UV irradiation and exposure to chemicals. They have been found at different concentrations in all parts of the plant. In a previous study (Amalfitano *et al*. [2000] *Phytopathologia Mediterranea* 39, 178–183), *trans*-resveratrol and ε-viniferin were detected in higher concentrations in the brown-red wood of esca-diseased *Vitis vinifera* cv. Sangiovese plants. We now report on two other minor metabolites related to resveratrol, one of which was not detected in healthy vine wood. The two metabolites were isolated from brown-red wood and their structures established on the basis of spectroscopic evidence. The spectroscopic data (H^1 and C^{13} NMR) of the metabolite not occurring in healthy wood suggested the structure was that of ampelopsin-B, a resveratrol dimer first isolated in a Vitacea (*Ampelopsis brevipeduncolata* var. *hancei*) (Oshima *et al*. [1990] *Tetrahedron*, 46, 5121–5126) or its stereoisomer. The other metabolite appeared strictly correlated to ε-viniferin, from which it differed by the stereochemistry (*cis*) of the double bond. This is the first report on ampelopsin-B in *Vitis vinifera*.

***Phaeomoniella chlamydospora* inhibits callus formation by grapevine rootstock and scion cultivars.** J. Wallace[3], J. Edwards[1, 2], I.G. Pascoe[1, 2] and P. May[3]. *[1]Co-operative Research Centre for Viticulture, P.O. Box 154, Glen Osmond, SA 5064, Australia. [2]Institute for Horticultural Development, Department of Primary Industries, Private Bag 15, Ferntree Gully Delivery Centre, Victoria, 3156, Australia. [3]Burnley College, Institute for Land and Food Resources, The University of Melbourne, 500 Yarra Boulevard, Richmond, Victoria 3121, Australia. E-mail:* jacky.edwards@nre.vic.gov.au

Phaeomoniella chlamydospora and *Phaeoacremonium aleophilum* are hyphomycete fungi that inhabit the xylem-tissues of grapevine wood. Both

have been implicated as causal agents of Petri disease in grapevines, symptoms of which are poor growth, decline and dieback. Anecdotal evidence has suggested that infected grapevine cuttings do not callus properly, resulting in poor planting material, and that some grapevine cultivars are affected less than others. To test this, we inoculated the bases of grapevine cuttings with 100 spores (20 ml of 5×10^3 spores ml^{-1}) of either *P. chlamydospora, P. aleophilum* or a mixture of both. Control treatments were no inoculation and inoculation with 20 ml water only. The cultivars used were seven rootstock varieties (Ramsey, 99 Richter, Schwarzmann, Kober 5BB, Paulsen, 101-14 Millardet and SO4) and five scion varieties (Merlot, Cabernet Sauvignon, Pinot Noir, Shiraz PT10 and Shiraz PT23). After inoculation, the cuttings were layered in boxes of moist vermiculite and maintained at 25°C to callus and form roots as per nursery practice. After 10 weeks, callus production, root initiation and internal symptom development were assessed. *P. chlamydospora* inhibited callus formation on all cultivars but *P. aleophilum* did not. Root initiation was not affected by either fungus. Cultivars differed with respect to internal symptom development. *P. chlamydospora* caused brown wood streaking in the rootstock cultivars but not the scion varieties. *P. aleophilum* did not cause visible internal symptoms in any of the cultivars used. These results suggest that *P. chlamydospora* is the more virulent of the two fungi.

Molecular diagnostics for industry: sources of Petri Disease in grapevine nurseries. H.J. Ridgway, S. Whiteman, M. Jaspers, and A. Stewart. *Soil, Plant and Ecological Sciences Division, P.O. Box 84, Lincoln University, Canterbury. E-mail:* ridgwh@lincoln.ac.nz

Phaeomoniella chlamydospora is a fungal pathogen of grapevines that causes Petri disease. The disease causes decline of vines and threatens the long-term sustainability of winegrape production in NZ. Research has suggested that the pathogen can be introduced into vineyards through planting of infected grafted cuttings. To reduce disease spread, a PCR-based test was developed to detect the pathogen at critical points in winegrape production such as propagative material, grafting and field soil. The test could detect low levels of pathogen DNA: 1 pg in wood cuttings, 50 fg in field soil and 5 fg in grafting solutions. This highly sensitive tool was used to detect spores in naturally infected rootstock mother vines, where it distinguished *P. chlamydospora* from grape DNA and other endophytes. However, the positive test did not correlate with a specific region in the mother vines and development of the diagnostic system should be complemented by analysis of the regions of the vine that are likely to harbour the pathogen. The diagnostic test was validated in a commercial grafting shed where it showed that *P. chlamydospora* was prevalent in solutions with repeated exposure to plant material. Analysis of artificially infested vineyard soil showed that the test could detect $<10^2$ conidia g^{-1} soil, demonstrating that the assay was suitable for testing the *P. chlamydospora* status of soil prior to vineyard establishment. The broad applicability of this PCR-based assay provides the potential for it to be further developed as a commercial diagnostic service to provide pathogen-free plant material for the viticulture industry in NZ.

Identification of potential sources of *Phaeomoniella chlamydospora* in the grapevine propagation process. S.A. Whiteman, M.V. Jaspers, A. Stewart and H.J. Ridgway. *National Centre for Advanced Bio-Protection Technologies, P.O. Box 84, Lincoln University, Canterbury, New Zealand. E-mail:* whitemas@lincoln.ac.nz

Phaeomoniella chlamydospora is the main causal agents of Petri disease in New Zealand. It has been hypothesized that young vines arrive at the vineyard having already become infected during the propagation process. A two-year field trial revealed that infection levels in own-rooted scion, own-rooted rootstock vines and grafted vines were 0, 42 and 18% respectively, proving that the use of cutting material collected from infected rootstock mother-vines can result in infected young vines. Cutting origin along an infected rootstock mother-vine shoot also had an effect on disease incidence of young vines. Infection levels were 81% higher in vines originating from cuttings taken 0–60 cm from the mother-vine head than in those further away. To determine *P. chlamydospora* contamination was

present in the propagation process, samples were taken from within a commercial nursery operation at five stages; washings of plant material, pre-storage rehydration/fungicide tanks, pre-grafting rehydration tanks, washings from grafting tools and callusing media, and tested using a nested species-specific PCR. Pathogen DNA was detected at all stages. The percentage of positive samples ranged from 15–50% and was high from both rehydration tanks, moderate from grafting tool washings and low from washings of callusing media. To determine the exact source of this contamination, the experiment was repeated using a single batch cuttings from infected mother-vines, following a protocol designed to eliminate contamination from other sources such as air borne conidia. *Phaeomoniella chlamydospora* DNA was again found at all stages of the process at low levels (10–15%) and the number of positive samples from plant material increased from 39% before processing to 70% afterwards. These results suggest that the source of contamination in the propagation process is the use of infected cutting material and repeated exposure of the process to this material results in build-up of inoculum. The re use of nursery beds may also provide a source of inoculum. The ability of soil to harbour the pathogen was proved when soil collected from around the trunk of known infected rootstock mother-vines tested positive for the presence of pathogen DNA. Standard operational procedures for industry are being developed to eliminate contamination sources with a view to reducing infection in young vines by improving nursery hygiene.

Occurrence of the pycnidial state of *Phaeomoniella chlamydospora* in Californian vineyards. A. Eskalen, S.N. Rooney Latham and W.D. Gubler. *Department of Plant Pathology, University of California, One Shields Ave., Davis, CA 95616, USA. E-mail:* aeskalen@ucdavis.edu

Phaeomoniella chlamydospora is considered to be the primary pathogen causing esca and Petri disease. Previous studies have shown that *Pa. chlamydospora* has the ability to produce pycnidia in culture. However, these structures had not been documented in Californian vineyards. Spore traps were placed in selected vineyards throughout the State. Spores of *Pa. chlamydospora* were trapped from coastal counties and were correlated with rainfall events. Grapevine tissues were collected from vineyards where spores were previously trapped. Tissues were washed and cultured on media. Results showed that spores of *Pa. chlamydospora* were found on grapevine cordons and on 2–3 year-old pruning wounds. Pycnidia were observed primarily on 2–4 year-old pruning wounds and beneath bark, particularly where injury resulted in exposed vascular tissue. These pycnidia were similar to those that were artificially produced in culture. Spores from pycnidia were shown to be viable. Pathogenicity tests of spores from artificially and naturally produced pycnidia were compared. ITS and ß-tubulin regions from pycnidiospores were compared to that of known isolates of the *Pa. chlamydospora* and showed the pycnidiospores to be those of *Pa. chlamydospora*.

Occurrence of esca in California vineyards and association with environmental conditions. W.D. Gubler, A. Eskalen and S.N. Rooney Latham. *Department of Plant Pathology, University of California, One Shields Ave., Davis, CA 95616, USA. E-mail:* wdgubler@ucdavis.edu

Esca in California has been a problem on both wine and table grape varieties for over 70 years. The on again - off again nature of the symptoms both on vines and in vineyards resulted in sporadic research efforts to try to identify the pathogen(s) involved but were generally not fruitful. For many years, symptoms of esca were rare in vineyards less than 10 years of age. However, in the last 7 years, we have observed esca symptoms on vines that were only 1–3 years of age. Though *Phaeomoniella chlamydospora* has only recently been identified as the probable, primary cause of esca in Californian vineyards, *Phaeoacremonium* spp. also seem to play a role in disease occurrence and symptom expression. Weather conditions appeared to be roughly correlated with the occurrence of esca symptoms, in that more severe symptoms were observed in years with higher than normal rainfall and high summer temperatures. Vineyard observations, spore trapping data and some speculation seemed to indicate that symptom expression occurred in a year when new infections occurred. Vineyard surveys over

the past two years however, seem to point to the possibility that symptom expression may not occur until the second year after infection. Correlations between symptom expression, rainfall and temperature data will be presented.

Grapevine trunk diseases caused by fungi in Japan. H. NASU[1], K. INOUE[1] and S. NAKAO[2]. [1]*Okayama Agricultural Experiment Station, Sanyo-cho, Okayama 709-0801, Japan.* [2]*Oita Prefectural Agricultural Research Center, Usa-city, Oita 872-0103, Japan. E-mail:* hideo_nasu@pref.okayama.jp

Five fungal species, *Botryosphaeria* sp. (black rot), *Sclerotinia sclerotiorum* (shoot blight), *Valsa vitis* (canker), *Diaporthe* sp. (swollen arm) and *Phomopsis viticola* (dead arm) have been causal agents of shoot/trunk diseases of grapevine in Japan. The two most serious diseases, black rot and swollen arm, are presented here. Black rot: it was characterised by leaf blight, shoot blight and cluster rot symptoms. The leaf blight caused leaves to droop after the petiole bases had browned off, mainly from short pruning. Shoot blight occurred mainly from long pruning. These symptoms developed more frequently in plastic houses and glasshouses, especially in early forcing culture, more than in open-field culture. We assumed that the primary infection source was conidia of *Dothiorella* sp., the imperfect stage of *Botryoshaeria* sp., infecting two year-old shoots. Swollen arm: symptoms included small black spots on grape berries and on the basal parts of one-year-old shoots in July, swellings of two- to three-year-old vines, and canker with xylem browning and occasional death in vines over four years old. The disease was most prevalent on the susceptible grapevine cultivar, Kyoho. The causal fungus produced two types of conidia: α-conidia were fusiform, hyaline, unicellular, 15–21×5–7 μm in size, while β-conidia were campanulate, 31–46×1–1.6 μm in size, larger than those reported to be formed by *Phomopsis* spp. The perithecium was obligate-globoid with a beak 1400–3000 μm in length. Fungal infection occurred between May and September, particularly between June to July, and the disease is spread from one area to another largely by infected seedlings. Dithianon, mancozeb, oxine-copper, and copper oxychloride were effective in controlling this fungus.

Epiphytic occurrence of esca and Petri disease pathogens on grapevine tissues. A. ESKALEN, S. ROONEY LATHAM, A.J. FELICIANO and W.D. GUBLER. *Department of Plant Pathology, University of Califonia, One Shields Ave., Davis, CA 95616, USA. E-mail:* aeskalen@ucdavis.edu

Spores of *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* have been successfully trapped in Californian vineyards. These fungi have been shown to be capable of producing aerially dispersed inoculum that can cause infection by water splashing onto susceptible pruning wounds. However, it is not known whether aerially dispersed inoculum is capable of surviving epiphytically on the surfaces of grapevines. Various grapevine tissues including roots, shoots, leaves, spurs, fruit, cordons, trunks, old pruning wounds and tendrils were collected from different vineyards showing esca symptoms to test for the epiphytic presence of these fungi. Soil samples and standing water were also collected. All samples were washed in sterile distilled water to detach any spores or propagules. Solutions were then filtered before culturing them onto potato dextrose agar amended with tetracyline. Plates were examined 10 days later. Various grapevine tissues from many counties tested positive for the presence of *Phaeoacremonium* spp. and *Pa. chlamydospora*. *Pa. chlamydospora* was isolated most commonly from the surface of spurs, cordons and trunks. Interestingly, it was also isolated from old lignified tendrils on wires as well as berry surfaces. *Phaeoacremonium* spp. were also commonly isolated from the surfaces of spurs. However, they were also found on the surfaces of roots, leaves, clusters and in soil. It is probable that many of the positive isolations were from tissues previously infected. However, since many of the tissues were asymptomatic or did not test positive for these fungi when surface sterilised and cultured, it is speculated that these fungi can survive epiphytically on grapevines.

***Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* can spread into grapevine canes from trunks of infected mother vines.** J. EDWARDS[1, 2], I.G. PASCOE[1, 2], S. SALIB[1, 2] and N. LAUKART[1, 2]. [1]*Co-operative Research Centre for Viticulture, P.O. Box 154, Glen Osmond, SA 5064, Australia.* [2]*Institute for Horticultural Development, De-*

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Petri disease, thought to be caused by the vascular pathogens *Phaeomoniella chlamydospora* and/or *Phaeoacremonium aleophilum*, is responsible for poor establishment in many newly-planted vineyards. In these cases, the young vines are infected prior to planting, but it is not known how the infection occurs. The infection courts of both *P. chlamydospora* and *P. aleophilum* are the xylem parenchyma and vessels of mature grapevine wood. It is suspected that infection may be passed from mother vines into progeny via spores carried in the sap flow. To test this hypothesis, we harvested 4–5 m long canes from infected Ramsey rootstock mother vines in order to map the occurrence of *P. chlamydospora* and *P. aleophilum* along the full length of the cane. The canes were processed into cuttings 400–500 cm long, recording the position of the cuttings along the cane length and tagging accordingly. To account for the full length of cane, the waste pieces from between the cutting lengths were surface sterilised, cut into 3 mm thick slices and plated onto potato dextrose agar. After 2–4 weeks any fungal growth was identified. The cuttings were callused, potted up and grown for 18 months in a glasshouse. Then they were destructively assessed for internal symptom development, whereupon all the stems were moist incubated and examined after 6–8 weeks for the presence of *P. chlamydospora* and *P. aleophilum*. We found both *P. chlamydospora* and *P. aleophilum* scattered randomly along the full length of the canes, suggesting that infection does indeed occur via propagules such as spores or fragments of hyphae carried in the sap flow, rather than by mycelial growth from the crown of the infected trunk into the canes.

The occurrence, distribution and control of *Botryodiplodia theobromae* on *Vitis vinifera* in California, Arizona and north Mexico. G.M. Leavitt. *University of California Cooperative Extension, 328 Madera Ave, Madera, CA 93637, USA. E-mail:* gmleavitt@ucdavis.edu

In California, Arizona and Hermosillo, Mexico, *Botryodiplodia theobromae* was found in cankers on *Vitis vinifera* (grape) in greater incidence than *Eutypa lata*. Spurs, arms, cordons and whole vines may be affected and slowly die. Two physiologic races, designated as high and low temperature races, exist. Only the high temperature race (optimum growth temperature 30–33°C) was found in the desert areas of California, Arizona and Hermosillo, Mexico. Both races exist in the central and northern parts of California, but the low temperature race (average optimum temperature 27°C) was most frequently isolated (95%). Three isolates of *B. theobromae*, representing the high and low temperature races, were used to inoculate fresh pruning wounds of grapevines in 1983. In 1990, 90.7% of the high temperature race and 77.3% of the low temperature race were recovered, fulfilling Koch's postulates. Arm death began four years after inoculation. The natural incidence of disease progress was observed for 5 years on young vines. Occurrence of the disease on vines 10 years and older was as great as 100% with several cankers per vine. Various materials were used as pruning wound applications to prevent infection. Most fungicides tested were effective in reducing disease incidence when used on fresh pruning wounds. Latex paint reduced infections but was significantly inferior in control to any fungicide. Springtails (*Entomobrya unostrigata*) were implicated as a possible vector carrying spores to pruning wounds as they feed on vine exudates following pruning.

Effects of temperature and moisture on disease incidence of swollen arm of grapevine and its chemical control. N. Tashiro and Y. Ide. *Saga Pref. Fruit Tree Expperimental Station, Ogi, Saga 845-0014, Japan. E-mail:* tashironobuya@pref.saga.jp

One of the most destructive trunk diseases of grapevine in Japan is the swollen arm caused by *Diaporthe kyushuensis* which causes black spot lesions on green shoots and swollen arm symptoms on nodes of 2–3 year old canes. The disease results in seriously retarded growth and ensuing death of grapevines in three to four years. In vineyards, spores were dispersed from late April to early November, particularly during the rainy season from early June to mid July. Infection oc-

curred when the temperature ranged between 15 and 32°C, although it was considerably lower at 15°C. The minimum free water period required to establish infection was approximately 12 hr at 25°C and 15 hr both at 22°C and 28°C. No effective cultural control measures were identified, but we found that preventive treatment of non-systemic surface chemicals (kresoxim-methyl, dithianon, oxine-copper, fluazinam and benomyl) was effective against the disease. Of all chemicals tested, kresoxim-methyl was the most effective, with the treated plants protected for 14 days as long as precipitation was not above 300 mm after treatment. Contrarily, the effective period of dithianon was 7 days under 200 mm and of oxine-copper 7 days under 100 mm precipitation. No lesions developed when kresoxim-methyl and azoxystrobin were applied within two or three days after infection, which is indicative of curative action of these chemicals.

Cultural requirements of *Phaeomoniella chlamydospora*. S.A. Whiteman[1], A. Stewart[1], M.C. Trought[2] and M.V. Jaspers[1]. [1]*Soil, Plant and Ecological Sciences Division, P.O. Box 84, Lincoln University, Canterbury, New Zealand.* [2]*Villa Maria Estate Ltd., P.O. Box 848, Blenheim, New Zealand. E-mail:* whitemas@lincoln.ac.nz

Phaeomoniella chlamydospora is a fungal pathogen of woody grapevine tissue believed to be the main causal agent of "Petri disease" or "Black Goo". In order to facilitate research into the epidemiology of this disease, a number of cultural requirements of *P. chlamydospora* were investigated. Mycelial growth and spore germination were determined over a range of temperatures (5, 10, 15, 20, 25, 30, 35, 40 and 50°C). Mycelial growth occurred at 10 to 35°C with an optimum of 25°C ($P<0.05$). At all temperatures some conidia had germinated 12 h after inoculation, but germination was significantly higher ($P<0.01$) in the range of 20 to 35°C with 75 to 79% of spores germinated. Mycelial growth and sporulation of *P. chlamydospora* was determined on five media. Colony diameter was 17% greater on potato dextrose agar than malt extract agar ($P<0.01$), whilst sporulation was 53% higher on malt extract agar ($P<0.05$) than on potato dextrose agar. A range of chemical compounds was added to agar to test their ability to suppress fungal contaminants in order to aid isolation of the pathogen. Lithium chloride and a combination of benomyl and 2-phenylphenol were shown to inhibit the growth of a range of common fungal contaminants of grapevine wood.

***Phaeomoniella chlamydospora* detection in the grapevine propagation process by species-specific PCR.** S.A. Whiteman, M.V. Jaspers, A. Stewart and H.J. Ridgway. *Soil, Plant and Ecological Sciences Division, P.O. Box 84, Lincoln University, Canterbury, New Zealand. E-mail:* whitemas@lincoln.ac.nz

Petri disease, caused by the fungus *Phaeomoniella chlamydospora*, results in poor growth and decline of grapevines. In cut woody tissue, brown/black streaking is observed in transverse and black tarry droplets form on cross sections. Most New Zealand commercial wine grapevines are grafted and this propagation process may contain sources of inoculum. To test this hypothesis, samples were taken from within a commercial nursery operation at five stages: washings from the surface of plant material, grafting tools and callusing media and samples from the pre-storage rehydration/fungicide treatment and the pre-grafting rehydration tanks. Samples were tested by nested species-specific PCR involving amplification of a large region of ribosomal DNA, followed by secondary amplification with species-specific primers Pch1 and Pch2. The assay was determined to be highly sensitive having a detection level of 10 conidia ml^{-1} and <5 fg of genomic DNA. DNA of the pathogen was detected at all stages of the grape propagation process with percentage of positive samples ranging from 15 to 50. Percentage of positive samples was high from rehydration tanks, moderate from plant and grafting tool washings and low from washings of callusing media. The aim of ongoing experiments is to determine primary sources of the contamination, which was detected in the propagation process. Probable sources are spores on the surface of infected grafting material and/or airborne spores from nearby rootstock blocks. The technology utilised in this research has the potential to form the basis of a commercial diagnostic service for the industry.

Proactive control measures for Petri disease in grapevine nurseries. P.H. Fourie[1] and F. Halleen[2]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland (Stellenbosch) 7602, South Africa.* [2]*Disease Management Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa. E-mail:* phfourie@sun.ac.za

Petri disease is associated with decline and dieback of young grapevines that are subjected to stress. A major means of spread of the causal organisms (*Phaeomoniella chlamydospora* and *Phaeoacremonium* spp.) is via infected propagation material. Since no curative control measures are known, proactive measures must therefore be taken in grapevine nurseries to control this disease. An extensive semi-commercial trial with naturally-infected Richter 110 and 101-14 Mgt rootstock material was performed in a grapevine nursery. Prior to omega-grafting with Cabernet Sauvignon, the rootstocks were treated as follows: 1 h drench in suspensions of benomyl, phosphoric acid, different bacterial and *Trichoderma* formulations, water, or hot water treated (HWT; 30 min at 50°C). Callused graftlings were planted in two commercial field nurseries and a greenhouse and uprooted 8 months later. None of the treatments affected callus or initial shoot growth, nor was percentage take, root or shoot mass of vines from the field nurseries and greenhouse significantly affected. Although *Phaeomoniella* and *Phaeoacremonium* incidences in graftlings and uprooted nursery vines, of which rootstocks received chemical or biological treatments, were in some instances significantly lower than that of the water treatment, the reduction was most significant in HWT rootstocks. It was also shown that HWT of dormant nursery vines effected a similar reduction in *Phaeomoniella* and *Phaeoacremonium* incidence to that of HWT prior to grafting. These results clearly indicate that HWT did not eradicate *P. chlamydospora* or *Phaeoacremonium* spp., but was effective in significantly reducing the incidence of these pathogens.

The effects of hot water treatment, hydration and order of nursery operations on cuttings of *Vitis vinifera* cultivars. H. Waite and P. May. *Institute of Land and Food Resources, University of Melbourne, Dookie College, Victoria, 3647, Australia. E-mail:* hwaite@unimelb.edu.au

This study examined the effects of hot water treatment (HWT) at 50°C for 30 minutes, order of HWT and storage (store/HWT and HWT/store), and 3 hydration times (0, 4 and 15 hrs) on root and shoot development and final condition in dormant cuttings of Cabernet Sauvignon and Chardonnay. Sound, healthy cuttings were collected from a registered source block at Irymple in the Murray Valley. Equal numbers of cuttings of each variety were randomly allocated to 0, 4 or 15 hours hydration. Equal numbers of cuttings from each hydration treatment were then allocated to pre- or post-storage HWT, or storage with no HWT. On removal from cold storage, the post-storage group received HWT and all cuttings were incubated in moist vermiculite at 27°C and 95% humidity for 14 days. Callus, root and shoot development were assessed. The cuttings were potted into cardboard plant bands and grown on in a protected environment to marketable size (early October) and assessed as "A" grade, "B" grade or dead. Callus development in Chardonnay was affected by an interaction between HWT protocols and hydration times. In Chardonnay, callus was least developed in cuttings hydrated for 15 hrs and stored before HWT. Callus development in all other treatments was significantly ($P<0.05$) greater regardless of HWT or hydration. By contrast, callus development in Cabernet Sauvignon was significantly ($P<0.05$) greater in HWT than in non-HWT cuttings regardless of hydration times or order of operations. Root development in Chardonnay was furthest advanced in cuttings hydrated for 15 hrs, regardless of HWT, and in HWT cuttings that were not hydrated. HWT was the only factor that affected root development in Cabernet Sauvignon. Root development was greatest in non-HWT cuttings regardless of hydration times and order of operations. There were no differences between any of the treatments in either variety at final assessment. The benign conditions of the protected environment may have enabled the cuttings to recover from the stresses imposed by the various treatments. Had the cuttings been grown on in a field nursery there may have been differences between treatments at final assessment.

Fungicide trials *in vitro* against pathogenic strains isolated from esca diseased grapevines (V*itis vinifera* L.). C. Redondo, M.L. Tello and E. Mateo-Sagasta. *Departamento de Investigación Agraria. Instituto Madrileño de Investigación Agraria y Alimentaria (I.M.I.A.). Finca EL Encín, Autovía de Aragón, km.38.200, Apdo correos 127, 28800 Alcalá de Henares, Madrid, Spain. E-mail:* cristina.redondo@imia.madrid.org

The control of the pathogenic fungi associated with esca disease is one of the most important factors in its study. The prohibition of sodium arsenite as a chemical control method in many countries, due to its high toxicity for humans and for the environment, demands the acquisition of alternative control methods. It would be desirable to develop more environmental friendly control methods against the fungi involved in the wood degradation process. In this study, three fungicides of different chemical families have been studied. These fungicides have different modes of activity and application and were tested against five pathogenic fungi species isolated from grapevines showing esca symptoms. Three fungicides and six concentrations were tested. Though the highest concentrations of fungicides can reduce the mycelial growth of many isolates, Triadimefon, even at lower concentrations, is more effective than the other treatments against the majority of pathogenic fungi tested. From the results it is possible to estimate the optimal treatment, the best application mode and the exact concentration in field trials in order to preserve its efficacy and contaminated as less as possible. A first attempt at biological control was made using *Clonostachys rosea* (Link: Fr.) Schroers, Samuels, Seifert & W. Gams as a biological control agent. We studied the possible antagonistic effect of *Clonostachys rosea* using methods previously carried out with *Trichoderma* sp. Depending on the results, we will do *in vivo* assays with one-year-old grapevines var. Tempranillo/140 Ru.

Microbial degradation of toxins as new biocontrol mechanism. Case study: Eutypa dieback and esca disease of grapevine. D. Christen[1], E. Abou-Mansour[2], M. Tharin[2], R. Tabacchi[2] and G. Défago[1]. *[1]Phytopathology Group, Institute of Plant Sciences, Swiss Federal Institute of Technology, Zürich, Switzerland. [2]Institute of Chemistry, University of Neuchâtel, Bellevaux 51, CH-2007 Neuchâtel, Switzerland. E-mail:* danilo.christen@ipw.agrl.ethz.ch

Eutypa dieback and esca disease are two serious trunk diseases of grapevine, characterised by a slow decline leading to the death of the plants. *Eutypa lata* is the causal agent of Eutypa dieback. *Phaeomoniella chlamydospora*, *Fomitiporia punctata* or *Stereum hirsutum* are some of the causal agents of esca disease. All these pathogens produce several toxins (eg. eutypine, D-3-phenyllactic acid, sterehirsutinal, 4-hydroxy-benzaldehyde) responsible for wood necrosis and aerial symptoms. No *Vitis* variety is resistant to these trunk diseases. Merlot is tolerant to Eutypa dieback and degrades eutypine, the *E. lata* toxin, to the non-toxic alcohol eutypinol. Beneficial microorganisms were screened for their ability to degrade trunk disease toxins. Four strains of *Fusarium lateritium* detoxified eutypine to eutypinol and eutypinate. The kinetics of eutypine degradation revealed several parallel detoxification pathways. No strain of *F. lateritium* was able to degrade D-3-phenyllactic acid, sterehirsutinal, 4-hydroxy-benzaldehyde. *Trichoderma album* metabolised eutypine only to eutypinol. The other toxins were not degraded. One strain of *T. atroviride* was able to degrade all the toxins tested and another strain all except D-3-phenyllactic acid. Experiments are under way to prove whether microbial degradation of pathogen toxins is a new effective biological control mechanism.

Hot water treatment of grapevine cuttings reduces incidence of *Phaeomoniella chlamydospora* in young vines. J. Edwards, I.G. Pascoe, S. Salib and N. Laukart. *Cooperative Research Centre for Viticulture, P.O. Box 154, Glen Osmond SA 5064, Australia. Institute for Horticultural Development - Knoxfield, Department of Primary Industries, Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia. E-mail:* jacky.edwards@nre.vic.gov.au

Young grapevines propagated from cuttings infected with *Phaeomoniella chlamydospora* may develop Petri disease when planted out into vineyards.

Currently there is no screening method available to identify infected planting material. Anecdotal evidence has suggested that vineyards planted with material that had been hot water treated at 50°C for 30 mins do not suffer losses from Petri disease. To test this proposition, cuttings were harvested from infected Zinfandel and Ramsey mother vines. Half were hot water treated and the rest left untreated. All were then planted in newly-established nursery beds. After 6 months, 100 Zinfandel plants were harvested from each treatment. The stems were cut open and any brown wood streaking symptoms were noted. The stems were then moist incubated and examined after 6–8 weeks for *P. chlamydospora*. After 12 and 18 months, 100 Ramsey plants were harvested from each treatment and assessed in the same manner. At 6 months, *P. chlamydospora* was detected in 13% of Zinfandel control plants but in none of the hot water treated plants. For Ramsey, *P. chlamydospora* was detected in 26% of the control plants harvested at 12 months as compared with 3% of hot water treated plants, and in 12% of the control plants harvested at 18 months as compared with 1% of hot water treated plants. These results suggest that routine hot water treatment of dormant cuttings may be an effective method of reducing the incidence of infected young vines and consequently reducing vineyard losses due to Petri disease.