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ABSTRACTS

Pathogen identification and detection

***Phaeoacremonium* and *Phaeoacremonium*-like fungi in the Calosphaeriales.** P.W. Crous, W. Gams and L. Mostert. *Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands*.
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Although it is now commonly accepted that the genus *Phaeoacremonium* has teleomorphs in *Togninia*, hardly anything is known about other fungi within the *Calosphaeriales*. During a survey of perithecial ascomycetes in New Zealand, two collections of a *Togninia*-like fungus were made on decayed wood. In culture, colonies produced a *Phaeoacremonium*-like anamorph. In order to reveal the phylogenetic relationships of the unknown fungus and its affinity to *Togninia* and other genera in the *Calosphaeriales*, sequences of nuclear LSU and SSU ribosomal DNA were obtained of several members of this order. These data, supported by morphological and cultural characteristics, confirm that the New Zealand fungus represents a new genus very close to *Calosphaeria*. The genus *Togniniella* is proposed to accommodate these collections, while *Phaeocrella* is established for their anamorphs. Furthermore, *Calosphaeria pulchella* was found to form a distinct *Acremonium*-like anamorph in culture, for which the genus *Calosphaeriophora* is proposed. Similarily the genus *Pleurostomophora* is proposed to accommodate anamorphs of *Pleurostoma*, which are accommodated in a new family, *Pleurostomataceae*. *Togninia* with its *Phaeoacremonium* anamorphs, together with *Jobellisia*, are closer to the *Diaporthales*, and represent yet another distinct family, for which the *Togniniaceae* is proposed. The presence of significantly different anamorphs in the *Calosphaeriales*, as well as obvious differences in teleomorph morphology of species accommodated in *Calosphaeria*, suggest that both the *Calosphaeriales* and *Calosphaeria,* as presently perceived, are polyphyletic.

Delimitation of new species in *Phaeoacremonium* and the development of an identification system. L. Mostert, J.Z. Groenewald, W. Gams, R.C. Summerbell, V. Robert and P.W. Crous. *Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands*.
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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*. 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*. Fourteen new species of *Phaeoacremonium* have been identified on the basis of cultural and morphological characters as well as DNA phylogeny of the partial ß-tubulin and actin genes. Of these new species the following have been isolated from *Vitis vinifera*: *Pm. alvesii*, *Pm. australiense*, *Pm. krajdenii*, *Pm. scolyti*, *Pm. subulatum*, *Pm. venezuelense* and a further two unnamed species. New species isolated from hosts other than grapevines include: *Pm. tardicrescens* (human), *Pm. griseorubrum* (human), *Pm. amstelodamense* (human) and another three, yet unnamed species (soil, *Theobroma grandiflorum*, *Cupressus macrocarpa*, *Pinus radiata* and *Desmoschoenus spiralis*). *Phaeoacremonium* species that were isolated from grapevines as well as human infections are *Pm. alvesii*, *Pm. krajdenii*, *Pm. parasiticum* and *Pm. venezuelense*. Re-examination of isolates obtained as *Pm. inflatipes* showed that those isolated from grapevines and humans had originally been misidentified and belong to either *Pm. aleophilum* or the new taxa described here. The status of *Pm. angustius* has been clarified with the designation of an epitype from the original type locality. Isolates representing *Pm. angustius* proved to be phylogenetically distinct from their sister species, *Pm. viticola*. Furthermore, a multiple-entry online electronic key based on morphological, cultural and ß-tubulin sequence data was developed to facilitate routine identification of species.

Occurrence of *Togninia fraxinopennsylvanica* perithecia and *Phaeoacremonium* species in California vineyards. A. Eskalen, S. Rooney-Latham and W.D. Gubler. *Department of Plant Pathology, University of California, Davis, CA 95616, USA*.
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Esca and Petri disease are two of the most destructive diseases of grapevines in California and many

other grape production countries. Esca has been a problem in California for over 70 years and is suspected of being caused by multiple species of *Phaeoacremonium* and *Phaeomoniella chlamydospora*. In our study, we isolated *Togninia fraxinopennsylvanica* multiple times from symptomatic grapevines. Furthermore, spore trapping studies suggest that spores of this fungus are aerially present in infected vineyards, and perithecia of *T. fraxinopennsylvanica* were identified in these vineyards. Molecular analyses of the ITS region of the nuclear ribosomal DNA confirmed that these perithecia were *T. fraxinopennsylvanica*. This work presents the first report of *T. fraxinopennsylvanica* on grapevines. Additionally, *Pa. rubrigenum*, *Pa. parasiticum,* and *Pa. angustius* were also isolated from symptomatic grapevines. Pathogenicity tests have shown that many of these fungi effectively colonise pruning wounds resulting in vascular discoloration. The probability of these fungi being non-injurious endophytes also exists.

Real-Time PCR-SYBR® Green detection of grapevine decline pathogens. B.E. Overton, E.L. Stewart, Xinshun Qu, N.G. Wenner, B.J. Christ and F.E. Gildow. *Department of Plant Pathology, Pennsylvania State University, University Park, PA 16802, USA. E-mail:* els4@psu.edu

Petri disease of grape and *Tomato ring spot virus* (TomRSV) have been shown to be associated with vine decline in commercial vineyards. Petri disease is caused by *Phaeomoniella chlamydospora* and species in the genus *Phaeoacremonium*. The primer pairs, Pmo1f + Pmo2r and Pac1f + Pac2r, were designed for genus-specific amplification of *Phaeomoniella chlamydospora* (Pch) and *Phaeoacremonium* spp. respectively, using real-time PCR. The primers were specific for each target genus and showed no primer dimers in the first 35 cycles. Pch was detected in roots, shoots, and young trunks of drill-inoculated vines. *Phaeoacremonium aleophilum* (Pal) was detected in trunk cross sections of naturally infected vines from which Pal had been isolated. TomRSV is a polyhedral nematode-transmissible virus (nepovirus) that causes various degrees of systemic necrosis and stunting of growth in many hosts. The primer pair Trv2f + Trv2r was designed based on sequences in Genbank from several hosts, including grape, for the one-step, reverse transcription (RT) and real-time PCR amplification of TomRSV. Cuttings were taken from grapevines naturally infected with TomRSV and grown under greenhouse conditions. After 6 months, cuttings were tested using ELISA and real-time RT-PCR. Samples that tested positive based on ELISA were also positive based on real time RT-PCR. The primers were specific and showed no primer dimers. Real-time PCR affords nursery owners and growers a non-destructive method for detecting the presence of pathogens involved in grapevine decline. The methods presented here could be utilized as detection systems in a clean vine certification program.

White rot symptoms in esca affected grapevine: further insights into the biodiversity, host range and molecular diagnosis of associated basidiomycetes. M. Fischer[1], F. Mela[2], L. Mugnai[2], F. Halleen[3], J. Edwards[4] and I. Pascoe[4]. *[1]Staatliches Weinbauinstitut Freiburg, Merzhauser Str. 119, D-79100 Freiburg. [2]Dipartimento di Biotecnologie Agrarie-Patologia vegetale, P.le delle Cascine 28, I-50144 Firenze. [3]Disease Management Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa. [4]Institute for Horticultural Development - Knoxfield, Department of Primary Industries, Private Bag 15, Ferntree Gully, Victoria 3156, Australia.*
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While the spectrum of fungal organisms associated with Petri disease seems to be pretty much uniform throughout wine-growing regions, available evidence suggests that the situation is more complex for esca related white rot basidiomycetes. For instance, *Fomitiporia mediterranea* (*Fmed*) is associated with esca disease of grapevine mainly in Europe. The geographic distribution of *Fmed* is distinct, covering different climatic zones in Europe and Asia; it is suggested to be closely linked with viticulture, and is likely to have spread in recent years. Besides *Vitis*, host plants include a variety of other hardwood genera. During the last century, increasing amounts of grapevine have been cultivated in non-European countries. In recent years, plantations throughout the world have been found to suffer from white rot symptoms, caused by so far unknown basidiomycetes. In general, fruiting structures of esca-related basidiomycetes are hard to find, or they may mostly occur on non-*Vitis* hosts, which are not well investigated. The

existence of these fungi can be demonstrated only by the vegetative mycelia that may be isolated from infected wood. In this study, molecular sequences (nuclear encoded ribosomal ITS region) were generated for basidiomycete isolates from esca affected grapevine in Europe, Australia and South Africa. In a phylogenetic approach the sequences obtained were compared with selected representatives of the Hymenochaetales. With these data, several taxa not easily assignable to already described species were detected. In addition, taxon-specific primers, prFmed1 and prFmed2, were developed. In a first-hand assay, these may help to resolve the question to which extent *Fmed* and/or closely related taxa are responible for the problem of esca-related white rot in grapevine.

Identification of basidiomycete species associated with wood decay symptoms of grapevine chlorotic leaf roll in Chile. J. Auger, N. Aguilera and M. Esterio. *Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004, Santiago, Chile.*
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The grapevine disease named chlorotic leaf roll in Chile is comparable to esca disease in Europe, despite some differences in the foliar symptoms and in the basidiomycete fungi associated with the wood decay symptoms. The *Phellinus* sp. associated with chlorotic leaf roll symptoms might indicate that this basidiomycete is involved in the disease. Correct identification of the basidiomycete may also be relevant in determining possible sources of inoculum in vineyards. Identification was carried out on the basis of morphological characteristics of the fruiting bodies, such as size and form of the pores, basidia and basidiospores, rate of growth and mycelial biochemical reactions, and sequence of the large subunit of ribosomal DNA, which was extracted from fruit bodies. From the morphological characteristics and phylogenetic analysis of the section sequenced, it was concluded that the hymenomycete fungi associated with symptoms of chlorotic leaf roll belong to the genus *Fomitiporella*. They differed from the species *Fomitiporella cavicola* (Kotl. & Pouz) T. Wagner & M. Fisher comb. nov. and *F. caryophylli* (Racib) T. Wagner & M. Fisher comb. nov. described for this genus. Genetic variability of the isolates was determined by means of the RAPD technique and revealed a phylogenetic association among isolates from the localities evaluated. This study demonstrated morphological and molecular differences between the basidiomycete species associated with grapevine trunk diseases, *Phellinus igniarius* and *Fomitiporia punctata*, and the Hymenomycete fungus associated with chlorotic leaf roll symptoms, which was named *Fomitiporella vitis* Auger, Aguilera & Esterio, sp. nov.

Molecular characterisation and identification of *Eutypa* spp. from grapevines in South Africa. S. Safodien[1], F. Halleen[1], P.W. Crous[2], W.A. Smit[1] and A. Botha[3]. *[1]ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa. [2]Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. [3]Department of Microbiology, University of Stellenbosch, Private Bag X1, Matieland (Stellenbosch) 7602, South Africa.*
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Eutypa dieback of grapevines holds enormous economic implications for the vine and wine industry of South Africa. The pathogen responsible for the disease, *Eutypa lata*, is an ascomycetous fungus that has a wide host range and has become a significant problem in grape growing areas worldwide. Since infected grapevines gradually decline and eventually die, infection with this pathogen is causing significant reductions in yield. Consequently, an investigation of local *Eutypa* populations occurring on grapevine was undertaken. Isolates were collected from grapevines and fruit trees with dieback symptoms, as well as from fruiting structures on infected wood. *Eutypa* isolates were also obtained from Australia and France. The isolates were analysed using sequence data from the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA, the ß-tubulin gene region and the large subunit to infer the phylogenetic relationship of local *Eutypa* populations. Analysis of the ITS sequence data revealed the presence of two groups: Group I designated for isolates that produced an ~600bp fragment, and Group II designated for those that produced an ~560bp fragment. The results grouped most of the isolates from South Africa and those obtained from Australia and France (Group I) in a well-supported clade with a bootstrap support value of 81% with *E. lata*, except for STE-U 5581, which was closely related to *E. leptoplaca* (bootstrap support value, 100%). The grape-

vine isolates STE-U 5561 and 5562 and the fruit tree isolates (STE-U 5550–5559) formed a separate cluster (Group II) showing sequence similarity to *Eutypella vitis*. The molecular data derived from the amplification of the three markers helped to elucidate the phylogenetic relationship of local *Eutypa* populations, and in particular revealed the presence of a second species, *E. leptoplaca,* which could be capable of causing disease.

Diagnosis of *Eutypa* infection by metabolite analysis. R.J. Molyneux[1], N. Mahoney[1], L.R. Smith[1], P.E. Rolshausen[2], R. Lardner[3] and E. Scott[3]. *[1]Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, CA 94710, USA. [2]Department of Plant Pathology, University of California, One Shields Avenue, Davis, CA 95616, USA. [3]Cooperative Research Centre for Viticulture, PO Box 154 and School of Agriculture and Wine, University of Adelaide, PMB 1, Glen Osmond, SA 5064, USA.*
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Dying arm disease in grapevines is responsible for vineyard production losses around the world, including California, and is believed to be due to acetylenic phenol fungal metabolites produced by *Eutypa lata*. In order to identify specific metabolites that could potentially be used for diagnosis of infection, eight *Eutypa* strains isolated from grapevines were grown *in vitro* on hot water extracts, fortified with sucrose, of the susceptible grape varieties Cabernet Sauvignon, Sauvignon Blanc and Syrah, and the tolerant varieties Merlot and Semillon. Analysis by HPLC showed that eutypinol was consistently produced in large amounts, together with smaller amounts of methyleutypinol and eulatachromene. Eutypine, the putative toxin, was rarely produced, and when present, amounts were low. Since differences did not correlate with susceptible and tolerant grape varieties, the time-course and profiles of metabolites produced by *E. lata* strains isolated from Cabernet Sauvignon and Merlot grown on identical media were examined to determine the effect of fungal source. Although the amounts of metabolites produced differed significantly between strains, the pattern of metabolites was quite similar, with eutypinol again predominating. The consistent production of eutypinol indicated that this was the most suitable metabolite as a diagnostic marker of *E. lata*. Analysis of leaves, fruits, stems and cordons of grapevines exhibiting symptoms of dieback failed to show the presence of metabolites. However, when cordon sections were placed in water and cultured for 5 days, eutypinol was readily detected in the aqueous solution; metabolites were not produced from uninfected tissue. This provides a method for detection of infected tissue and indicates that the toxic metabolites react at the point of production, disrupting the vascular structure, rather than being translocated to symptomatic tissues.

Further studies into *Botryosphaeria* as pathogens of grapevine in California. J.R. Urbez[1], G.M. Leavitt[2] and W.D. Gubler[1]. *[1]Department of Plant Pathology, U.C. Davis, Davis, CA 95616, USA. [2]Farm Advisor, UCCE, 328 Madera Ave. Madera, CA 93637, USA.*
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Botryosphaeria Ces. & De Not (1863) constitutes a complex fungal genus with many taxonomic and nomenclature problems. Different species of *Botryosphaeria* occur on grapevines causing a wide variety of diseases and symptoms in various geographic regions worldwide. In this study, in order to determine the occurrence of *Botryosphaeria* spp. in the main grape-producing areas in California, one hundred vineyards of different ages among the predominant table and wine grape varieties from eleven different counties (Mendocino, Sonoma, Napa, San Joaquin, Stanislaus, Madera, Fresno, Monterey, Tulare, Kern and Riverside) were sampled throughout the state. *Botryosphaeria* was found associated with the internal wedge-shaped canker at a different percentage in every grape-growing area surveyed in California. Preliminary results, based on morphological colony characters and phylogenetic analysis of the internal transcribed spacer (ITS) of the rDNA and a partial sequence of the ß-tubulin gene, showed that at least four different species of *Botryosphaeria*: *B. obtusa*, *B. rhodina*, *B. dothidea* and the unidentified *Botryosphaeria* sp1, occur in grapevines in California. *B. obtusa* was the most common species isolated in California. Only *B. rhodina* was found in vineyards sampled in southern California (Riverside County). This study represents the first report describing the population and distribution of *Botryosphaeria* species on Californian grapevines.

Variability of *Cylindrocarpon* spp. associated with black foot disease of grapevine. C. Rego[1], T. Nascimento[1], A. Cabral[2], P. Talhinhas[2], A. Phillips[3] and H. Oliveira[2]. [1]*Laboratório de Patologia Vegetal "Veríssimo de Almeida", Tapada da Ajuda, 1349-017 Lisboa, Portugal.* [2]*Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal.* [3]*Universidade Nova de Lisboa, Monte da Caparica, 2829-516 Caparica, Portugal.*
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Black-foot disease caused by *Cylindrocarpon* species is a serious problem of grapevine in many countries. Seventy *Cylindrocarpon* isolates, obtained from young grapevines with black foot symptoms and from rootstocks showing decline symptoms in the nursery, were analysed for morphological, cultural, pathogenicity characteristics and random amplified polymorphic DNA (RAPD). Culture morphology on PDA, especially colony colour, was highly variable among the isolates studied. However, no meaningful pattern of variation was detected. Analysis of morphological structures in SNAY and CLA media revealed that the majority of isolates formed abundant round slimy conidial heads and abundant chlamydospores. In contrast, a small group of isolates formed longer and wider conidia and produced few chlamydospores. Of the isolates, only eight grew at 34°C and the optimum temperature for growth was 24°C. Pathogenicity experiments on rooted cuttings of grapevine cv. Periquita under greenhouse conditions revealed that all isolates induced black foot symptoms and the majority significantly (P=0.05) decreased plant height, number of internodes, number of roots and root elongation. The percentage of pathogen re-isolations varied from 50 to 96%. RAPD profiles clearly distinguished two main groups, and a third represented by a single isolate. These main RAPD groups were not clearly correlated with the morphological characteristics and pathogenicity tests. However, they indicated the existence of a high degree of genetic variability (similarity coefficient 0.29) within the *Cylindrocarpon*-complex on grapevines. It remains to be clarified if this variability is intra- or inter-specific.

***Fomitiporia australiensis*, a new species of *Fomitiporia* from Australian grapevines found associated with heart rot of grapevines.** M. Fischer[1], J. Edwards[2,3], J.H. Cunnington[3] and I.G. Pascoe[2,3]. [1]*Weinbauinstitut Freiburg, Merzhauser Str. 119, D-79100 Freiburg, Germany.* [2]*Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond, South Australia 5064, Australia.* [3]*Primary Industries Research Victoria – Knoxfield Centre, Department of Primary Industries, Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia.*
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Phylogenetic species recognition allows identification of a new basidiomycetous species, *Fomitiporia australiensis*, associated with white heart rot of esca-affected grapevines in Australia. Microscopic characters of fruit bodies are very similar to those of the closely related species, *F. punctata* and *F. mediterranea*. *Fomitiporia australiensis* is distinct in forming both resupinate and pileate fruit bodies and in the sequences of its ribosomal ITS region. Fruit bodies of this species are rarely found in the field; it occurs mostly as vegetative mycelium. No definite statements are possible regarding the exact geographic distribution or host range of the species.

Characterisation and identification of the basidiomycetous fungus associated to 'hoja de malvón' grapevine disease in Argentina. L. Bettucci[1], S. Lupo[1], A. Pérez[1], S. Martínez[1], C. Césari[2], G. Escoriaza[2] and M. Gatica[2]. [1]*Facultad de Ciencias / Ingeniería, Laboratorio de Micología, Universidad de la República, J. Herrera y Reissig 565, 11300, Montevideo, Uruguay.* [2]*INTA, EEA Mendoza, San Martín 3853, Mayor Drummond, 5507, Luján de Cuyo, Mendoza, Argentina.*
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The pathogenic fungus *Inocutis jamaicensis* (Murrill) Gottlieb, J.E. Wright & Moncalvo was identified as the basidiomycetous species associated with hoja de malvón grapevine disease. Macro and micromorphological characteristics of fruiting bodies collected from grapevine trunks corresponded to those of this fungus. Cultural characters of the isolates analysed also corresponded to isolates from Uruguayan specimens of this species from *Eucalyptus globulus* and other native plant species. In order to compare the strains, polymerase chain reaction (PCR) of the internal transcribed spacer (ITS) regions was performed. RFLPs from this region generated by restriction digestion with *Alu* I, *Hae* III, *Taq* I, *Msp* I and *Hha* I were compared

with RFLPs of the product amplified by the same primers from DNA obtained from cultures of *I. jamaicensis* from decayed wood of *E. globulus*. The profile of some enzymes matched those obtained from *I. jamaicensis* associated with *E. globulus*. Moreover, an amplification product generated with specific primers (INO1 and INO7) was observed for the isolates of *I. jamaicensis* used in this study. The PCR products revealed a fragment of approximately 333 bp, which was expected. In addition, the aligned partial sequence of LSU rDNA showed a high percentage of identity. Differences found in some restriction patterns could reflect a certain degree of variability between strains, probably related to a particular lifestyle, host specificity or geographic origin. A somatic incompatibility test and other methods used to identify genotypes are necessary to clarify these differences.

Fungi isolated from failing graft unions with necrotic lesions. L. Morton[1], N.G. Wenner[2], E.L. Stewart[2] and B.E. Overton[2]. *[1]Box 208, Broad Run, VA 20137, USA. [2]Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA.*
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Declining vines in three Virginia vineyards exhibited fissured and failing graft unions with internal necrotic lesions. External symptoms included marginal leaf chlorosis, total leaf reddening, lack of fruit, severe cordon dieback, trunk mortality, and vine death. Graft union deformation was visible accompanied by abnormal regenerative callus growth beneath the bark resembling crown gall. Symptomatic vines were randomly scattered in planting blocks of Merlot, Cabernet Sauvignon, and Cabernet Franc on SO4, 5 BB, 3309, and Riparia Gloire rootstocks. Vines originated from four different nursery sources and were 3, 4, 5, and 20 years old. Single and double-trunked vines in this study grew to full size (on lyre or VSP, at 2.1 m and 1.2 m between vines respectively). A 6-month study, from October 2003-March 2004, investigated the fungi associated with these failing vines. The vineyards sampled were in three different geographically- and geologically-distinct regions at elevations of 10 m, 150 m, and 300 m. Vine samples were cross-sectioned into disks on a band saw, and surface sterilized using a brief ethanol dip and flaming. Discolored wood was aseptically excised and plated onto 2% AMA. Isolates were identified to genus using morphological and molecular means. The most common fungi isolated from the declining vines were *Botryosphaeria* spp. 7/9, *Clonostachys* 5/9, *Fusarium* spp. 6/9, *Phaeoacremonium* spp. 4/9, *Phaeomoniella* 5/9, and Phialophora-like isolates. The necrotic wood and bark was often infected with *Clonostachys*. Peeling back bark tissue showed necrotic phloem, new callus growth and necrotic lesions bordered by a blackish film similar to symptoms associated with "Diplodia dieback." The climate in Virginia includes summers with high humidity, summer rainfall, and heavy morning dew. This study suggests that graft unions infected with *Botryosphaeria* and other fungi represent a latent threat to vine health. In areas with cold winters, the external symptoms may be confused with cold injury-induced crown gall disease.

Fungi associated with grapevine wood decay and young vine decline in Chile. J. Auger[1], I. Pérez[1], M. Esterio[1], V. Navia[2], W.D. Gubler[3] and A. Eskalen[3]. *[1]Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004, Santiago, Chile. [2]Bayer CropScience, Santiago, Chile. [3]Department of Plant Pathology, University of California, Davis, CA, 95616, USA.*
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The incidence of grapevine wood decay and young vine decline in Chile has increased continously over the last few years. Although Chile is one of the world's largest table grape exporters and the wine grape growing area has been increasing in the last few years, no systematic analysis has yet been carried out to evaluate the presence and incidence of fungal pathogens associated with trunk diseases of grapevine. In response to grower concern, a survey of vineyards was initiated to determine the status and causes of grapevine decline in the country. A significant number of samples of rooted cuttings of different varieties grafted on different rootstocks, ready for out-planting, or planted and declined a few months or years later, were collected or sent to our laboratory to investigate the presence of phytopatogenic fungi. Two- to nine-year-old and older vines were also examined. Additionally, plant and soil samples were taken to ascertain the presence of fungi and levels of grapevine viruses, nematodes, and soil fer-

tility. Cross sections of declining vines revealed brown wood-streaking, wedge-shaped discolouration and pockets of white rot fungi. Tissue explants from symptomatic plants were transferred to acidified PDA or Malt agar. *Phaeomoniella chlamydospora* (Pc) and *Phaeoacremonium* spp., which included *P. aleophilum* (Pa), *P. rubrigenum* (Pr) and *P. parasiticum* (Pp), were isolated and identified. These pathogens were associated with young vine decline and with brown wood streaking in Pinot Noir, Cabernet Sauvignon, Ruby Seedless, Thompson Seedless, Flame Seedless, Autumn Royal, Red Globe and the rootstocks Kober 5BB, C3309 and Harmony from 27 locations in the V, VI, VII and Metropolitan regions of Chile. Pc occurred more frequently than Pa. *Botryosphaeria obtusa* was isolated and identified. It was always associated with wedge-shaped wood discolouration or dead-arm symptoms, and mainly from the table grape cultivars Red Globe, Ruby Seedless and Thompson Seedless. A basidiomycete identified as *Fomitiporella vitis* sp. nov. was always isolated from wood with white soft rot and chlorotic leaf roll symptoms.

Occurrence of fungi associated with Petri disease in bench-grafted vines. A. Aroca and R. Raposo. *INIA, Ctra. Coruña km. 7.5, 28040 Madrid, Spain.*
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Bench-grafted vines were sampled at the time of their delivery from Spanish grapevine nurseries to growers (March 2004) to evaluate the presence of fungi associated with Petri disease and wood decay. A total of 34 samples (135 plants) were collected. Each sample consisted of 3–5 plants taken at random from a batch of bench-grafted vines. Internal examination was done by cutting the rootstocks transversely at different intervals (roots, internodes and graft union). Six wood sections 1–2-mm thick were obtained at each interval to carry out fungal isolations. All plants showed wood browning or blackening around the pith at the basal end of the rootstock, decreasing towards the apex. The fungi most frequently isolated from the rootstocks were: *Botryosphaeria* spp. (isolated from 46% of plants), *Phaeomoniella chlamydospora* (39%), *Phaeoacremonium* spp. (26%), *Phomopsis* spp. (17%), and *Cylindrocarpon* spp. (16%). Sixty-nine plants out of 135 (51%) were infected with at least one of these fungi. With respect to the distribution of pathogens within the plants, *Phomopsis* and *Botryosphaeria* were found mainly in the upper part of the rootstock, while *Phaeomoniella chlamydospora* was isolated from the lower half. Surprisingly, *Cylindrocarpon* spp. were isolated from the graft union in 15% of pathogen isolations. None of the rootstocks sampled (110R, 140Ru, 161-49C, 41B and SO4) were free from pathogens.

Molecular phylogenetics of grapevine decline fungi from Pennsylvania and New York. B.E. Overton, E.L. Stewart, and N.G. Wenner. *Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA.*
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Twenty-seven vineyard sites comprising more than 200 ha of vines were examined over three growing seasons (2001–03) to quantify the extent of vine decline and to isolate fungi from declining vineyards. Three hundred and sixty-two fungal isolates were recovered from vines exhibiting decline symptoms in Pennsylvania (PA) and New York (NY). Twenty-nine percent of the fungi isolated were those associated with Petri disease. *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum*, *Phaeoacremonium mortoniae*, *Phaeoacremonium rubrigenum* and *Phaeoacremonium angustius / viticola* were identified by morphological and molecular characters. *Phaeoacremonium inflatipes* and *P. paristicum* were not isolated from declining vines in PA or NY. Twenty-four percent of the fungi isolated were Phialophora-like, referable to the genera *Cadophora*, *Harpophora*, and *Phialophora* s.s. Twenty-four percent were pycnidial fungi, with 8% identified to the genus *Phomopsis*. To date, the ITS rDNA gene region has been sequenced for 96 of the 362 isolates. Data obtained from the ITS rDNA region will be used to determine target strains for further phylogenetic study, including phylogenetic analyses based on other gene regions. *Cadophora* and *Harpophora* species were isolated with high frequency from declining vines and consequently will be included in the overall sequencing project. The end result will be an established culture collection that can be used for ecological and plant pathological studies; and the generation of sequence data can be used for regional phylogenetic analyses, for identifica-

tion of potentially new species, and for developing pathogen detection systems (real-time PCR).

New teleomorph findings for species in the genus *Phaeoacremonium*. L. Mostert, W. Gams and P.W. Crous. *Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.*
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Several species of *Phaeoacremonium* (*Pm.*) are associated with Petri disease symptoms, namely *Pm. aleophilum*, *Pm. angustius, Pm. mortoniae, Pm. parasiticum*, *Pm. rubrigenum* and *Pm. viticola*. Teleomorphs of *Phaeoacremonium* have been established in the genus *Togninia* (*T.*). Three teleomorphs have thus far been identified in the genus, namely *T. minima* (*Pm. aleophilum*), *T. novae-zealandiae* and *T. fraxinopennsylvanica*. To investigate possible teleomorph associations, isolates of various *Phaeoacremonium* spp. were mated *in vitro* on sterile grapevine canes. Perithecia were observed for *Pm. parasiticum*, *Pm. viticola*, *Pm. krajdenii*, *Pm. rubrigenum* and two as yet unnamed species of *Phaeoacremonium*. Of these species *Pm. parasiticum*, *Pm. viticola*, *Pm. krajdenii* and one unnamed species have been isolated from grapevines. The dominant mating system was heterothallic, though one taxon was homothallic. DNA sequence data from the partial ß-tubulin and actin genes confirms the link of *T. fraxinopennsylvanica* with *Pm. mortoniae*, a species originally described from grapevines in California.

Characterisation of *Phomopsis* spp. infecting grapes in the midwestern and northeastern United States. O. Erincik[1], L. Castlebury[2], A.M.C. Schilder[3], A. Rossman[2], and M.A. Ellis[1]. *[1]Dept. Plant Pathology, Ohio State University, 1680 Madison Ave, Wooster, OH 44691, USA. [2]Systemic Botany and Mycology, USDA-ARS, 10300 Baltimore Ave, Beltsville, MD 20705, USA. [3]Dept. Plant Pathology, 104 CIPS Building, Michigan State University, East Lansing, MI 48824, USA.*
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Phomopsis cane and leaf spot is an economically important disease of grapes (*Vitis* spp.) in the eastern US. The objective of this study was to determine which *Phomopsis* spp. are associated with the disease in this region. Eighty isolates of *Phomopsis* were collected from grapevines in Ohio, Michigan, New York, and Pennsylvania. Isolates were also obtained from California, Canada, France, and Italy. The isolates were grouped on the basis of DNA sequences from intron regions in the translation elongation factor 1-A and calmodulin genes. According to DNA sequence comparisons with the type isolate, almost all isolates were found to be *P. viticola*. One isolate resembled *Diaporthe phaseolorum* while another isolate was *Phomopsis eucommicola,* a tentative species. Both of these isolates had significantly higher mycelial growth rates and shorter conidia than the *P. viticola* isolates. Neither of these species has been previously reported on *Vitis,* and they were only slightly or not pathogenic to *Vitis*. Thirty representative isolates were evaluated for morphological characters and for pathogenicity on grapevine (*Vitis* interspecific hybrid 'Seyval') leaves and canes, and 13 of these isolates were also evaluated for pathogenicity on fruit and rachises. All isolates of *P. viticola* caused disease on grape. However, they differed in virulence on the different grape tissues, suggesting some degree of specialisation with respect to host tissue. Virulence on the fruits and rachises, but not on the leaves and canes, was positively correlated with the rate of mycelial growth of the *P. viticola* isolates.

***Phomopsis* spp. on grapevines: characterisation and pathogenicity.** J.M. van Niekerk[1], J.Z. Groenewald[2], D.F. Farr[3], P.H. Fourie[1], F. Halleen[4] and P.W. Crous[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, The Netherlands. [3]USDA-ARS, Systematic Botany and Mycology Lab., Rm 304, Bldg 011A, Beltsville, MD 20705, USA. [4]ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa.*
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The fungi in the genus *Phomopsis,* with teleomorphs in *Diaporthe,* are pathogens of a wide variety of crops. Due to wide host ranges of species, morphological overlap between different species and morphological plasticity, within species, morphological and cultural characteristics must be combined with DNA sequence data to distinguish taxa. Ten species have previously been reported from grapevines, of which *P. viticola* (the causal

organism of Phomopsis cane and leaf spot) and *P. vitimegaspora* (causal organism of swelling arm of grapevine) have been confirmed as pathogens. However, several unidentified *Phomopsis* species were isolated from diseased grapevines, and from symptoms atypical of the known *Phomopsis* diseases on this crop. This prompted the need to reassess *Phomopsis* species occurring on grapevines. Sixty-one isolates obtained from grapevines were subjected to DNA analysis. A phylogenetic analysis of sequence data obtained from the rRNA operon's internal transcribed spacer regions (ITS-1, ITS-2) and the 5.8S rRNA gene, combined with morphological and cultural studies, differentiated at least fifteen *Phomopsis* species, of which 12 occurred on grapevines in South Africa. Pathogenicity tests indicated that of the 12 species from South Africa, *P. viticola* and *P. amygdali* caused the most severe lesions on green grapevine shoots. *P. amygdali* is frequently isolated from grapevines, but *P. amygdali,* a known peach pathogen in the USA, has been isolated only twice from grapevines in South Africa.

Characterisation and pathogenicity of *Botryosphaeria* species occurring on grapevines. J.M. van Niekerk[1], P.W. Crous[2], J.Z. Groenewald[2], P.H. Fourie[1] and F. Halleen[3]. *[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa. [2]Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. [3]ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa.*
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Several *Botryosphaeria* spp. are isolated from grapevines, frequently from symptoms traditionally associated with other diseases such as cane and leaf spot (caused by *Phomopsis viticola*) and Eutypa dieback (caused by *Eutypa lata*), making field diagnosis very difficult. Nevertheless, uncertainty still remains as to the species identity and pathogenicity of the various *Botryosphaeria* species. The aim of this study was to characterise *Botryosphaeria* species from grapevines with regard to species identity and pathogenicity. A total of 112 *Botryosphaeria* isolates from diseased grapevines, pruning debris and symptomless nursery plants were studied. Based on morphology and DNA sequences (ITS 1, 5.8S, ITS 2 and EF1-α) 11 *Botryosphaeria* species were distinguished of which 8 species occurred in South Africa. Several of these species were new host records, while *Diplodia porosum, Fusicoccum viticlavatum* and *F. vitifusiforme* are described as new. From *in vitro* and *in vivo* pathogenicity trials on grapevine shoots it was concluded that *B. australis, B. parva, B. ribis* and *B. stevensii* were more virulent than the other species studied.

Identification and characterisation of *Botryosphaeria* fungi in New South Wales vineyards. S. Savocchia[1], E.N. Laurent[1], B.J. Stodart[2,3], A. Somers[2,4], C.C. Steel[1] and M. Priest[2,5]. *[1]National Wine and Grape Industry Centre, School of Wine and Food Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia. [22]New South Wales Department of Primary Industries, Tocal Agricultural College, Paterson, NSW, 2421, Australia. [3]Wagga Wagga Agricultural Institute, PMB Pine Gully Road, Wagga Wagga, NSW 2650, Australia. [4]Tocal Agricultural College, Paterson, NSW 2421, Australia. [5]Orange Agricultural Institute, Forest Road, Orange, NSW 2800, Australia.*
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Botryosphaeria fungi were isolated from declining grapevines in the Hunter Valley and Mudgee grape-growing regions of New South Wales, Australia, during a survey of 4- to 122-year-old grapevines across 21 vineyards. Grapevines infected with *Botryosphaeria* produced symptoms in the wood similar to those caused by *Eutypa lata*. Loss of spur positions, dead arms, cankers and wedge-shaped lesions in the wood were observed in infected vines. Field observations suggest that Chardonnay is most susceptible to infection by *Botryosphaeria* fungi. We are currently in the process of identifying which species are present in these vineyards, and whether they are responsible for the symptoms observed. To date, two species, *B. obtusa* and *B. lutea*, have been identified amongst the 30 strains examined. Pathogenicity studies revealed that both these species can infect wounded and non-wounded grape berries and one-year-old canes *in vitro*. Symptoms on infected grape berries include the presence of black pycnidia, splitting and collapse of the berry skin. Lesions were observed in infected one-year-old canes of Chardonnay and Shiraz. Studies are also in progress to determine the ability of *Botryosophaeria* fungi to infect glasshouse-grown grapevines (cv. Chardonnay).

Black dead arm and basal canker of *Vitis vinifera* cv. Red Globe caused by *Botryosphaeria obtusa* in Chile. J. Auger, M. Esterio, G. Ricke and I. Pérez. *Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile. Casilla 1004, Santiago, Chile.*
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Vitis vinifera cv. Red Globe vines, over 6 years old, started to show disease symptoms about ten weeks after bud break. Symptoms first appeared on the leaves at the base of the shoots and then spread to the other leaves, continuing to appear throughout the growing season. Two forms of the disease were observed. The mild form led to premature leaf drop, while the severe form was characterised by dieback of one or more shoots, accompanied by leaf drop, and the shrivelling and drying of fruit clusters. The mild form was characterised by wine-red spots on the leaf margins or on the leaf blade, which coalesced to form large zones of necrosis between the veins and the margins of the leaf. Fruit clusters may wither. If the bark was scraped off, a brown streak 1–2 cm wide was often seen in the wood. The streaking began at the base of the affected shoot and extended to the ground level, eventually resulting in a basal canker. *Botryosphaeria obtusa* (Schwein.) Shoemaker (anamorph = *Sphaeropsis malorum* Berk.) was isolated from 86% of samples from 6- to 10-year-old vines from 12 locations in the IV, V, VI and Metropolitan regions of Chile. Isolations were made from the brown streaked wood. Isolates were identified based on previous description and by internal transcribed spacer (ITS1-5.8-ITS2) rDNA sequences identical to those of *B. obtusa*. Pathogenicity tests were done by inoculating 20 µl of mycelial suspension via injection into the pith of 16 single-node, rooted cuttings of *V. vinifera* cv. Red Globe. Sixteen control cuttings were injected with an equal volume of sterile distilled water. Twenty weeks after inoculation, all *B. obtusa*-inoculated cuttings exhibited brown streaks in the wood extending 50 to 60 mm from the point of inoculation. The wood streaking observed in inoculated plants was identical to symptoms in naturally infected black dead arm vines in the vineyard. No symptoms were observed in the controls. *B. obtusa* was reisolated from the region of brown streaking in all the inoculated cuttings. *B. obtusa* was not isolated from the water-treated controls. This is the first report of *B. obtusa* causing black dead arm and basal canker on Red Globe grapevines in Chile.

***Cryptovalsa ampelina* on grapevine: identification and pathogenicity.** J. Luque[1], F. Garcia[2], E. Torres[2] and D. Sierra[3]. *[1]Dep. 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. [3]Dep. Biologia Vegetal (Botànica), Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal 645, E-08028 Barcelona, Spain.*
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In recent years, *Cryptovalsa ampelina* (Diatrypaceae) has been found repeatedly on grapevine canes in several countries, including Australia, South Africa and Spain. Surveys conducted in 2003–2004 in Catalonia (NE Spain) have shown this fungus to be very abundant on old pruned canes, although it has been recorded only rarely from cankered wood of living plants. The polysporous asci and slightly pigmented ascospores of *Cryptovalsa ampelina* distinguish the fungus from *Eutypa lata*, the causal agent of Eutypa dieback. However, cultures of both anamorphic forms are practically indistinguishable by their morphology alone. Therefore, our first objective was to design species-specific primers to be used in a PCR-based diagnostic test for *C. ampelina*. A primer pair was designed to amplify part of the ITS1-5.8S-ITS2 rDNA region and its specificity was later confirmed on *E. lata*, several other fungi occurring on *Vitis*, and the host plant. To test the pathogenicity of *C. ampelina*, artificial inoculations were conducted on one-year-old potted grapevine plants of Ull de llebre (red) and Macabeu (white) cultivars. Wounds made on the bark of canes were inoculated with a mycelial plug of the fungus and the plants were then maintained in a greenhouse with regular watering. Vascular lesions were measured twice during the experiment, at six and ten months after the inoculations. The results of the pathogenicity test showed that *C. ampelina* has low virulence, confirming some previous reports on the pathogenicity of this fungus.

Biochemical detection of *Eutypa lata* in grapevines. R. Lardner[1], N. Mahoney[2], E. Scott[1] and R. Molyneux[2]. *[1]Cooperative Research Centre for*

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Eutypa dieback of grapevines, caused by *Eutypa lata*, is a major threat to the sustainability and productivity of vineyards worldwide. Foliar symptoms, believed to be caused by translocatable toxins produced by the fungus in the xylem tissue, do not become visible until several years after infection. Infected vines may not display symptoms every season, or may display symptoms only at the start of a season. The aim of this research was to develop methods based upon the detection of fungal metabolites in grapevine sap or foliage to facilitate early diagnosis of the disease. High performance liquid chromatography (HPLC) was used to identify metabolite(s) that were both specific to *E. lata* and ubiquitous. *E. lata* isolates and other fungi were grown in media derived from grapevine wood. Although *E. lata* produces a range of secondary metabolites in culture, one compound, eutypinol, appeared to be predominant. This compound was not detected in any other fungal species. Because cultivars of grapevine exhibited varying degrees of tolerance to eutypa dieback, we examined differences in metabolite production following growth of selected isolates of *E. lata* on media derived from various cultivars of grapevine. Preliminary results suggested that cultivar has no influence on secondary metabolite production *in vitro*. Grapevine tissue-culture plantlets were inoculated with *E. lata* to assess metabolite production *in planta*. To examine metabolite uptake and to optimise extraction protocols for detecting *E. lata* metabolites *in planta*, shoots excised from tissue-culture plantlets were immersed in purified culture filtrates of *E. lata*. To date, eutypinol is the only compound which has been detected by HPLC in plantlets inoculated with *E. lata*, although eulatachromene, eutypine and 2-*iso*prenyl-5-formyl-benzofuran were also detected in excised shoots treated with culture filtrates of *E. lata*.

Molecular diagnostic tools for the detection of Eutypa lata in grapevines. R. Lardner, B. Stummer and E. Scott. *Cooperative Research Centre for Viticulture, PO Box 154 and School of Agriculture and Wine, University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia.*
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Eutypa dieback, caused by *Eutypa lata*, is a major threat to the sustainability and productivity of vineyards worldwide. The pathogen is difficult to identify on the basis of colony morphology and is often out-competed by other fungi when isolated from wood. Incubation for 1–2 months may be required to confirm the identity of cultures suspected to be *E. lata*. To facilitate diagnosis of the disease, we designed SCAR primers specific to *E. lata* and constructed a genomic DNA library from which an *E. lata*-specific probe was identified and sequenced. The SCAR primers were used to amplify DNA of *E. lata* directly from mycelium without the need for prolonged incubation or DNA extraction. These primers could also detect the pathogen in infected grapevine wood, although only when DNA was isolated using the Bio-101 FastDNA® SPIN kit for soil (Qbiogene). The *E. lata*-specific DNA probe was used to detect the pathogen in grapevine wood using a slot blot assay. The probe was validated for the detection of *E. lata* in wood using total DNA isolated from 198 grapevine trunks, in association with culturing of wood chips to enable morphological identification of *E. lata*. Comparison of data obtained using molecular and culturing techniques showed that the DNA probe was more sensitive than culturing, with *E. lata* detected in 54% of samples using the probe and in 38% of the same samples when wood chips were cultured on agar. The primers and probe can be used to gather information on epidemiology and to assess the efficacy of potential control agents for eutypa dieback.

Detection of *Eutypa lata* from grapevines by reverse dot blot hybridisation. S. Safodien[1], F. Halleen[1], P.W. Crous[2], W.A. Smit[1] and A. Botha[3]. [1]*ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa.* [2]*Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.* [3]*Department of Microbiology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa.*
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Eutypa dieback of grapevines, caused by the fungus *Eutypa lata*, has been recognised as a serious disease of grapevines worldwide. The disease is

responsible for a slow decline of vineyards by reducing growth and yield, and eventually killing the grapevine. Infection occurs primarily through pruning wounds. After infection the disease develops slowly and initial symptoms may even appear only after several years. Consequently, it is important to develop detection techniques to identify the presence of the disease as early as possible. Fast, accurate and reliable detection techniques could be used to screen grapevine in an effort to provide clean and healthy grapevine material to the grapevine industry. With the aim of fulfilling these criteria, a method using reverse dot blot hybridisation was developed. This method makes use of species-specific oligonucleotides derived from the internal transcribed spacer (ITS) region, which are blotted onto a membrane. In a PCR amplification with genomic DNA as the template, universal primers and digoxigenin-dUTP, the ITS region was simultaneously amplified and labelled. An aliquot of this PCR product was used as a probe for hybridisation to a membrane containing the immobilised oligonucleotides. A positive signal was observed when hybridisation occurred between the labelled DNA sample and the immobilised specific oligonucleotide. In this study, species-specific oligonucleotides were designed for *E. lata* and *E. leptoplaca*. The oligonucleotides developed to detect *E. lata* hybridised to pure DNA preparations of *Eutypa* isolates and work is currently being done to make the method amenable to the identification of *Eutypa* directly from environmental samples such as woody tissue. The oligonucleotide designed to identify *E. leptoplaca* hybridised to *E. lata* isolates as well, therefore, more work is needed to improve the specificity of the probe by optimising the hybridisation conditions. Once the method has been fully optimised it would be a rapid, consistent, reliable and highly reproducible PCR-based assay for the routine diagnosis of this plant disease.

PCR-based detection of *Eutypa lata* and *Eutypella vitis*. M. Catal, S.A. Jordan, S.C. Butterworth and A.M.C. Schilder. *Department of Plant Pathology, Michigan State University, 104 Center for Integrated Plant Systems, East Lansing, MI 48824, USA.*
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Eutypa dieback, caused by *Eutypa lata*, affects grapevines around the world, including the United States, Europe, Australia and South Africa. This disease is also common in older 'Concord' vineyards in Michigan. The objective of this study was to develop a rapid and reliable procedure for the detection of the causal agent. However, two fungi, *E. lata* and *Eutypella vitis,* were consistently isolated from 'Concord' grapevines with Eutypa dieback symptoms, sometimes from the same vine. These fungi are difficult to distinguish morphologically, but are genetically distinct, as is shown by sequencing the internal transcribed spacer (ITS) region of their ribosomal DNA. It is not known whether *E. vitis* is pathogenic on grapes. Isolates of both species were collected from vineyards in southwestern Michigan, and the ITS region of 25 *E. lata* and 15 *Eutypella vitis* isolates was sequenced. The sequences were compared to each other and to existing sequences in GenBank using the BLAST search program. *Eutypa lata* sequences were more variable than those of *Eutypella vitis*. Specific PCR probes were designed for both species and evaluated for specificity against a range of fungal isolates from grapes and other fruit crops. The specific primers were selective for each species. A multiplex PCR protocol was developed that detected each fungus in culture, in sawdust from cankers, and in young infected canes.

First detection of *Eutypa lata* (Pers.:Fr.) Tul. with PCR directly out of grapevine trunks in Germany. P. Schwappach[1] and M. Grimm[2]. *[1]Bavarian State Institute for Viticulture and Horticulture, Section for Grapevine Protection, Herrnstrasse 8, D-97080 Veitshoechheim, Germany. [2]Julius-Maximilians-University Wuerzburg, Institute for Biochemistry, Am Hubland, D-97074 Wuerzburg, Germany. E-mail:* peter.schwappach@lwg.bayern.de

In 1999, symptoms of *Eutypa lata* were discovered in a Frankonian vineyard and have been observed there since then every year in all rootstocks. In 1999, only 16% of all vines showed the typical symptoms of eutypa dieback but by 2003 the incidence of symptomatic vines had increased to 58% and almost one third of all vines (29%) were dead or already removed. Another 11% developed poorly and 18% showed typical signs of eutypa dieback. When cutting the grapevine trunk vertically the extent of the typical dark regions inside of the trunk was equivalent to the severity of the external symptoms. Up to now, no direct method exists to control

this disease. Therefore, a non-destructive method is needed to detect infected plants prior to external symptom expression so that they can be eradicated from the vineyards. Small pieces of wood were taken from the dark region of infected grapevine trunks for molecular analysis. After isolating the DNA by means of a purification kit and an extensive denaturing step, species-specific ITS-primers were added. DNA of any *E. lata* that may have been present in the eluate was amplified by means of a polymerase chain reaction (PCR). Following PCR, fragments of DNA were separated by agarose gel electrophoresis. A prominent band at 385 bp was typical for positive detection of *E. lata*. The DNA of these bands was excised from the gel, phosphorylised and, after ligation in a pUC19-vector, transformed into *E.coli*-DH5α. Thus we obtained sufficient material for DNA sequence analysis. Ninety-nine percent of the tested DNA was identical to the reference-sequence of *E. lata* as described in the literature. We furthermore found the same conformity between the tested grapevine DNA and the DNA of an *E. lata* control isolate.

Development and validation of a protocol for molecular detection of *Phaeomoniella chlamydospora* in grapevine wood. E. Retief, U. Damm, J.M. van Niekerk and P.H. Fourie. *Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch, 7602, South Africa.*
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Petri disease, caused mainly by *Phaeomoniella chlamydospora*, is a serious decline and dieback disease of young grapevines. Pathogen detection and accurate diagnosis are currently based on fungal isolation from artificial growth media. The fungus is, however, extremely slow-growing and cultures are often overgrown by saprophytes before it can be identified: this may lead to false negative results. The aim of this study was to develop a time-efficient and cost-effective protocol for molecular detection of *Pa. chlamydospora* in grapevine wood. Different published methods for extraction of fungal DNA were tested on grapevine wood, some were modified, and certain steps combined. A protocol involving the pulverisation of wood samples in liquid nitrogen and DNA extraction in a hexadecyltrimethyl ammonium bromide (CTAB) extraction buffer was most effective. Bovine serum albumin was added to enhance the subsequent PCR reaction with species-specific primers (Pch1 and Pch2). As little as 1 pg of *Pa. chlamydospora* genomic DNA could be detected from spiked wood samples. For validation of the protocol, different combinations of grafted grapevines were sampled after uprooting, hot water treated (HWT; 50°C for 30 min) or left untreated. Isolations were made from the basal ends of rootstocks on potato dextrose agar amended with streptomycin. The same section was also used for molecular detection. The identity of the PCR products was confirmed by digestion with the restriction enzymes *Aat* II and *Mlu*N I. *Pa. chlamydospora* DNA was detected in 80.9% of samples, compared to only 24.1% positive detections by means of isolations. However, *Pa. chlamydospora* DNA was detected from 100% of samples that tested positive after isolation. Although none of the isolations from HWTed plants yielded *Pa. chlamydospora*, molecular detection was not adversely affected. The applicability and robustness of the molecular detection protocol will be evaluated in subsequent epidemiological studies.

Molecular detection of grapevine trunk disease pathogens in nursery and vineyard soils in South Africa. U. Damm, E. Retief and P.H. Fourie. *Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland (Stellenbosch) 7602, South Africa.*
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Management of grapevine trunk diseases such as Petri and black foot is difficult, especially because the disease cycles are currently poorly understood. Contaminated soil was reported as a source of inoculum, especially for black foot in nursery vines at susceptible stages after grafting. Use of molecular detection of these pathogens in the soil is still limited because of PCR-inhibiting soil components and the high costs of commercial DNA extraction kits. The aim of this study was therefore to develop a cost-effective, easy and sensitive method for extraction of fungal DNA from the soil and to use this method to determine whether South African nursery and vineyard soils are contaminated with grapevine trunk disease pathogens. Soil samples were collected from 27 nurseries, 16 rootstock motherblocks and 7 vineyards in the Western Cape province. DNA was extracted using a method based on sodium dodecyl sulphate buffer, the

FastPrep, instrument (Bio101) and a spin column for removal of PCR inhibitors. The extracted DNA was tested for *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Cylindrocarpon* spp. by means of PCR reactions with specific primers. *Pm. aleophilum* was not detected in any of the soils tested. However, *Pa. chlamydospora* and *Cylindrocarpon* spp. were frequently detected in the soils. This is the first time *Pa. chlamydospora* was detected in soils in South Africa. This fungus occurred very often (66%) in homogenously low amounts in soils, independent of the locality and the history of grapevine cultivation. While the overall incidence of *Cylindrocarpon* spp. was similar (66%), results suggest these pathogens might be more prevalent in certain areas, but further research needs to be conducted to substantiate these findings. Soils should therefore be regarded as potential inoculum sources of grapevine trunk disease pathogens. Crop rotation, soil fumigation, composting or the use of antagonistic microorganisms should be considered as means to reduce inoculum levels in soils.

Fungi associated with black foot disease in South African vineyards and nurseries. F. Halleen[1], H.-J. Schroers[2, 3], J.Z. Groenewald[3] and P.W. Crous[3]. *[1]ARC Infruitec-Nietvoorbij, P. Bag X5026, Stellenbosch, 7599, South Africa. [2]Agricultural Institute of Slovenia, Hacquetova 17, p.p. 2553, 1001 Ljubljana, Slovenia. [3]Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.*
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Cylindrocarpon and *Cylindrocarpon*-like taxa isolated from *Vitis vinifera* plants in nurseries and vineyards were compared morphologically and phylogenetically with other *Cylindrocarpon* taxa. LSU, ITS and ß-tubulin sequences were used for phylogenetic inferences. *Cylindrocarpon* species clustered mainly in three groups: a monophyletic group consisting of three subclades comprising (i) members of the *Cylindrocarpon destructans* complex; (ii) a *Cylindrocarpon* species newly described as *C. macrodidymum*; and (iii) an assemblage of species closely related to strains identified as *C. cylindroides*, the type species of *Cylindrocarpon*. This monophyletic group excluded two other groups, (i) comprising members of the *Neonectria mammoidea* complex; and (ii) comprising two undescribed *Cylindrocarpon*-like species. The latter two clades formed a paraphyletic group by LSU analysis, but ITS and ß-tubulin gene analysis made them a monophyletic group. Strains of the *C. destructans* complex isolated from grapevines matched *C. destructans* in morphology and DNA sequences. *C. macrodidymum* formed micro- and macroconidia, but rarely formed chlamydospores. Its mostly 3-septate macroconidia were more or less straight, minutely widening towards the tip, and had an apical cell slightly bent to one side. Its teleomorph, *Neonectria macrodidyma*, was obtained in mating experiments, and was characterised by smooth to finely warted ascospores, smooth to finely warted perithecia, and moderately sized angular to subglobose cells in the outer region of the perithecial wall. The two undescribed *Cylindrocarpon*-like species were characterised by mostly 3–5-septate, curved macroconidia, and lacked microconidia. For these species a new genus, *Campylocarpon*, was established. It comprised the new species *Campyl. fasciculare* and *Campyl. pseudofasciculare* respectively. Inoculation of 6-month-old potted grapevine rootstocks (cv. Ramsey) with selected isolates of *C. destructans*, *C. macrodidymum*, *Campyl. fasciculare*, and *Campyl. pseudofasciculare* resulted in a reduced root and shoot mass of inoculated plants.

Surveys of Oklahoma grapevines for *Xylella fastidiosa*. S.L. von Broembsen and B. Olson. *Department of Entomology and Plant Pathology, 127 Noble Research Center, Oklahoma State University, Stillwater, OK 74078, USA.*
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Xylella fastidiosa (XF), a xylem-limited bacterium with a wide host range, is indigenous to the Gulf Coast Region of the USA and is vectored by xylem feeding insects. Grape strains of XF cause a vascular wilt, Pierce's disease of grapevines (PD), which often results in the death of susceptible cultivars, especially those of *Vitis vinifera*. Although Oklahoma (OK) grape production is still quite limited, it has dramatically increased in the past five years and supports a fledgling regional wine industry. XF has not previously been recorded on native or cultivated hosts in OK, but has been reported from grapevines in Texas counties that border OK to the south. A number of potential vectors of XF have been recorded in OK. Seventeen vineyards located primarily in southern

OK were surveyed in 2003. During 2004, 18 vineyards were surveyed that were more generally distributed throughout OK production areas. During both surveys, all grapevines in the second leaf or greater in these vineyards were inspected and petiole samples from visually unhealthy vines for which no apparent cause was evident were taken for laboratory analysis. Products resulting from DNA amplification using polymerase chain reaction technology (PCR) were elucidated on gels in 2003, but real time PCR was used for the 2004 analyses. XF was not detected in any samples in either 2003 or 2004. Surveys in 2005 will include native grapevines and other potential hosts.

Host-pathogen interaction

Consequences of esca on carbohydrate physiology in French vineyards. A.N. Petit[1], M. Boulay[2], F. Baillieul[1], C. Clément[1] and F. Fontaine[1]. *[1]Université de Reims Champagne-Ardenne, UFR Sciences, Laboratoire Stress, Defenses et Reproduction des Plantes, BP 1039, 51 687 Reims Cedex, France. [2]Moet et Chandon, 6 rue de Bussy, 51 200 Epernay, France.*
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Esca is a complex wood disease characterised by symptoms on the plant organs and in the woody tissue of grapevines. Several fungi are involved in esca and the disease is not yet fully elucidated. In the last few years a dramatic upsurge of esca has occurred in all grape-growing areas, including the Champagne. Sodium arsenite, which used to be the only mode of control against esca, was banned in 2001 and other chemical or alternative control methods need to be developed rapidly. For this purpose it is necessary to know more about this disease. The aim of this work was therefore to characterise the physiological impact of esca on vineyards in the Champagne area. We focused particularly on the effects of foliar symptoms on two parameters: 1. carbon nutrition, which is the key factor for grapevine yield and vigour, was determined. In this context gas exchanges were measured, and the biochemical analysis of photosynthetic pigments and carbohydrate reserves was undertaken. Moreover, we followed the expression of a gene encoding an oxygen-evolving enhancer, which is a protein involved in photosystem II. 2. Secondly, the activation of defence mechanisms was monitored in order to confirm whether grapevines infected with esca are able to react. Here we observed the expression of genes encoding for proteins involved in this process. The results of vineyard experiments carried out in 2003 and 2004 indicate that esca disease leads to grapevine physiological changes relating to foliar symptoms. First, carbon nutrition damage was observed resulting in a reduction in net photosynthesis and in carbohydrate reserves in the canes during winter dormancy. This reduction could induce losses in yield and grapevine vigour, thereby affecting plant longevity. With regard to defence mechanisms, grapevines seemed to detect infection by esca pathogens. The vines react by inducing several genes involved in defence mechanisms, such as chitinase and lipoxygenase.

Purification and characterisation of extracellular laccase from liquid culture of *Fomitiporia mediterranea*. E. Abou-Mansour[1], R. Pezet[2] and R. Tabacchi[1]. *[1]Laboratory of Analytical Organic Chemistry, University of Neuchâtel, CH-2007 Neuchâtel, Switzerland. [2]Agroscope-RAC, Swiss Federal Agriculture Research of Changins, Route de Duiller, CH-1260 Nyon, Switzerland.*
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The common internal symptom in chronic esca syndrome is white rot, which gradually changes the hard wood to a soft, friable, spongy mass. The wood decay symptoms of esca have been most commonly associated with the basidiomycete fungus *Fomitiporia mediterranea*, which produces various extracellular lignin-degrading enzymes, including peroxidases and laccases. Laccases are involved in several physiological functions, such as lignin biosynthesis, plant pathogenesis, insect sclerotisation, and degradation of lignocellulosic materials. Laccases are also involved in both the polymerisation and depolymerisation processes of lignin. In this study we report for the first time on the purification and characterisation of a 60-kDa extracellular laccase enzyme from a liquid culture of *F. mediterranea*. Using gel filtration and ion exchange chromatography, a 2006-fold purification and a yield of 9.6% of the laccase was obtained. Using 2.6-DMP as the substrate, the enzyme exhibited an optimum pH of 3 and an optimum temperature of 40°C against ABTS, and 50°C for DMP, and its

K_m was 25 mM. Substrate specificity and inhibitor studies indicated the enzyme to be a typical fungal laccase.

Chemical characterisation of stilbenic polyphenols from esca diseased wood and their role in defence mechanisms. C. Amalfitano[1], F. Peduto[2], 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 Università 100, 80055 Portici, Italy.* [2]*Dipartimento di Biotecnologie Agrarie, Sezione di Patologia Vegetale, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy.*
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It has been demonstrated that stress metabolites such as resveratrol (3,5,4'-trihydroxystilbene) and its oligomers (viniferins) accumulate in grapevine tissues in response to fungal infection. In this work we report on the purification of three resveratrol tetramers extracted from the brown-red wood of esca diseased *Vitis vinifera* cv. Sangiovese by extensive chromatographic methods (CC and HPLC). Resveratrol, *cis* and *trans* ε-viniferin and ampelopsin B were detected in these grapevines in previous studies. The tetramers were identified mainly by ^{1}H and ^{13}C mono and bidimensional NMR spectroscopy, ESI-MS spectrometry and optical activity, and were found to be hopeaphenol, isohopeaphenol and ampelopsin H. Resveratrol and its oligomers were also found in higher concentrations in diseased, discoloured wood than in healthy wood. This suggests that some of these metabolites could be involved in the plant defence response. Some of the isolated polyphenols were assayed *in vitro* and compared with resveratrol in germination tests against the fungi involved in esca (*Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*). No or low antifungal activity was found at the assayed concentrations. However, to fully elucidate the biological activity of such compounds, more *in vitro* tests (including activity on hyphal growth, sporulation and spore vitality) are under way to elucidate the role of stress metabolites in the plant/pathogen interaction.

Fungal identification and biochemical analysis of wood canker caused by *Eutypa lata*. P.E. Rolshausen[1], J.M. Labavitch[2] and W.D. Gubler[1]. [1]*Department of Plant Pathology, University of California, Davis, CA, 95616, USA.* [2]*Department of Pomology, University of California, Davis, CA, 95616, USA.*
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Eutypa lata is a vascular pathogen of grapevines. The fungus spreads by ascospores, infects open wood vessels and slowly decays the wood. The goal of this study was to identify the fungal population inhabiting wood cankers and to determine the polymer targets and products of degradation of the cell wall degraded by *E. lata*. The cordons of naturally infected grapevine cv. Merlot and Cabernet Sauvignon, which are reported to be respectively tolerant and susceptible to the disease, were sampled for two consecutive years in California. The sapwood was separated into three zones, healthy, cankered, and canker margin. Isolations were conduced from all three wood zones on PDA medium and fungi were identified based on their mitosporic stage, or by BLAST search in the GenBank database when morphological characters were missing. Wood from all three zones parasitised by *E. lata* only was ground to powder and the hemicellulose fraction of the cell wall was analysed by gas chromatography, while lignin, pectin, and cellulose levels were determined by colorimetric assays. The microbial population of cankered tissue showed frequent association of *E. lata* with one or more additional parasitic fungi (*Botryosphaeria obtusa, Phomopsis viticola, Phaeoacremonium aleophilum, Phaeomoniella chlamydospora, Verticillium* sp.) in a complex of fungi. *Diatrypell*a sp., a fungus closely related to *E. lata*, was also found in some wood cankers, indicating that other diatrypaceous fungi may be associated with grapevine dieback. Biochemical analysis of the wood revealed no degradation of cellulose and pectin. However, glucose in the hemicellulose fraction of the cell wall was targeted by fungal attacks. Moreover, a higher lignin content was measured for cv. Merlot as compared with Cabernet Sauvignon in all three wood zones, suggesting that phenolic compounds may be involved in disease resistance.

Isolation of esca-associated fungi, chemical composition of xylem exudate from bleeding spurs of infected grapevines and annual trend of sap flux. G. Bruno and L. Sparapano. *Dipartimento di Biologia e Patologia vegetale, Università di Bari, Italy.*
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Phaeomoniella chlamydospora (*Pch*), *Phaeoacremo-*

nium aleophilum (*Pal*) and *Fomitiporia mediterranea* (*Fme*) cause at least a part of the external symptoms of esca by phytotoxic metabolites produced in the discoloured or decayed woody tissue. Both *Pch* and *Pal* produced *in vitro* several phytotoxic compounds: exopolysaccharides (EPS) (pullulans) and two pentaketides (scytalone and isosclerone). The aim of this study was to: 1. isolate fungal pathogens; 2. detect phytotoxic pullulans and pentaketides; 3. detect proteins, glycoproteins and polyphenols of the host response; 4. assess the annual trend of sap flux and its chemical composition. During vine bleeding (early spring: 2000–2004), the xylem sap was collected from 'Sangiovese' vines showing severe symptoms of brown wood-streaking, with or without white rot. Aliquots of sap were poured on malt-agar dishes or filtered and assayed on leaves of grapevine cv. Italia and Sangiovese. Numerous phialidic conidia were found in the xylem sap from esca-affected grapevines; isolated in pure culture, these conidia gave origin to colonies of *Pch* and *Pal*. In addition, strains of *Sphaeropsis malorum*, *Alternaria* sp., *Diplodia* sp., *Fusarium* sp. and bacteria were also isolated. Symptoms on the leaves after absorption of the xylem sap solutions were: irregular, pale green areas located among the main veins or at the leaf margins, yellow-brown or red-brown necrotic spots. Significant differences in the trend of the parameters examined each year (flux, pH, and organic compounds) were recorded. The main components of the EPS were pullulans, galactomannans, glucogalactomannans, and arabinogalactans. Proteins and glycoproteins were detected ranging from 14 to 60 kDa. Benzoic acid derivatives, stilbenes and flavonol-*O*-glycosides were the dominant polyphenols of the xylem sap. The results of this study provide new information on the production of toxic metabolites *in planta* by *Pch* and *Pal* associated with esca disease. Conidia of *Pch* and *Pal*, their metabolites and host response compounds were found to occur in the xylem sap of naturally infected vines, indicating that the pathogens, their by-products and defense substances were translocated from the infected woody tissue of the trunk to the aerial part of the affected vines.

Differential virulence effects of single and dual inoculations of *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Pm. inflatipes* on young grapevines. L. Gaforio, E. Aparicio, G. Hernaiz and M.L. Tello. *Instituto Madrileño de Investigación Agraria. Autovía A-2, Km. 38,200. Alcalá de Henares E-28800- Madrid, Spain.*
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Petri disease is a serious problem in newly planted vineyards, causing decline and dieback of young grapevines. Being a complex syndrome, its aetiology is still partly unknown. The aim of this work was to acquire a better understanding of the aetiology of this disease through analysis of the relationships between the main causal agents. Two year old asymptomatic grapevines of var. Tempranillo clone RJ-78/140-Ruggeri clone 101 were infected with the fungi *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Pm. inflatipes*. Inoculations were carried out by inserting plugs, removed from actively growing colonies, into shoot wounds (5 mm diam. × 2–4 mm depth). The three strains were inoculated separately and in dual combinations, introducing pairs of fungi in the same wound, with an interval of fifteen days. Lesion lengths were measured weekly for three months and the differences between wounds inoculated with each fungus or combination of fungi were compared. *Pa. chlamydospora* was the most virulent fungus causing the largest lesions, both in single inoculations and in combinations, whenever it was inoculated first. However, it had the lowest percent isolation due to its less competitive behaviour compared with other saprophytic fungi. *Pm. aleophilum* showed a synergistic effect with *Pa. chlamydospora* when it was inoculated last. *Pm. inflatipes* did not seem to have a predominant role in the rot. These results point to *Pa. chlamydospora* and *Pm. aleophilum* as the causal organisms of Petri disease.

The influence of *Phaeomoniella chlamydospora* and other fungi on the strength of the graft union and biomass of young grapevines. A.B. Reed[1], L.D. Melton[1], B.G. Smith[1] and P. Johnston[2]. *[1]Food Science Programme, Department of Chemistry, University of Auckland, Private Bag 92019, Auckland, New Zealand. [2]Mycology Department, Landcare Research, University of Auckland (Tamaki Campus), Auckland, New Zealand.*
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During nursery propagation, some grafted vines die and weak grafts were observed in otherwise

healthy looking 8-month-old vines. Petri disease, caused by *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp., is associated with decline and dieback of young grapevines that are subjected to stress. The effects of inoculating spore suspensions of *P. chlamydospora* into the graft wound were examined. Root and shoot dry weights were measured as well as the tensile strength of the graft assessed. Field trials were conducted in conjunction with a commercial viticulture nursery, using Sauvignon Blanc and Pinot Noir grafted onto 101-14 Mgt. Vines were inoculated with *P. chlamydospora* spore suspensions (10^2, 10^3, 10^4, 10^6 and 10^8 cfu ml^{-1}) or water into the omega graft wound at the time of grafting. Stock-takes of young vines growing in the nursery were performed at 10 and 24 weeks. The average root and shoot biomass was recorded (n=10). After 8 months, the graft tensile strength was assessed as well as the development of black streaking in the xylem tissue. Results indicated that at least 10^4 cfu ml^{-1} of *P. chlamydospora* was necessary to significantly reduce root and shoot biomass and weaken grafts. Some inoculated (up to 10^6 cfu ml^{-1}) vines, did not show obvious symptoms of vine decline, but all inoculated vines developed black streaking in the xylem of both rootstock and scion. The extent of black streaking varied with the inoculation dosage.

Ethylene metabolism in grapevine plants infected by *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*. P. Gómez[1], A. G. Báidez[1], A. Ortuño[1], J.A. del Río[1], A. Carballo[2] and V. Frías[3]. [1]*Department of Plant Biology, Faculty of Biology, Campus Espinardo, University of Murcia, 30100 Murcia, Spain.* [2]*Department of Genetic, University of Sevilla, 41080 Sevilla, Spain.* [3]*Department of I+D of Agrométodos S.A., Alamos 1, Urb. Monteclaro, 28223 Pozuelo de Alarcón, Madrid, Spain.*
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Petri disease is caused by different fungi such as *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*. The disease causes decline and dieback of young grapevines because the xylem becomes blocked by tyloses, which form when the xylem lumen is invaded by the protoplasm of the adjacent parenchyma cells. This invasion is possible because of the softening of the xylem walls, which is caused by various wall hydrolitic enzymes. In this study, we demonstrated the involvement of ethylene in the first phase of the development of Petri disease in grapevine plants growing *in vitro*, which had been infected with *Pa. chlamydospora* and *Pm. aleophilum*. The presence of Brotomax in the culture media decreased the biosynthesis of ethylene and reduced the progress made by these fungi. The results suggest that the increase of natural defences brought about by Brotomax can reduce the incidence of these pathogens in grapevine plants.

Bioactive secondary metabolites from *Phomopsis viticola*, a fungus responsible for grapevine excoriosis. R. Tabacchi, M.-L. Goddard, N. Mottier and E. Abou-Mansour. *Laboratory of Analytical Organic Chemistry, University of Neuchâtel, CH-2007 Neuchâtel, Switzerland.*
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Phomopsis cane and leaf spot, also known as excoriosis, is widely distributed throughout the viticultural world. The causal agent, *Phomopsis viticola* (Sacc.), infects all green parts of vines such as the canes, leaves and fruits. The symptoms are black necrotic lesions on the lowest internodes of the shoots and small black spots on the leaves and berries. Crop losses are due to weakening canes and poor fruit development or fruit loss. In a continuing effort to identify phytotoxins from pathogens implicated in grapevine trunk diseases, we screened several strains of *P. viticola* for cytotoxicity, antifungal, and antimicrobial activities. From three different strains, a variety of bioactive compounds, including Phomopsides B, four new pyranones and a new xanthenone, were characterised. We describe here the isolation of the fungi, the culture conditions, the structure elucidation and the biological activity of the isolated compounds.

Antagonistic behaviour of *Phaeomoniella chlamydospora* and *Phaeocremonium* spp. vs. *Fomitiporia mediterranea*: isolation, purification, chemical and biological characterisation of active compounds. G. Bruno and L. Sparapano. *Dipartimento di Biologia e Patologia vegetale, Università di Bari, Italy.*
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Recent advances in research on esca of grapevine shed further light upon the aetiology and patho-

genesis of this disease complex. The pathogenicity of the three fungi most commonly associated with the disease, the thracheiphilous hyphomycetes *Phaeomoniella chlamydospora* (*Pch*) and *Phaeoacremonium aleophilum* (*Pal*), and the wood-rotting basidiomycete *Fomitiporia mediterranea* (*Fme)* has been clearly demonstrated. The non-synergistic, competitive association of the two hyphomycetes and the inhibition of *Fme* growth have been depicted. The objectives of this study were to: 1. assess the toxigenicity of *P. angustius* (*Pan*), *P. inflatipes* (*Pin*), *P. parasiticum* (*Ppa*), *P. rubrigenum* (*Pru*), *P. viticola* (*Pvi*) and their inhibition of *Fme* growth; 2. study how *Pch, Pal* and *Fme* interact with each other and with host tissue; 3. isolate and characterise chemically and biologically the substances involved, showing how the mycelium of each fungus functions and how the fungi co-ordinate their activities. The culture filtrates of all strains, assayed on detached leaves of 'Italia' or 'Sangiovese' grapevines, were phytotoxic. Isosclerone was produced by *Pal*, *Pch*, *Pin*, *Pan*, *Pru, Pvi*, whereas scytalone was produced by *Pal*, *Pch*, *Pin*, *Pan* and *Ppa*. Pullulan was also produced by *Pan*, *Pin*, *Ppa* and *Pvi*. In dual cultures, the antagonism against *Fme* was clearly shown on malt-agar plates: the margin of the *Fme* colonies turned brown, became thicker, and aerial hyphae formed a ridge-like barrier between the two fungi. This experiment showed that the antagonistic effect against *Fme* was probably caused by strains of *Phaeoacremonium* spp. and *Pch* producing substances that spread through the medium and affected the *Fme* colony. All *Phaeoacremonium* spp. and *Pch* strains were used for the concentration and desalting of culture filtrates through an anisotropic membrane. Most of the growth inhibitor activity of the crude culture filtrate was located in the 3–10 kDa or up to 3 kDa fractions. These fractions were found to contain peptides and proteins that suppressed *Fme* growth.

Preliminary studies on micro and macro elements in esca diseased leaves of *Vitis vinifera* cv. Cardinal. C. Amalfitano[1], V. Cozzolino[1], D. Agrelli[1], V. Di Meo[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 Università 100, 80055 Portici, Italy. [2]Dipartimento di Biotecnologie Agrarie, Sezione di Patologia Vegetale, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy.*
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Changes in the host metabolism or in metabolic and catabolic processes following pathogen attack can result in localised variations in the micro and macro elements content. Such changes are often accompanied by an increase in the resistance or tolerance to a given pathogen. In a disease such as esca, whose foliar symptoms are similar to symptoms of nutrient deficiency, an investigation was started on the mineral content of leaves from healthy and esca diseased vines, and on the main biochemical and physiological pathways of the defence response in which those minerals were involved. Leaves from healthy vines and vines showing chronic symptoms (leaves showing only interveinal chlorotic spots, and leaves showing chlorotic and necrotic interveinal areas) of the table grape cv. Cardinal were collected at the end of flowering (middle of May), at the end of colour change (beginning of July) and just after harvest (middle of September). The content of Ca, Cu, K, Fe, Mg, Mn, Mo, Na, P and Zn were determined by spectroscopic methods (A.A. and UV-Vis spectrometers), and C, H, N and S by elemental analysis (HCNS elemental analyser). Significant differences were found in the mineral content of leaves between diseased and healthy vines. While such differences could be simply the result of physiological events linked to the early senescence of leaves in diseased vines, triggered by toxic compounds produced by the esca pathogens, the possibility cannot be excluded that the imbalance in nutrients in some way caused these leaf symptoms.

Effect of fungi associated with decline symptoms on growth curves and berry development of *Vitis vinifera* cv. Red Globe. J. Auger[1], M. Esterio[1], C. Carreras[1], P. Naulin[2] and M. Pinto[3]. *[1]Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile. [2]Departamento de Silvicultura, Facultad de Ciencias Forestales, Universidad de Chile [3]Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004, Santiago-Chile.*
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Table grape is the main exported Chilean fruit-bearing species and the table grape cultivar Red Globe is the second most important cultivar grown.

This cultivar is subject to a decline syndrome which has been associated with *Botryosphaeria obtusa* and *Phaeoacremonium* sp. The symptoms are: delayed budding, poor shoot and berry growth and uneven ripeness; other symptoms are: short internodes, wine-red spots on the leaf margins or on the leaf blade, and dark wood streaking of the xylem. To study the effect of this pathology on berry development, healthy and diseased plants were compared by evaluating grape berries for size, colour, soluble solid content, fresh and dry weight, calcium and potassium, and number of functional vascular bundles. Diseased plants had a 2-week delay in growth, ripeness and xylem closing compared with healthy plants. After xylem closing, there was a decrease in the calcium content of both plants, but the potassium content decreased only in diseased plants, which suggested a phloem deterioration. At harvest time, berries from diseased plants had a much lower sugar content.

Pathogenicity testing of *Phialophora*, *Phialophora*–like, *Phaeoacremonium* and *Acremonium* species isolated from vascular tissues of grapevines. F. Halleen[1], L. Mostert[2] and P.W. Crous[2]. [1]*ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa.* [2]*Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.*
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Several *Phialophora*, *Phialophora*-like, *Phaeoacremonium* and *Acremonium*-like species were recently isolated from vascular tissues of grapevines in South Africa. In most cases their status as pathogens was unknown and pathogenicity studies were therefore conducted to determine their potential as decline pathogens. The fungi included *Acremonium* cf. *charticola*, *A.* cf. *strictum*, *Cadophora luteo-olivacea*, *Massarina* sp., *Phialemonium* cf. *curvatum*, *Phialophora richardsiae*, four new *Phaeoacremonium* species (*Pm. krajdenii*, *Pm. venezuelense*, *Pm. subulatum* and *Pm.* Sp. 3). *Phaeomoniella chlamydospora*, *Pm. aleophilum, Pm. parasiticum*, and *Pm. viticola* were also included. Data obtained from the glasshouse trial three months post inoculation were difficult to interpret due to the small lesions, and the similarity of lesions from different species. However, *Pa. chlamydospora* produced the largest lesions and proved to be the most aggressive pathogen. *A.* cf. *charticola* and *A.* cf. *strictum* are considered to be non-pathogens or endophytes due to their small lesions and low re-isolation percentages. Field trials after 14 months confirmed *Pa. chlamydospora* as the most aggressive pathogen, since it produced the largest lesions in the trunks, as well as on inoculated pruning wounds. It was also re-isolated more frequently than any other fungus, especially from the inoculated pruning wounds. The *Massarina* sp. was the only fungus that could not be re-isolated from any of the inoculated trunks or pruning wounds and the wounding reaction did not differ from the control treatment. The *Massarina* sp. should therefore be regarded as a non-pathogen or endophyte. All the other fungi infected and colonised the vines and produced lesions statistically different from those caused by the water control and the non-pathogen in the field trial.

The influence of *Phaeomoniella chlamydospora* on the production of polyphenols and polyphenol oxidase (PPO) in nursery grown grapevines. A.B. Reed, L.D. Melton and B.G. Smith. *Food Science Programme*, *Department of Chemistry, University of Auckland, Private Bag 92019, Auckland, New Zealand.*
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Petri disease, caused by *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp., is associated with vine decline and with the development of black streaking in the xylem of young grapevines subject to stress. Plants produce some polyphenols such as resveratrol, *trans*-piceid and the oligomer viniferin, in response to fungal invasion. Polyphenols are oxidised by polyphenol oxidases (PPO), resulting in the formation of brown/black pigments. Both grapevines and fungi are known to produce PPO enzymes. It is not known whether the brown/black streaking is as a result of PPO activity on the endogenous polyphenols. Field trials conducted in conjunction with a commercial viticulture nursery were carried out using Sauvignon blanc grafted onto 101-14 Mgt. Vines were inoculated at the time of grafting on the omega graft wound with *P. chlamydosporum* spore suspensions (10^6 cfu ml^{-1}) or water. After 10 weeks, polyphenols were extracted from the xylem of rootstocks (n=10) and analysed by the HPLC and Folin-Ciocalteu methods. PPO was also extracted and the activity of the crude extract of both inoculated vines and controls

was measured using several polyphenol substrates. The amount of resveratrol, *trans*-piceid and several other oligomeric polyphenols was greater in the inoculated vines than in the controls. PPO extracted from both inoculated and control grapevines produced brown/black products when catechol, resveratrol and chlorogenic acid were used as substrates. Little initial activity was measured by fungal PPO alone, with no formation of brown/black pigments.

***Phaeomoniella chlamydospora*: infection ability and survival in soil.** L. Gaforio, S. Pastor, C. Redondo and M.L. Tello. *Instituto Madrileño de Investigación Agraria (IMIA). Autovia del Nordeste A-2, km. 38.200, E-28800 Alcala de Henares, Madrid, Spain.*
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Petri disease affects young grapevines. The main causal agent is the fungus *Phaeomoniella chlamydospora*, which causes decline and dieback. Infection, as proposed by several researches, occurs by penetration through pruning wounds, by the use of infected propagation material, and by the infection of rooted cuttings through the soil. This last way of transmission could well be occurring in propagation plots in nurseries. The aim of this work was to test the ability of *P. chlamydospora* to infect un-rooted cuttings from the soil; the ability of the fungus to survive in the soil; and to determine its infection rate inside plant tissues. Healthy cuttings of *Vitis vinifera* var. Tempranillo, with no roots, were planted in pots that were artificially-inoculated with a spore suspension of 1.3×10^7 sp ml^{-1} and incubated under semi-controlled conditions (22°C, 30% RH, 14-hr light). The inoculum was applied to the substrate (peat/vermiculite 1:1) before planting the cuttings. Samples from substrate and cuttings (wood) were collected periodically over 3 months, and the pathogen was reisolated from them. Even though the cuttings remained asymptomatic, the fungus was isolated from plant tissue throughout the assay, penetrating the plant tissue up to 10 cm from the cutting base at 90 days after inoculation. The progression rate was also confirmed by microscopic observation of tissue slides and estimated as 16.7 mm/day in the first month, 3.3 mm/day in the second month and 13.3 mm/day in the third month. Tylose formation started during the second month. The fungus was also isolated from the substrate. Results confirmed that *P. chlamydospora* infected un-rooted cuttings of grapevine from the substrate and kept its viability throughout the assay. The tissue colonisation rate was moderate but sufficiently severe to render future use of infected propagation material unadvisable.

Epidemiology

Esca – a disease or a case of commensalism? T. Gräfenhan[1], R. Zare[2] and W. Gams[3]. *[1]Federal Biological Research Centre for Agriculture and Forestry (BBA), Institute for Plant Virology, Microbiology and Biological Safety, Königin-Luise-Str. 19, 14195 Berlin, Germany. [2]Plant Pests and Diseases Research Institute (PPDRI), Department of Botany, PO Box 1454, Tehran 19395, Iran. [3]Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.*
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Esca is a complex disease of increasing importance around the globe. Its causal agents are well known and now have a cosmopolitan distribution. Reasons for their spread are found in modern viticultural practices: in the worldwide trade of scions and rootstocks without certification, and in current techniques of grafting and high-density planting. When looking for unspoiled situations of viticulture with an ancient history, we expected that Iran would offer insights into a traditional, apparently primitive style of wine growing. A high diversity of indigenous cultivars has been preserved and own-rooted grapevine plants are grown in a traditional way with limited commercial incentive. Therefore we were particularly interested to study the distribution of esca and its causal agents under these conditions. We assumed that the most natural growing conditions would be found in the foothills of the Minor Caucasus, on the north-western border of Iran, where notable populations of wild grapevine (*Vitis sylvestris*) still occur. During a summer visit, few diseased plants with clear-cut external and internal esca symptoms were found in various vineyards in the provinces of Ardabil and West Azarbaijan and the pathogens of these plants were isolated. During a spring visit, symptoms mainly consisting of yellowing were seen in some vines in the provinces of Fars, Qom, Qazvin and Semnan, and the fungi con-

cerned were isolated. Apoplexy has neither been seen nor reported in Iran. Wild grapevine hardly showed any symptoms, but some apparently endophytic fungi were isolated. Obviously grapevine trunks are a suitable biotope for a diversity of fungi, including species that are pathogenic in intensified viticulture, but they cause little harm in more natural situations.

First report of naturally occurring *Togninia minima* perithecia in California vineyards. S. Rooney-Latham, A. Eskalen and W.D. Gubler. *Department of Plant Pathology, University of California, Davis 95616, USA.*
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Esca can be a severe disease of grapevines in California, capable of causing serious economic damage to table, raisin and wine grape cultivars. One of the most commonly isolated fungal pathogens associated with esca-diseased vines in California is *Togninia minima* (*P. aleophilum*). Although the perfect stage was recently identified *in vitro* by successful crossings of compatible isolates, the natural occurrence of perithecia in vineyards was hitherto unknown. The existence of perithecia in vineyards was recently suggested in California and Australia, after *T. minima* perithecia developed on naturally infected, moist-incubated grapevine wood after extended periods of time. Vineyards throughout California, shown to contain aerial propagules of *T. minima* by spore trapping, were surveyed for the source of these spores. Perithecia morphologically identical to those of published *T. minima* descriptions and to those produced in culture, were identified on grapevines in multiple vineyards in California. After thorough moistening, perithecia forcibly discharged their ascospores, as previously documented for *T. minima* perithecia produced *in vitro*. Furthermore, molecular analyses of the ITS region of the nuclear ribosomal DNA confirmed these perithecia to be *T. minima*. Additional details regarding ascospore release and where these perithecia reside on the grapevines will be discussed. This is the first report of the occurrence of *T. minima* perithecia on standing grapevines and suggests the importance of ascospores as a source of inoculum for new grapevine infections.

Infested soil as a source of inoculum for *Phaeomoniella chlamydospora*, causal agent of Petri disease. S.A. Whiteman, M.V. Jaspers, A. Stewart and H.J. Ridgway. *National Centre for Advanced Bio-Protection Technologies, PO Box 84, Lincoln University, Canterbury, New Zealand.*
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Phaeomoniella chlamydospora is believed to be the primary causal agent of Petri disease of grapevines in New Zealand. A number of researchers have hypothesised that planting young vines in soil infested with the fungus can result in infection. DNA was extracted from soil samples collected from beneath rootstock mother-vines known to be infected with *P. chlamydospora*, using a SDS/phenol/chloroform method and then tested using a nested species-specific PCR assay. The detection level of the assay was determined to be 10^3 conidia g^{-1} when a conidial suspension was added to sterilised soil samples, and 5 fg when *P. chlamydospora* genomic DNA was added directly to the PCR reaction. Of the 20 bulked samples tested, 20% were positive. In order to determine whether infested soil was a source of inoculum, young vines were planted in soil that was artificially infested with a conidial suspension of *P. chlamydospora* for which an isolate-specific genetic marker had been developed. Use of this isolate allowed differentiation between natural infection and infection due to the introduced inoculum. Three months after planting, samples of woody tissue from all vines were plated on agar. DNA was extracted from all recovered *P. chlamydospora* isolates using a commercially available kit and amplified using an isolate-specific primer pair. A restriction digest was performed on the resulting products. Identification of the inoculated isolate was possible by the observation of a specific banding pattern following digestion. Of the 40 vines planted in infested soil, 5% were found to be infected with the inoculated isolate. *P. chlamydospora* was not reisolated from any of the control vines. The results of this work confirm that *P. chlamydospora* is present in rootstock mother-vine block soil and has limited potential to infect young vines. Options for further research to determine the importance of this inoculum source will be discussed.

Susceptibility of grapevine spring wounds to *Eutypa lata*: further results and present epidemiological status. P. Lecomte, S. Cardon, N. Bastien, S. Giry Laterriere and D. Bailey. *In-*

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The susceptibility of grapevine to Eutypa dieback by the contamination of wounds caused when excess shoots are pruned and removed in spring may play a role in the epidemiology of this wood decline disease. Preliminary findings, following inoculation of young Cabernet Sauvignon plants grown in sheltered conditions and a 2-week incubation period, led us to hypothesise that this kind of wound provides an infection court for ascospores of *E. lata*. To further understand the conditions that affect the colonisation of spring wounds, a number of natural or artificial infection studies were undertaken in Bordeaux area between 1999 and 2004. The infection efficiency (IE, percentage recovery of *E. lata*) was examined for different growth conditions (controlled or natural), contamination conditions (artificial or natural), wound type (desuckering or disbudding), inoculum density (0, 100 or 1000 spores per wound) and incubation period (two weeks or one year). All inoculations were performed on Cabernet Sauvignon. The presence of *E. lata* was diagnosed by morphological assessment assisted by PCR. Results were highly variable. For plantlets grown under controlled conditions, the IE after one year incubation was lower than had been reported previously. Moreover, the overall IE recorded in this study was lower than that reported generally for winter pruning analysed under the same conditions. In this study, we found no consistent effect of inoculum density or wound type on IE. However, the IE was significantly higher with artificial than with natural contamination. With artificial contamination, the IE was higher for wounds assessed after 1 year than after two weeks. Variability between results obtained from artificially infected wounds was most likely due to assay limitations such as the buildup of other micro-organisms. On the whole, these results, combined with relatively low levels of ascospore release during spring, suggest that the epidemiological role of spring wound contamination is less important than that caused by winter pruning.

Eutypa dieback: disease progress and losses in 'Concord' grapes. S.C. Butterworth, S.A. Jordan and A.M.C. Schilder**.** *Department of Plant Pathology, Michigan State University, 104 CIPS Building, East Lansing, MI 48824, USA.*
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The objective of this study was to monitor 'Concord' vines with Eutypa dieback over several years and assess losses in yield and quality due to the disease. A 'Concord' vineyard in southwest Michigan was monitored from 1999 to 2002. The incidence of vines with typical symptoms (cupped leaves, chlorosis, and stunted shoots) was 1% in 1999, 0.6% in 2000, 4% in 2001, and 17% in 2002. None of the vines showed foliar symptoms every year and a few vines died. To determine the effect on yield on a per-shoot basis, individual shoots on infected vines were categorized as having no, mild, intermediate, or severe symptoms in 1999–2001. Compared to nonsymptomatic shoots on the same vine, severe infection resulted in significant reductions in fruit weight per shoot, fruit weight per cluster, number of berries per cluster, number of clusters per shoot, and weight per berry. Even in mildly infected shoots, significant reductions were observed in fruit weight per shoot, fruit weight per cluster, and number of berries per cluster. Nonsymptomatic shoots on infected vines had higher cluster weights and a greater number of berries per cluster than similar shoots on apparently healthy vines, suggesting compensation by infected vines. The disease had only a slight effect on pH, and the Brix, and tartaric acid content of the juice of infected vines. On a whole-vine basis, vines with mild symptoms showed an average yield reduction of 21% (2003) and 15% (2004) compared to apparently healthy vines. Vines with intermediate symptoms showed an average yield reduction of 27% (2003) and 30% (2004). Severely symptomatic vines had an average yield reduction of 59% (2003) and 83% (2004). These results show that Eutypa reduces yield considerably, even in mildly symptomatic vines. Variability in symptom expression from year to year will make it more difficult for growers to monitor their vines for infection based on foliar symptoms.

Investigation into the occurrence of *Botryosphaeria dothidea* in grape propagative material and pathogenicity studies on different woody plants. I.C. Rumbos. *NAGREF, Plant Protection Institute of Volos, Volos 380 01, Greece.*
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The fungus *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not. is the causal agent of black dead arm of grapevine and is associated with branch and trunk dieback, wood necrosis, brown wood streaking and incomplete grafts. An extensive study was carried out during 2002 and 2003 covering the entire grape-growing area of Greece in order to investigate the incidence of *B. dothidea* and other wood destroying fungi in the grape propagative material that was produced in Greece or other European countries. Four groups of vine material were examined: a) 12,000 unrooted rootstock cuttings from governmental or private mother nurseries, b) 3000 rooted rootstock cuttings from Greek nurseries or from other European countries, c) 5000 scion cuttings, d) 5000 bench-grafted rooted vines from Greek nurseries or from other European countries. The sampling strategy was carried out by 33 agronomists according to our instructions. In total, over 20,000 cuttings or grafted rooted vines were examined and over 80,000 isolations were made. *B. dothidea* was not isolated from any unrooted rootstock or scion cutting. The fungus was isolated in a low percentage (0.3%) from rooted rootstock cuttings and in a higher percentage from grafted rooted vines (3.5%). In some grafted rooted cultivars from other European countries the percentage was significantly higher (12.5%). It was concluded that planting material colonised by *Botryosphaeria dothidea* at the nursery was one reason for the decline of young grapevines. Pathogenicity studies with isolates from kiwifruit (*B. parva*), olive (*Botryosphaeria* sp.), walnut (*B. dothidea*), *Ilex aquifolium* (*B. parva*), grapevine (*B. dothidea*) and pistachio (*B. dothidea*) on grapevine (different cultivars and rootstocks) and other woody hosts (kiwifruit, olive, *Ilex aquifolium*, walnut, quince, pear, almond, plum, cherry), showed that all isolates from any host would infect other hosts although considerable variation in aggressiveness was noticed. No evidence for host specificity was observed. The growth rate of *Botryosphaeria* isolates from different hosts at different temperatures (5, 10, 15, 20, 25, 30, 33, 37°C) on PDA was also studied.

Relation of esca foliar symptoms to rainfall and rainfall-related parameters. P. Braccini[1], F. Calzarano[2], A. Dalla Marta[3], S. Di Marco[4], G. Marchi[5], L. Mugnai[5], F. Peduto[5], S. Orlandini[3], F. Osti[4] and G. Surico[5]. [1]*A.R.S.I.A. Regione Toscana, Via Pietrapiana 30, 50100 Firenze, Italy.* [2]*Dipartimento di Scienze degli Alimenti, Università di Teramo, Via Spagna 1, 64023 Mosciano S.A., Teramo, Italy.* [3]*Dipartimento di Scienze Agronomiche e Gestione del Territorio Agro-forestale, Università di Firenze, Piazzale delle Cascine 18, 50144 Firenze, Italy.* [4]*Istituto di Biometeorologia, Sezione di Bologna, CNR, via Gobetti 101, 40129, Bologna, Italy.* [5]*Dipartimento di Biotecnologie Agrarie, Sezione di Patologia vegetale, Piazzale delle Cascine 28, 50144 Firenze, Italy.*
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Outbreaks of esca were monitored in Tuscany, Emilia Romagna and Marche, Italy. Data and analyses were consistent with previous observations. Random or near-random distribution of diseased vines was observed in vineyards with low disease incidence (<10%). In vineyards with a disease incidence over 10–15%, infected vines were nearly uniformly distributed or tended to aggregate in clusters. None of the analyses detected a preferential spread of the disease from plant to plant along columns or in any other direction. But though diseased vines did not often influence immediately adjacent plants, infected vines did appear to be aggregated within 10–12 plant-spacings of each other in all directions. A single-point source of outbreaks could not be identified: several foci were present in the vineyards surveyed. From the time the outbreaks started (in some vineyards two or three years after establishment) disease incidence increased in almost linear progression. Weather data were compared with esca incidence over 4-to-10-year periods in all vineyards. to look for a relation between annual esca incidence and the number of days with rain, total rainfall during the entire growth period, and rainfall in single months. The results seem to confirm what was previously reported, that cool, rainy growing seasons are more favourable to the emergence of foliar esca symptoms.

Enviro-spatial distribution of grapevine trunk pathogens in South Africa. J.M. van Niekerk[1], W. Bester[1], U. Damm[1], F. Halleen[2], P.W. Crous[3] and P.H. Fourie[1]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa.* [2]*ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch*

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Recent studies have identified several trunk pathogen complexes from grapevines in South Africa. These include *Eutypa lata, Phaeomoniella chlamydospora*, *Phaeoacremonium*, *Botryosphaeria* and *Phomopsis* spp. Climatic differences between grape growing regions are likely to affect the demographics of regional pathogen populations. In order to develop management strategies for specific regions, it was therefore necessary to determine the distribution of grapevine trunk pathogens in the different grape-growing regions of South Africa. During 2003 and 2004, a survey of visually healthy grapevines was conducted in 15 wine (Cabernet Sauvignon) and 15 table grape (Dan-ben-Hannah) vineyards that were 10 years and older. These vineyards were situated in the summer-, intermediate- or winter-rainfall regions of South Africa. The occurrence of trunk pathogens was determined by making isolations from areas with wood decay symptoms, observed in cross sections of the samples taken (distal portions of arms). Symptoms observed were brown internal necrosis (BIN), black (Bl-st) and brown (Br-st) streaking, esca-like yellow (E-Y) and brown (E-B) soft rot, V-shaped necrosis (V-N) and watery necrosis (W-N). Results indicate that *Pa. chlamydospora* was isolated significantly more often in the winter-rainfall region (19%) than in the intermediate-rainfall (8.9%) and summer-rainfall (3.1%) regions. In the winter-rainfall region, the fungus had a broad symptom profile, whereas in the other regions it was isolated mainly from BIN and Bl-st. *Phaeoacremonium* spp. had similar distribution patterns and symptom profiles, but were isolated at slightly higher levels in the summer rainfall region. *Botryosphaeria* spp. were isolated most frequently from the intermediate-rainfall (12.4%) and summer-rainfall (5.9%) regions and mostly from BIN, V-N and Bl-st. *Phomopsis* spp. were isolated mostly from the winter- (3.4%) and intermediate-rainfall (2.8%) regions, with a varying symptom profile between regions. *E. lata* was infrequently isolated and only from the winter-rainfall region. Once all isolates have been identified to species level, regional management strategies will be formulated accordingly.

Black foot disease in South African vineyards and grapevine nurseries. F. Halleen[1], P.H. Fourie[2] and P.W. Crous[3]. [1]*ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa.* [2]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa.* [3]*Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.*
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The survival rate of grapevine cuttings in nurseries, and young grapevine plants in newly established vineyards in South Africa is being increasingly compromised by root and trunk pathogens. *Cylindrocarpon* spp., which cause black foot disease of grapevine, were associated with 52, 22 and 29% of diseased grapevines during 1999–2002, 2002–2003 and 2003–2004 respectively. However, very little information is available regarding the aetiology and epidemiology of the pathogens believed to be involved in black foot disease. The primary aims of research have been (1) to conduct nursery surveys in order to determine which fungi are involved in the decline phenomenon, (2) to identify the organisms believed to be the causal organisms of black foot disease, and (3) to develop management strategies to prevent or eradicate these infections. The diversity of species associated with black foot disease was confirmed by this study and four *Cylindrocarpon*-like species were identified: *Cylindrocarpon destructans*, *C. macrodidymum, Campylocarpon fasciculare* and *Campyl. pseudofasciculare*. The observation that these species infect grapevine cuttings in nursery soils has clearly placed the emphasis on the importance of suitable measures to prevent or eradicate these infections. However, none of the chemical and biological treatments evaluated in this study prevented infection of nursery grapevines. On the other hand, the reduction of infection in uprooted dormant nursery grapevines caused by hot water treatment clearly demonstrated the potential of this control measure. Apart from these pro-active management strategies in grapevine nurseries, no curative strategy is known for declining grapevines in vineyards. Producers are therefore urged to prevent or correct predisposing stress situations, such as unbalanced root development, soil compaction and poor drainage.

Petri disease: potential inoculum sources in South African grapevine nurseries. E. Retief, U. Damm, A. Mcleod and P.H. Fourie. *Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland (Stellenbosch) 7602, South Africa.*
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Petri disease of grapevine is primarily caused by *Phaeomoniella chlamydospora*. This pathogen affects mostly young grapevines, but is also implicated in esca disease of older grapevines. Little is known about the disease cycle of this fungus. Infected propagation material was identified as a major means of dissemination of the pathogen. Recently, the pathogen was also detected from soil in South Africa, and airborne conidia have been trapped in vineyards. The aim of this study was to use a molecular detection technique to test different samples (water, soil, rootstock and scion cuttings and callusing medium) collected from nurseries in South Africa at different nursery stages for the presence of *Pa. chlamydospora*. All samples were tested using a DNA extraction and species-specific PCR protocol that was previously developed and validated. *Pa. chlamydospora* was detected from the rootstock cuttings, hydration water and soil. These media can therefore be considered possible inoculum sources of the pathogen. Management strategies should include pruning-wound protection of rootstock mother plants, pathogen eradication from rootstock cuttings (e.g. by hot water treatment), biological or chemical amendments in hydration water and prevention of wound-infection of grafted cuttings in nursery soils.

The occurrence of Petri disease and esca and their causal organisms in Australia. J. Edwards[1,2] and I.G. Pascoe[2]. [1]*Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond, South Australia 5064, Australia.* [2]*Primary Industries Research Victoria – Knoxfield Centre, Department of Primary Industries, Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia.*
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Over a five year period (1998–2002), 124 samples of diseased grapevines sent to the Department of Primary Industries, Knoxfield, in Victoria were diagnosed with the grapevine decline diseases, Petri disease and esca. The proportion of grapevines with Petri disease (82%) proved to be much more than those with esca (18%). In addition, *Phaeomoniella chlamydospora* was isolated from all but two of the samples, whereas *Phaeoacremonium aleophilum* was isolated from only nineteen samples. Other fungi associated with grapevine decline diseases, such as species of *Phomopsis, Cylindrocarpon, Botryosphaeria* and heart-rotting basidiomycetes, were also isolated but not consistently. Thirty-two different grapevine cultivars were represented, and samples were received from most major grape-growing regions of New South Wales, South Australia, Victoria and Western Australia. The majority (62%) were ungrafted grapevines. *Pa. chlamydospora* was also found to be present in grapevines with no external symptoms of disease, including mother grapevines used for propagation.

Stone fruit trees as alternative hosts of grapevine trunk disease pathogens. U. Damm[1], P.W. Crous[2] and P.H. Fourie[1]. [1]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa.* [2]*Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.*
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Grapevine (*Vitis vinifera*) and stone fruits (several *Prunus* species) are often grown in close proximity to each other. Both species of woody plants are attacked by fungal trunk disease pathogens, for example *Botryosphaeria*, *Phomopsis* and *Eutypa* species. The aim of this study was to determine whether stone fruits are inhabited by known grapevine trunk disease pathogens as either pathogens or saprobes, and whether stone fruits could act as alternative hosts of grapevine trunk disease pathogens. A survey of wood-inhabiting fungi of plum and peach trees from the Western Cape province in South Africa was conducted by sampling living wood with dieback, canker or necrotic symptoms as well as pruning debris. Fungi occurring in/on the samples were isolated and morphologically and phylogenetically characterised. DNA sequences of the 5.8S ribosomal gene with the two flanking internal transcribed spacers (ITS-1, ITS-2) were compared to those of known taxa currently available in GeneBank. The genus *Botryosphaeria* dominated among the fungi isolated from necrotic plum wood, and its fruiting structures were often observed on dead peach wood. Ribosomal ITS sequences of the isolates were the same as or very similar to those

isolated from grapevine and belonging to *B. obtusa*, *B. australis*, *B. rhodina*, *B. stevensii* and *Fusicoccum vitifusiforme*. Based on sequence comparison analysis we also identified *Togninia minima* (anamorph: *Phaeoacremonium aleophilum*) and *Phomopsis amygdali* from necrotic plum wood. All these species are reported to be pathogenic on grapevine. According to these results, stone fruit orchards should be considered a potential inoculum source of grapevine trunk disease pathogens. Further examinations will include similar surveys in climatically different areas of South Africa and pathogenicity trails on grapevine and stone fruit wood.

Spatial distribution of esca symptomatic plants in Dão vineyards (Centre Portugal) and isolation of associated fungi. J. Sofia[1], M.T. Gonçalves[2] and H. Oliveira[3]. *[1]DRABL, CEVDão, Quinta da Cal, 3520-090 Nelas, Portugal. [2]Dep. Botânica, FCTUC, Universidade de Coimbra, 3000 Coimbra, Portugal. [3]Laboratório de Patologia Vegetal "Veríssimo de Almeida", Tapada da Ajuda, 1399 Lisboa Codex, Portugal.*
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In recent years there has been a noticeable increase in the incidence of esca disease in the Portuguese wine region of Dão. Since wine grape production is the most important agricultural activity of this region the occurrence of esca is of great concern and deserves our attention. The objectives of this study were to evaluate the spatial distribution of symptomatic plants and to investigate the fungi associated with diseased wood in these grapevines. The plot studied, with an area of ca. 4000 m^2, comprised 1296 grapevines cv. Touriga Nacional grafted on SO4 rootstock and was located in a characteristic 11 ha vineyard. Vines were planted in 1989. During three years (2002–2004) the presence/absence of foliar symptoms of esca in each vine was recorded as well as any dead vines. Surveys took place after the first symptoms on the leaves were noticed, especially between June and September, with an average of one visit per month. Occurrence maps were drawn each year, and cumulative maps in the last two years. After the harvest, dead and strongly symptomatic grapevines were uprooted; transversal slices of the trunk were cut off and small portions of deteriorated wood tissues used for mycological analysis. Annual disease incidence increased over the three-year sampling period: 2.4% in 2002, 3.55% newly infected vines in 2003 and 5.25% in 2004 (until September). The fungi most frequently isolated were *Phaeoacremonium* spp., *Phaeomoniella chlamydospora*, *Fomitiporia punctata* and *Fusicoccum* sp. Annual and cumulative field maps will be presented as well as an index of dispersion of diseased plants.

Relationship between incidence of esca and black dead arm foliar symptom expression in the vineyard, ecophysiological indicators and cultural practices. L. Guérin-Dubrana[1], A. Destrac-Irvine[1], J.P. Goutouly[2], A. Letouze[2] and J.P. Gaudillère[2]. *[1]U.M.R Santé végétale INRA / ENITAB, CR Bordeaux, BP 81 33 883 Villenave d'Ornon, France. [2]U.M.R Oeno-Ampélologie INRA / Faculté d'œnologie, Université Bordeaux II, CR Bordeaux BP 81 33 883 Villenave d'Ornon, France.*
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Esca or black dead arm (BDA) foliar symptoms were recorded at different frequencies in vineyards of the same variety and similar age in the same area of production. This may be explained as due to variation in (i) inoculum dispersal of the fungi associated with these syndromes, (ii) contamination-related factors, (iii) the physiological status of the vine before and after infection and (iv) cultural practices. In order to evaluate variations in symptom expression of esca and BDA and to study its relationship to host and environmental factors, a 5-year field survey was set up around Bordeaux in 2004. Twenty-four vineyards of about 15-year-old Cabernet Sauvignon vines trained to a Guyot Double trellis system were selected. The frequency and severity of BDA and esca symptoms on 2000 vines in each vineyard were recorded at two dates in summer. Pedological and ecophysiological indicators were measured to estimate the water, nitrogen and carbon nutrition status of the vines and their vegetative vigour. Cultural practices were also recorded. Assessed disease results show a large range of vines with BDA and esca foliar symptoms: from 0 to 12.9% and from 0 to 30.8% respectively. To investigate the relationship between the incidence of diseases with physiological and site traits, collected data were analysed by means of principal component analysis (PCA).

Long term vineyard monoculture influences the rhizosphere microbial communities. M.

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Root colonising *Pseudomonas fluorescens* strains, which produce 2,4-diacetylphloroglucinol (Phl), are biocontrol agents against fungal pathogens in different pathosystems. The secondary metabolite Phl is toxic to certain bacteria, fungi and nematodes and plays a key role in plant protection. Four vineyards were identified where cultures of up to 1653 years old were growing next to others of 9–70 years. Long-term cultivation increases (i) the diversity of the soil bacteria in the deep (60–120 cm) soil layers, (ii) the content of calcite in these deep layers and (iii) the frequency of the grape root associated pseudomonads carrying the key biocontrol gene *phlD*. The copper added during cultivation accumulated at the top of the soil profile following the soil organic matter. This project is funded by the Swiss National Competence Centre for Research "Plant survival".

Disease management

Integrated strategies for pro-active management of grapevine trunk diseases in 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.*
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Phaeomoniella chlamydospora and *Phaeoacremonium* spp. (causal organisms of Petri disease), *Cylindrocarpon* and *Campylocarpon* spp. (causal organisms of black foot rot), *Botryosphaeria* spp. (causal organisms of black dead arm) and *Phomopsis* spp. (causal organisms of *Phomopsis* cane and leaf blight and dead arm) are economically important grapevine trunk disease causing pathogens, which can infect grapevines through wounds made during nursery processes or through unprotected pruning wounds at later vineyard stages. Given long periods prior to external symptom expression in plants and/or stress predisposition of grapevines to these diseases, internal wood symptoms are usually far advanced by the time they are noticed. No curative control measures for these diseases are known and remedial management strategies in established and mature grapevines are expensive and in most cases only marginally effective. Research into the management of these diseases has therefore focused on pro-active management during the nursery stages in order to prevent and eradicate contamination and infection of propagation material and nursery grapevines, and consequently to ensure the production of healthy, pathogen-free grapevines. Several semi-commercial nursery trials were conducted to study the effect of various treatments on the occurrence of natural infections of grapevine trunk disease pathogens in graft unions, rootstocks and/or roots of nursery grapevines. Results from these projects culminated in an extensive management strategy that integrated pruning wound protection in rootstock mother blocks, chemical or biological amendments in hydration water, hot water treatment of rootstock cuttings and/or dormant nursery plants, hygienic grafting, wound protection during callusing and in field nursery soils and soil amendments with *Trichoderma* formulations.

The effects of hot water treatment on grapevine cutting physiology and cell ultrastructure. H. Waite[1,2], J. Faragher[3] and G. Jaudzems[4]. [1]*Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond, South Australia,506, Australia.* [2]*Institute of Land and Food Resources, University of Melbourne, Dookie Campus, Dookie College, Victoria, 3647, Australia.* [3]*Department of Primary Industries, Knoxfield Centre, Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia.* [4]*Monash Microimaging, School of Biological Sciences, Monash University 3800, Victoria, Australia.*
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Hot water treatment (HWT; 50°C/30min) of dormant grapevine cuttings is a useful measure to control endogenous pathogens including *Phaeomoniella chlamydospora*. However, there are reports of sporadic losses of HWT cuttings. To determine the underlying cause of cutting failure, the effects of HWT on respiration and cell ultrastructure were investigated. Emission of CO_2

in Cabernet Sauvignon, Chardonnay and Pinot Noir cuttings collected in early, mid- and late winter was measured before, and 1 and 24 hours after HWT and after 14 weeks cold storage. In early and mid season the pre-HWT respiration rate of Chardonnay was higher ($P<0.05$) than that of Pinot Noir and Cabernet Sauvignon, but by late season the respiration rate of Pinot Noir was highest and Cabernet Sauvignon lowest ($P<0.05$). One hour after HWT, there was a rise ($P<0.05$) in all respiration rates except that of early-season Chardonnay. After 24 hours the respiration rates of all cuttings had fallen ($P<0.05$). Respiration rates after cold storage were variable. Ethanol emission was measured in Cabernet Sauvignon and Pinot Noir cuttings 24 hours and 1 month post HWT and detected in HWT cuttings 24 hours post HWT. High post-HWT respiration rates and emission of ethanol by HWT cuttings indicated that HWT tissue became anoxic in the immediate post-HWT period. Preliminary transmission electron microscopy examination of ray tissue in Cabernet Sauvignon 24 hours post HWT showed ruptured amyloplasts, fractured cell walls and increased ribosomal activity in HWT cuttings +/- hydration and hydration only. We conclude that HWT is a significant stress for grapevine cuttings and that therefore post-HWT handling is likely to be critical to cutting viability.

Experiences with amelioration treatments trialed on Petri disease in Australian vineyards. J. Edwards[1,2] and I.G. Pascoe[2]. [1]*Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond, South Australia 5064, Australia.* [3]*Department of Primary Industries, Knoxfield Centre, Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia..*
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This paper reports on the results of four field trials investigating amelioration treatments for Petri disease of grapevines. Treatments included applications of compost, nutrient fertilisers, extra water, phosphonates and Brotomax. Vine health was monitored for two to five years. To summarise our experience with field trials to date, development of management strategies to deal with infected vineyards has been very difficult because no single treatment has proven effective. Despite this, the vines recovered from Petri disease over time. The evidence suggests that stress is a major factor in development of the disease, particularly stress due to inadequate irrigation and overcropping before the vine has established a decent root system, and if the stress is relieved the vines will recover. It is still unknown, however, whether recovery is permanent or whether disease expression will re-occur some time in the future.

Degradation of trunk disease toxins by fungal antagonistic strains as a new prospect in biocontrol strategies against Eutypa dieback and esca disease on grapevine. D. Christen[1], M. Tharin[2], S. Perrin-Cherioux[2], E. Abou-Mansour[2], R. Tabacchi[2] and G. Défago[1]. [1]*Phytopathology Group, Institute of Plant Sciences, Swiss Federal Institute of Technology, Zürich, Switzerland.* [2]*Analytical and Structural Organic Chemistry, University of Neuchâtel, Switzerland.*
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Eutypine, 4-hydroxybenzaldehyde and 3-phenyllactic acid are some of the phytotoxins produced by *Eutypa lata* causing Eutypa dieback, and by *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp. and *Fomitiporia mediterranea* the causal agents causing esca. In order to develop a biocontrol strategy for disease prevention, known biocontrol agents such as *Fusarium lateritium* and *Trichoderma* sp. were tested for their ability to degrade these toxins. Detoxification processes were investigated by incubating these potential biocontrol agents in a liquid medium containing the individual pure toxins. An HPLC-based method using UV- and MS-detection was developed to analyse toxin metabolisation, and a quantification of degradation products was performed. The results show that the aldehyde function was reduced to the corresponding alcohol and oxidised to the acid form simultaneously for eutypine and for 4-hydroxybenzaldehyde. While the *Trichoderma* strains degraded completely every toxin tested, the *F. lateritium* strains degraded eutypine and 4-hydroxybenzaldehyde but not 3-phenyllactic acid. In biological assays on cells of *Vitis vinifera* cv. Chasselas, the degradation products exhibited a lower toxicity than the toxins. The possibility of developing new control strategies against trunk diseases of grapevine based on microbial detoxification is discussed.

Wood-inhabiting fungi isolated from New Zealand grapevines and the potential for protective control with *Trichoderma*. I.C. HARVEY[1] and J.S. HUNT[2]. *[1]Plantwise Services Ltd, PO Box 181, Lincoln, New Zealand. [2]Agrimm Technologies Ltd, PO Box 13-245, Christchurch, New Zealand.*
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The incidence of disease-causing fungi in samples received from the major winegrowing regions of New Zealand was assessed from August 2000 until December 2003. The pathogens were isolated from sections taken from the roots, rootstocks, graft unions, bud wood and stems of grapevine wood in specimens showing vineyard symptoms and sent to the Lincoln laboratory for diagnosis. The four most common pathogens isolated from all 37 samples were *Botryosphaeria* spp. (68%), *Phomopsis* (40%), *Cylindrocarpon destructans* (27%) and *Phaeomoniella chlamydosphora* (19%). The *in vitro* biocontrol potential of a cohort of 8 *Trichoderma harzianum* and *T. atroviride* antagonist strains were assessed in a dual inoculum culture system against five species of *Botryosphaeria* isolated from these samples (*B. dothideae*, *B lutea, B. obtusa, B. parva* and *B. stevensii).* Morphological changes observed in the mycelium of these *Botryosphareria* spp. at the interface with the *Trichoderma* included abutment and coiling associated with myco-parasitism by the *Trichoderma* as well as evidence of cell lysis. There was no evidence of antibiosis in any of the pathogen/antagonist combinations. Sauvignon blanc vines on SO4 rootstock were inoculated at the graft union with *Trichoderma* at the time of grafting (14 reps), rooted and compared with the untreated controls after growing under glasshouse conditions. Vines were destructively analysed (2 reps of each group) by sampling each plant in 10 locations from 5 cm below the graft union to 5 cm above. *Trichoderma* was found to have grown 5 cm into the rootstock wood and for varying distances into the scion wood. *Trichoderma* was not isolated from the new cordon growth or from any of the control samples. *B. parva*, *Phomopsis* spp. and *Alternaria alternata* were isolated from the graft union and scion wood of both control plants. These results suggest that a) *Botryosphaeria* and *Phomopsis* spp. and *C. destructans* may be more prevalent in New Zealand vineyards than previously thought; b) *Trichoderma* inoculation of grafted material may have potential in protecting against wood-infecting pathogens vines intended for vineyard planting stock.

Biological control of Eutypa dieback in Australia: field evaluation of fungal antagonists. S. JOHN[1], J. MALONE[1], E.S. SCOTT[1], T.J. WICKS[2] and J.S. HUNT[3]. *[1]The University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Australia. [2]South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001, Australia. [3] Agrimm Technologies Ltd, PO Box 13-245, Christchurch, New Zealand.*
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Eutypa dieback, caused by *Eutypa lata*, reduces the longevity of premium vineyards in Australia. Since ascospores of the pathogen enter the vine via wounds, wound protection offers a means of control. Preliminary research on the biological control of *E. lata* using *Trichoderma harzianum* and *Fusarium lateritium* was presented at the 3rd International Workshop on Grapevine Trunk Diseases (February 2003, Christchurch, New Zealand). Five trials were established over 3 years using mature vines of cv. Cabernet Sauvignon, Shiraz, Rondella and Palomino at various sites in South Australia. One-year-old canes were pruned in winter (July–August), treated with *T. harzianum* or *F. lateritium* within an hour of pruning, and inoculated with spores of *E. lata* 1 or 14 days later. *T. harzianum* was applied as a spore suspension or in commercial formulations (Trichoseal Spray™ or Vinevax™), *F. lateritium* as a spore suspension only. Colonisation of the spurs by *E. lata* was assessed by isolation after 10 weeks to 11 months. Also, the colonisation of vines of cv. Nyora by *T. harzianum*, applied as Trichodowels inserted into the trunk, was assessed over 20 months. Interactions of *T. harzianum* and *F. lateritium in vitro* were examined to assess the potential for dual application of the antagonists. Recovery of *E. lata* from inoculated controls varied and was greatest (77% of spurs) when wounds were inoculated 1 day after wounding in July (mid-winter). Pruning wounds treated with *T. harzianum* or *F. lateritium* showed reduced incidence of infection by *E. lata* in most experiments when the wounds were challenged 1 and 14 days after treatment with the antagonist. *T. harzianum* spread as far as the crown of the Nyora vines and persisted for at least 20 months in some

vines. *T. harzianum* inhibited growth of *F. lateritium in vitro*. *T. harzianum* and *F. lateritium,* applied separately, have potential to protect wounds from infection by *E. lata*. The spread and persistence of *T. harzianum* in the vine suggest that it may also offer systemic protection.

Field evaluation of pruning wound treatments for control of Eutypa dieback. M.R. Sosnowski[1,2], M.L. Creaser[1,2] and T.J. Wicks[2]. *[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.*
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Eutypa dieback causes a gradual decline of grapevine production in many wine regions around the world. Ascospores of the fungus *Eutypa lata* enter via pruning wounds and colonise the wood tissue in the cordons and trunks. Pruning wound protectants can be used to reduce infection, especially on larger wounds. In Australia, the only product currently registered for this purpose is Vinevax™ pruning wound dressing (*Trichoderma* sp., formerly Trichoseal®) and this study may lead to the registration of additional products. Trials were conducted on Cabernet Sauvignon vines in the Barossa Valley, South Australia, between 2000 and 2003 to evaluate the efficacy of various fungicides, a biocontrol agent and paint as wound protectants against *E. lata* infection. Pruning wounds were made in winter (August) on 1-year-old canes, and the treatments applied with a paintbrush within 1 hour. Wounds were inoculated with 500 ascospores of *E. lata* either 1 day before or 1, 7 or 14 days after treatment. Canes were removed in the following winter and the presence or absence of *E. lata* was determined by isolation on potato dextrose agar with streptomycin. Benlate® (benomyl), Bavistin® (carbendazim), Solucuivre® (copper and carbendazim), Garrison® (cyproconazole and iodocarb in paste) and ATCS Tree Wound Dressing (acrylic paint) reduced infection by *E. lata* at all inoculation times. Fungaflor® (imazalil sulphate), Scala® (pyrimethanil), Cabrio® (pyraclostrobin), Bayfidan® (triadimenol), Teldor® (fenhexamide) and Topas® (penconazole) reduced infection, but not at all inoculation times. Trichoseal® (*Trichoderma* sp.) reduced infection when the pathogen was applied 14 days after treatment. With the recent withdrawal of benomyl products from the market, alternatives such as carbendazim fungicides or acrylic paint are suitable for preventing infection of pruning wounds by *E. lata*. Furthermore, our study identified several other fungicides which have potential as pruning wound protectants and further testing is under way.

Effect of fosetyl Al foliar applications towards esca fungi in grapevine. S. Di Marco and F. Osti.
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This study reports on the activity and effect of fosetyl Al applications on esca and associated pathogens. Different trials were conducted treating potted grafted vines with fosetyl Al formulations. *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora* were then inoculated into the rootstock. Assessments were made by measuring the average length of wood discolouration and investigating *in vitro* the viability of the fungi. Field trials were carried out either before or soon after the appearance of the disease in properly managed vineyards with vigorous vines and abundant foliage. About 4500 vines in three vineyards were assessed annually by visual inspection of foliar symptoms, vine mortality and esca incidence. Six fosetyl Al treatments were given annually for 4 to 6 years with the routine plan for downy mildew control and were compared to vines that were never treated with the fungicide. Commercial formulations of fosetyl Al in combination with mancozeb and cymoxanil, and/or copper oxychloride were used. The effects of fungicide application on plant physiology were continuously assessed by a gas exchange measurement system. Most of the greenhouse trials showed a reduction in the average length of internal necrosis, especially *P. aleophilum* necrosis, in fosetyl Al treated plants compared with the control. Both fungi were re-isolated from the treated vines. Field trials generally showed that mortality of grapevines was not influenced by fosetyl Al treatment. Fungicide application generally led to a decrease in the number of vines showing esca symptoms in the year compared to the control. The incidence of esca in the fosetyl Al treated vineyards at the end of the trials was lower than that in the control. The net photosynthetic rates of treated plants were depressed, especially when fosetyl Al

+ mancozeb + cymoxanil was applied. This decrease in photosynthesis probably affected parameters associated with transport inside the plants. Although the production of foliar symptoms is not yet well understood, a relationship between physiological effects caused by fosetyl Al and foliar symptom expression cannot be excluded. Our findings indicate that fosetyl Al decreased the severity of esca. The activity seemed to be linked to the favourable combination between the fungicide mode of action and the particular condition of esca-affected vines.

Agronomical approach to reduce esca disease spread in the vineyard. G. Scalabrelli[1], G. Ferroni[1], C. D'Onofrio[1] and A. Felicioli[2]. *[1]Dipartimento di Coltivazione e Difesa delle Specie Legnose, Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italy. [2]Dipartimento di Anatomia Biochimica e Fisiologia Veterinaria, Università di Pisa, Viale delle Piagge, 56124 Pisa, Italy.*
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Pruning wounds are one of the main infection courts for the fungal agents of esca. A trial was set up to evaluate the outcome of suitable cultural practices aimed at limiting or avoiding contamination during pruning. A 22-year-old cv. Sangiovese vineyard (2 ha) was divided in two plots of 1 ha in order to set up two treatments. In the first plot, pruning was carried out using pneumatic scissors mounted on a tractor equipped with a pressurised container that sprayed a water solution (1/5 by volume) of sodium hypochlorite (8%) on the cutter to keep the cut surface clean. The second plot, taken as the control, was pruned in the same way but no spray was applied. The treatment was repeated for 7 years, and every year the vines showing leaf and shoot symptoms of esca were recorded. After seven years the vines showing clear symptoms of esca (leaf discoloration, necrosis and shoot drying) were removed and replaced the following year with new vines. In the control plot symptomatic vines were not removed. Disease incidence at the beginning of the trial was around 3% in both plots, and after three years it reached about 16%, with no difference between treatments. Starting from the fourth year, however, the control plot showed a rapid increase of symptomatic plants, while in the sprayed plot the incidence increased more slowly, so that after 7 years it was 27% in the treated plot and 63% in the control plot. The annual percentage of symptomatic plants followed a different pattern between the two treatments: in the pruned sprayed plots it was represented by a quadratic model ($y= -0.6463x^2 + 9.3639x – 6.6939$; $R^2=0.9916$), while the control plot showed a power pattern ($y=2.7507x^{1,593}$; $R^2=0.9992$). This difference suggests that spraying the cutter and the pruning cuts significantly reduced the number of symptomatic esca plants. This technique deserves to be better evaluated as a means to reduce esca spread in the vineyard.

Survival of fungi associated with grapevine wood declines in pruned material following composting. P. Lecomte[1], G. Louvet[1], B. Vacher[1] and P. Guilbault[2]. *[1]Institut National de la Recherche Agronomique, UMR Santé Végétale (INRA-ENITAB), CR Bordeaux-Aquitaine, Avenue Edouard Bourleaux, BP 81, 33883 - Villenave d'Ornon cedex, France. [2]Chambre d'Agriculture de la Gironde, 39 rue Michel Montaigne, BP 115, 33294 - Blanquefort, France.*
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In viticulture, a range of management practices exist to deal with canes or undesirable debris removed during the pruning period in winter. Among these, grinding, composting and reapplication in the vineyard are the most efficient methods for recycling this waste. However, such practices may be thought to pose a risk of recontamination with wood decline fungi. To examine the survival of four ascomycete fungi (*Eutypa lata, Botryosphaeria obtusa, Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*) in composted cane material, a 3-year-study was initiated in 2002 in a commercial vineyard located at Pauillac (Médoc). In each of the three years, about 140 m^3 of pruned shoot material was ground and mixed with 125 m^3 of sheep dung and 60 m^3 of stalks and garden residues (leaves, grass). The internal temperature of the compost increased to 75°C a few hours after mixing and thereafter fluctuated between 50°C to 75°C during the six-month period of composting. The compost was regularly wetted. To assess the effect of composting on the survival of fungi, naturally or artificially infested material (labelled pieces of wood) were incorporated into the compost prior to composting in 2003 and 2004. Samples were examined by classical isolation methods with or

without disinfection with calcium hypochlorite. Results were consistent over the years. Before composting, only *B. obtusa* was isolated from ground canes and all introduced infested samples were viable. However, after composting, no mycelial development was observed from ground cane material or introduced samples for any of the fungi (except some colonies of *B. obtusa* in 2003 from one piece of wood). These results clearly showed that composting eradicates the target fungi. We conclude that composting not only reduces inoculum pressure and thereby the incidence of some grapevine declines, but can also be safely recommended to growers as not furnishing a potential source of inoculum for wood declines.

Growth control of *Inocutis jamaicensis* by antagonistic fungi *in vitro*. G. Escoriaza[1], C. Césari[1], M. Césari[2] and M. Gatica[1]. [1]*Instituto Nacional de Tecnología Agropecuaria, EEA Mendoza, San Martín 3853, Mayor Drummond, 5507, Luján de Cuyo, Mendoza, Argentina.* [2]*Instituto Tecnológico de Buenos Aires, Av. E. Madero 399, 1106, Capital Federal, Argentina.*
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The effectiveness of *Trichoderma harzianum*, *Clonostachys rosea* and *Epicoccum purpurascens* against *Inocutis jamaicensis* (Murril) Gottlieb, J.E. Wright & Moncalvo, the basidiomycetous species associated with hoja de malvón grapevine disease in Argentina, was investigated. *In vitro* assays to determine the forms of antagonism were carried out. The fungi were grown on malt extract agar (MEA). Colony growth was measured and results were expressed as colony diameters and percentage inhibition of radial growth. All fungi were found to have an antagonistic effect by competition and produced soluble antifungal metabolites against the target fungus at varying distances. Statistical analysis indicated that *T. harzianum* strain II produced the greatest inhibition in dual culture. It also produced volatile and non-volatile compounds inhibiting mycelial growth. Microscopic examination by phase contrast revealed lysis of the phytopathogenic fungal mycelium only by *T. harzianum* strain II. Further experiments to improve the biocontrol of this strain in mature field-grown grapevines are under way.

Study of the possibility to control *Eutypa lata* (Pers. Fr.) Tul. in grapevine. V.A. Bourbos and E.A. Barbopoulou. *NAGREF, Institute of Olive Tree and Subtropical Plants of Chania, Laboratory of Plant Pathology and Ecotoxicology of Plant Protective Products, Agrokipio, 73100, Chania, Greece.*
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In Crete (Greece) during the last few years, serious damage has been caused by the wood fungus *Eutypa lata* (Pers. Fr.) Tul. (=*E. armeniacae*) to table grape as well as wine grape varieties. Apart from precautionary and cultural practices, the only effective chemical method after the ban on sodium arsenite is the utilisation of benomyl in the form of a paste. However, this drastic substance is difficult to apply and is also prohibited. This trial examined whether the pathogen responsible for Eutypa dieback could be controlled by carbendazim (100 gr hl^{-1} of the commercial product Bavistin 50 WP), fluazinam (150 ml hl^{-1} of the commercial product Ohayo 50 SC) or the biological product Promot, containing *Trichoderma harzianum* Rifai and *T. koningii* Oudem (100 g hl^{-1}). These products were used either alone or in combination with the biological product in two applications at a 12-day interval. Pruning wounds were artificially contaminated with a solution of 1.2×10^4 ascospores one hour after cutting and were sprayed with the products one day later. Products were judged effective if they prevented the appearance of circular sectors of infected wood. Best results were observed when fluazinam was used alone at one or two applications and carbendazim at two applications (100%). The biological product alone controlled the pathogen with an effectiveness of 40–54.6% after one application and 80–81.8% after two applications. The combination of the biological product with the fungicides achieved an effectiveness ranging from 81.8 to 100%.

Managing grapevines infected with Eutypa dieback using remedial surgery. M.R. Sosnowski[1,2], M.L. Creaser[1,2] and T.J. Wicks[2]. [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.*
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Eutypa dieback, caused by the wood colonising fungus *Eutypa lata*, is a major disease of grapevines worldwide. Characteristic symptoms include stunt-

ed shoots and a wedge of discoloured tissue in the wood. The disease reduces productivity and longevity in established vineyards, particularly of premium red cultivars such as Shiraz, Cabernet Sauvignon and Grenache. Two methods of remedial surgery were evaluated in 11 trials established in commercial vineyards across South Australia; i) the "cut and train" method, which involves cutting off the trunk 30–40 cm above the ground and training a watershoot to replace the lost canopy and ii) the "train and cut" method, where cordons are removed and the lowest healthy shoot on the trunk is trained to form a new canopy, followed by removal of the infected trunk. Watershoot production and recurrence of foliar symptoms of eutypa dieback were monitored over 1–5 years. Watershoot production was 40% greater for Pinot Noir and Malbec than for Shiraz and Cabernet Sauvignon when the cut and train method was used. Watershoot production was 30% less on vines subjected to the cut and train method compared to vines that were trained and then cut. Foliar symptoms first appeared 4 years after surgery in vines cut and trained (0.1–1.4% of vines). In one trial, foliar symptoms were observed on 3% of vines 1 year after train and cut surgery. The presence of infected wood remaining below the watershoot may influence the reappearance of foliar symptoms. Hence, regardless of the remedial surgery method, if the pathogen is not completely excised, the longevity of the reworked vines may be limited. Cultivar and environment may also be important, however. Long term monitoring and more trials are necessary to test this hypothesis.

Effect of Brotomax™ applied to Eutypa dieback-affected grapevines in South Australia. M.R. Sosnowski[1,2], M.L. Creaser[1,2] and T.J. Wicks[2]. *[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.*
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Eutypa dieback, caused by the wood infecting fungus *Eutypa lata*, is a major disease of grapevines worldwide causing a gradual decline in vineyard production. Characteristic symptoms include stunted shoots and a wedge of discoloured tissue in wood. Disease management strategies are generally aimed at preventing infection. Options for restoring infected vines are limited to removal of infected tissue. The ability of Brotomax™, a liquid fertiliser that stimulates the synthesis of phenolic compounds in fruit and vegetable crops, to alleviate foliar symptoms and increase yield was evaluated. Brotomax™ was applied to own-rooted Shiraz (planted 1971) and Cabernet Sauvignon (1960) vines on two commercial properties in Eden Valley and McLaren Vale, South Australia over three growing seasons between 2001 and 2004. At both sites, vines naturally infected with *E. lata* were sprayed i) when shoot length reached 20–30 cm, ii) 20–25 days later, iii) at bunch closure, and iv) 15–20 days after harvest. All sprays were applied at a rate of 10 ml l^{-1} to leaves and trunks with a backpack-spraying unit that dispensed approximately 1.5 l per vine. Disease severity was assessed on a rating scale from 0 (no foliar symptoms) – 4 (severe stunting). Since the number of vines for each level of severity was not equal, the data were subjected to Restricted Maximum Likelihood analysis. Eutypa dieback was well established in both vineyards. In the first year of the study, 68% of the vines in Eden Valley and 53% of the vines in McLaren Vale produced symptoms of the disease. In the first 2 years, yield from Brotomax™-treated vines was marginally higher than that in untreated vines, but the difference was not significant. In the third year of treatment, there was a significant yield increase of 20% for vines treated with Brotomax™ compared to untreated vines. However, the severity of foliar symptoms of eutypa dieback was not reduced following 3 years of treatment. Application of Brotomax™ and monitoring of vines is continuing.

Protection of grapevine pruning wounds against fungal infections. F. Halleen[1] and P.H. Fourie[2]. *[1]ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa. [2]Department of Plant Pathology, University of Stellenbosch, Matieland, 7602, South Africa.*
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Eutypa dieback, caused by *Eutypa lata*, is one of the most destructive diseases of grapevines worldwide. Infection occurs through unprotected wounds and protection of wounds directly after pruning is recommended to prevent the disease. However no fungicide is registered for control of this disease in South African vineyards. The aim of this study was

therefore to evaluate the effectiveness of fungicides and biological control agents against *E. lata* infections under field conditions. The effect of the treatments on other prominent wood-invading pathogens was also recorded. The study was conducted in two Cabernet Sauvignon vineyards. Treatments were sprayed onto pruning wounds directly after pruning in August 2001 and 2002. Treated wounds were inoculated after 24 h with a 1×10^3 suspension of *E. lata* ascospores. Treatments were benomyl, flusilazole, *Bacillus subtilis, Trichoderma* formulations A, B and C, an inoculated control and an uninoculated control. The incidence of trunk disease pathogens in treated wounds was determined 11 months after inoculation by making isolations from dissected pruning wounds. Flusilazole (5.5%) and benomyl (5%) were the most effective treatments against *E. lata* compared to the inoculated control plants (48.5%). The *B. subtilis* strain did not inhibit *E. lata*. The *Trichoderma* treatments were less effective than the fungicides, especially during the 2001–2002 season, but results indicated that *Trichoderma* survived in pruning wounds and colonised them. To what extent this colonisation might prevent or inhibit later infections is unclear at this stage. Isolations from the untreated, uninoculated control plants also revealed the presence of several other trunk disease pathogens including *Phomopsis* (37.5%), *Botryosphaeria* (18.5%) and *Phaeomoniella chlamydospora* (19.5%). Flusilazole and benomyl reduced infection of *P. chlamydospora* by 82 and 77%, respectively, whilst flusilazole reduced *Phomopsis* infection by 53%. The results clearly showed that pruning wound protection could contribute to lower levels of infections from trunk disease pathogens under vineyard conditions.

Proactive management of black foot disease in South African grapevine nurseries. F. Halleen[1], P.H. Fourie[2] and P.W. Crous[3]. *[1]ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa [2]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa. [3]Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.*
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Black foot disease (BFD), caused by species of *Cylindrocarpon* and *Campylocarpon*, is a relatively unknown disease that affects mainly young grapevines. The causal fungi infect grapevine cuttings planted in infested nursery soil. Control methods should therefore focus on preventing or eradicating infection at the basal ends of these cuttings. No fungicides are registered for control of BFD. *In vitro* studies revealed that benomyl, flusilazole and prochloraz manganese chloride were the most effective fungicides. Nursery trials were conducted to evaluate the effectiveness of various physical, chemical and biological treatments protecting the basal ends of rootstocks against infection. After callusing, the basal ends of grafted cuttings were dipped in various treatments prior to planting. Additional treatments involved soil amendments with *Trichoderma* formulations and hot water treatment of dormant nursery grapevines. Nursery plants were uprooted after eight months. None of the treatments affected root or shoot mass negatively, although some of the treatments caused a reduction (7.63% to 15.92%) in the number of certifiable nursery grapevines produced compared to the control plants. Isolations from the roots of uprooted plants revealed very low incidences (4.1%) of BFD pathogens and no significant differences were observed between treatments. Infection levels at the basal ends were substantially higher. BFD pathogens were isolated from 45.33% (2002-2003) and 16.83% (2003-2004) of the basal ends of control plants. The incidence of BFD pathogens at the basal ends was not significantly or consistently reduced by the majority of chemical and biological treatments. However, no BFD pathogens were isolated from the plants that were subjected to hot water treatment. It is therefore recommended that hot water treatment of dormant nursery grapevines be included in an integrated strategy for the proactive management of BFD in grapevine nurseries.

Black foot of grapevine: sensitivity of *Cylindrocarpon destructans* to fungicides. C. Rego[1], L. Farropas[2], T. Nascimento[1], A. Cabral[2] and H. Oliveira[2]. *[1]Laboratório de Patologia Vegetal "Veríssimo de Almeida", Tapada da Ajuda, 1349-017 Lisboa, Portugal. [2]Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal.*
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Black foot disease of grapevine is caused by *Cylindrocarpon* spp., with *Cylindrocarpon destructans* being the main pathogen isolated from vine cut-

tings and young vineyards in Portugal. Few recommendations for black foot disease control are presently available, and they are not easy to implement in commercial nurseries. In this study, 14 fungicides were evaluated for their effects on the radial growth and spore germination of four field isolates of *C. destructans*. Mycelial growth of the pathogen was inhibited by DMI fungicides, prochloraz (EC_{50} <0.09 mg l^{-1}), to a lesser extent by tebuconazole and difenoconazole (EC_{50} <5.66 mg l^{-1}), by the benzimidazole fungicide benomyl (EC_{50} <0.35 mg l^{-1}), and by the mixtures cyprodinil + fludioxonil and carbendazim + flusilazol, which gave EC_{50} <0.75 mg l^{-1}. Among these, only cyprodinil + fludioxonil (EC_{50} values < 0.15 mg l^{-1}), the strobilurin fungicides, azoxystrobin and tryfloxistrobin (EC_{50} <2.27 mg l^{-1}) and the sulphamide fungicide tolyfluanide (EC_{50} <0.54 mg l^{-1}) were effective in reducing spore germination. Results from *in vivo* studies carried out on potted grapevine plants (cultivar Periquita) showed that benomyl, tebuconazole and the mixtures carbendazim + flusilazol and cyprodinil + fludioxonil significantly (P=0.05) improved plant growth (plant height, number of roots and root elongation) and decreased disease incidence compared with non-treated plants.

Fungicide sensitivity of selected Botryosphaeria species from grapevine. W. Bester and P.H. Fourie. *Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland (Stellenbosch) 7602, South Africa.*
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Protection of wounds against infection by trunk disease pathogens is the most efficient and cost-effective means to prevent grapevine trunk diseases. Studies done to determine the effectiveness of chemical pruning wound protectants have mostly focused on the control of Eutypa dieback. However, other important wound pathogens, such as *Phaeomoniella chlamydospora*, *Phomopsis* and *Botryosphaeria* species, pose just as significant a threat to sustainable grape production. Fungicide sensitivity studies have been conducted for *Pa. chlamydospora*, *P. viticola* and *Eutypa lata*. However, no such studies have been conducted on the pathogenic *Botryosphaeria* species from grapevine. Ten fungicides were therefore tested *in vitro* for their efficacy on mycelial inhibition of four *Botryosphaeria* species, *B. australis, B. obtusa, B. parva* and *B. rhodina*. Iprodione, pyrimethanil, copper ammonium acetate, kresoxim-methyl and boscalid were ineffective in inhibiting mycelial growth at the highest concentration tested (5 μg ml^{-1}; 20 μg ml^{-1} for copper ammonium acetate). Benomyl, tebuconazole, prochloraz manganese chloride, flusilazole and fenarimol were the most effective, with EC_{50} values for the different species ranging from 0.36–0.55, 0.07–0.17, 0.07–1.15, 0.04–0.36 and 0.45–3.01 μg ml^{-1} respectively. All these fungicides, except prochloraz manganese chloride, are registered for use on grapes in South Africa and are also reported to be effective against *Pa. chlamydospora, P. viticola* and *E. lata*.

The effects of different post hot water treatment cool down protocols on dormant cuttings of *Vitis vinifera* cultivars. H. Waite[1,2], S. Beggs[2], H. Dark[2], P. Kwak[1] and G. Murrells[1]. *[1]Cooperative research Centre for Viticulture, PO Box 154, Glen Osmond, South Australia, 5064, Australia, [2]Faculty of Land and Food Resources, University of Melbourne, Dookie College, 3647 Victoria, Australia.*
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Hot water treatment (HWT) of dormant grapevine cuttings controls a number of pathogens including *Phaeomoniella chlamydospora*. Dormant cuttings are plunged into cold water immediately following hot water treatment (50°C/30 min) to quickly lower the temperature and minimise heat damage to the tissue. Water used in commercial cool down tanks is chlorinated, but it is not sterile and it is a potential source of microbial contaminants including *Phaeomoniella chlamydospora*. It would be desirable to eliminate this stage of the HWT process allowing cuttings to air-cool provided that the slower cooling did not affect cutting viability. The effects of post HWT air-cooling versus water-cooling on dormant cuttings of 4 *V. vinifera* cultivars, Pinot Noir, Chardonnay, Semillon and Cabernet Sauvignon were examined. Treatments applied were: HWT plus water-cooling (30 min immersed in cold water at 12°C), HWT plus air-cooling (30 min cooling on laboratory bench at 17°C), and untreated control. Cuttings were incubated at 28°C for 21 days and assessed for percentage callus, root number and length and bud development. All cuttings of all varieties, regardless of treatment, were alive and viable at callusing. In all varieties

both HWT treatments tended to stimulate callus and bud development. Although variable, root development in all treatments of all varieties was adequate for cutting establishment, indicating that it may be possible to eliminate water-cooling of HWT cuttings and reduce contamination by water borne micro-organisms. However these results should be tested in a commercial setting before changes to HWT protocols can be recommended.