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ABSTRACTS

UP-PCR analysis of Australian isolates of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*. E. Cottral[1,2,4], H. Ridgway[3], I. Pascoe[1,2], J. Edwards[1,2] and P. Taylor[4]. *[1]Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond, SA 5064, Australia. [2]Agriculture Victoria - Knoxfield, Private Bag 15, Scoresby Business Centre, Victoria 3176, Australia. [3]Soil, Plant and Ecological Sciences Division, P.O. Box 84, Lincoln University, Canterbury, New Zealand. [4]Institute of Land and Food Resources, Melbourne University, Royal Parade, Parkville, Victoria 3052, Australia.*

Phaeomoniella chlamydospora (*Pch*) and *Phaeoacremonium aleophilum* (*Pal*) are commonly isolated from vines showing esca symptoms and also from vines infected with Petri grapevine decline (also known as black goo). Although *Pch* is easily identified by morphological characters, *Pal* isolates and *Pal*-like isolates have variable morphology. The universally primed polymerase chain reaction (UP-PCR) was used to study the genetic diversity of *Pch* and *Pal* isolates from different wine-growing regions in Australia and also to compare these isolates with those from other countries. Using six UP-PCR primers, comparisons were made between 22 Australian, 5 Italian and 5 New Zealand *Pch* isolates, and between 8 Australian, 6 Italian and one Yugoslavian (CBS 246.91) *Pal* isolate. Strains from the Centraalbureau voor Schimmelcultures (CBS) of *P. angustius* (CBS 249.95), *P. inflatipes* (CBS 391.71), and *P. rubrigenum* (CBS 498.94) were also included for comparison with the *Pal* strains. Two distinct groups of *Pal* were identified with the UP-PCR primers. In one group the isolates shared similar band profiles with the five Italian isolates and the Yugoslavian *Pal* isolate. The second group produced profiles that were distinct from the other *Pal* isolates. The ITS region of the second group is being sequenced to determine whether these isolates are actually *Pal*. Genetic variation within *Pch* isolates (including the isolates from Italy and New Zealand) was low. Re-extraction of the fungal DNA is currently under way and the UP-PCR analysis will be repeated to confirm the findings.

Genetic variation within New Zealand populations of *Phaeomoniella chlamydospora*. B. Pottinger, H. Ridgway, B. Sleight and A. Stewart. *Soil, Plant and Ecological Sciences Division, P.O. Box 84, Lincoln University, Canterbury, New Zealand.*

Phaeomoniella chlamydospora has been implicated as the causal agent of Petri vine decline. This disease causes serious economic loss by severely retarding the productivity of young and established vines throughout the world. *P. chlamydospora* has only recently been recognised in New Zealand and little is known about the genetic composition of New Zealand populations. This study used the molecular technique of universally primed polymerase chain reaction (UP-PCR) to investigate polymorphism within 38 *P. chlamydospora* isolates. The isolates originated from four distinct geographic locations encompassing five vineyards throughout the North and South Islands and were compared with 6 reference isolates obtained from Italy. Relatively little genetic variation was observed, with 5 of the 11 UP-PCR primers identifying 2 genetic groups and 1 primer (3–2) identifying 3 groups. Genetic variation was present within an individual vine and within and between New Zealand vineyards. New Zealand isolates were similar to Italian isolates; however, one of the groups distinguished by primer 3–2 did not resemble any of the Italian isolates tested. Assessment of the genetic variation within the pathogen population will deepen understanding of the population composition within New Zealand vineyards and may provide insight about the origin(s) of New Zealand strains.

Diversity of basidiomycetous fungi associated with esca diseased vines. MICHAEL FISCHER. *Staatliches Weinbauinstitut, Merzhauser Str. 119, D-79100 Freiburg, Germany.*

Diversity of fungal organisms is well examined in Europe and North America. For these regions, only few basidiomycetes have been reported as growing on vine, the ones mostly mentioned *Stereum hirsutum*, *Phellinus igniarius*, and, recently, *Fomitiporia punctata* (= *Phellinus punctatus*). In Europe, the latter taxon has been shown as associated with esca disease, data are less conclusive for non-European vine growing countries.Thanks to colleagues from Argentina, Chile, and Australia, the author was provided with a considerable number of fungal isolates and/or fruit bodies isolated from or collected on vine from non-European countries. Molecular and, if applicable, microscopical studies have been performed with this material. Together with data based upon well-defined European samples of esca-related basidiomycetes, the obtained results were combined in one major data set. Conclusions are presented referring to the diversity of basidiomycetes occurring in vine-growing areas.

Detection of *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. from soil and host tissue with Nested-PCR. A. ESKALEN, S.N. ROONEY, and W.D. GUBLER. *Department of Plant Pathology, University of California, Davis, CA 95616, USA.*

A Nested-PCR method has been developed for the detection *of Phaeomoniella chlamydospora* and *Phaeoacremonium* spp., the causal agents of black measles or esca and Petri disease (syn: young vine decline) of grapevine. Work was started by amplifying Internal Transcribed Spacers (ITS) of the ribosomal DNA (rDNA) subunit repeat from previously cultured *P. chlamydospora* (11 isolates) and *Phaeoacremonium* sp. (8 isolates) in the UCD Plant Pathology Collection. Isolates were then purified with a QIAquick PCR Purification Kit and utilized for sequencing purposes. All sequences were aligned and compared with ITS sequences of other fungi in Gene Bank. Remarkable homology was noticed among sequences of *Phaeoacremonium* spp. and a similar homology was also seen between *Phaeoacremonium* spp. and *P. chlamydospora*. In view of this finding, nested primers were designed from regions that were non-homologous parts of the pathogens, and their specificity to the species was verified in standard PCR prior to carrying out all tests. External primers were the ITS4 and ITS5 universal primers for nested PCR. DNA was extracted from both artificially and naturally infected soil, the latter taken from crown areas below black measles vines. DNA was successfully amplified with ITS4 and ITS5 universal primers which gave an approximately 620-bp PCR product. The second round of amplification was carried out by using amplified DNA with internal primers that amplified a 300-bp PCR product from *P. chlamydospora* and a 400-bp product from *Phaeoacremonium* spp. The work presented here illustrates the potential for detecting fungi directly from infected host tissue and infested soil. This approach is particularly well suited to those organisms that are difficult to identify and isolate because of the presence of inhibitors in both vine tissue and the soil.

Basidiomycetes isolated from esca-like heart rots of grapevines in Australia. J. EDWARDS[1,2], I. PASCOE[1,2], N. LAUKART[1,2], J. CUNNINGTON[2] and M. FISCHER[3]. *[1]Cooperative Research Centre for Viticulture, P.O. Box 154, Glen Osmond, South Australia 5064, Australia. [2]Agriculture Victoria - Knoxfield, Private Bag 15, Scoresby Business Centre, Victoria 3176, Australia. [3]Staatliches Weinbauinstitut, Merzhauser Str. 119, D-79100 Freiburg, Germany.*

Esca is one of the most destructive diseases of grapevines in Europe, reducing both productivity and longevity of the vines. When the trunk of an esca-affected vine is cut open, the internal symptom is a soft white heart rot bordered by black necrotic wood. At least two pathogens are thought to be associated with esca: a mitosporic fungus, *Phaeomoniella chlamydospora,* responsible for the black necrosis, and a basidiomycete, *Fomitiporia punctata,* responsible for the white heart rot. Certainly, *F. punctata* is the predominant basidiomycete isolated from white heart rot of grapevines in parts of Europe such as Italy, but there have also been reports of other basidiomycetes, such as *Phellinus ignarius* and *Stereum hirsutum*, associated with esca-like heart rots. Over the past two years, we have isolated at least five basidiomycete species from esca-like heart rots of grapevines in Aus-

tralia, and have found several basidiocarps on vines in the field. At this stage, it is thought that one of the basidiomycetes is *F. punctata,* but the others are possibly *Inonotus* or related species, perhaps unique to Australia. Further morphological examination and DNA sequencing is under way in order to confirm the identity of these fungi associated with white heart rot of grapevines in Australia.

Initial survey results of grapevine decline in Pennsylvania and New York. E. Stewart[1], J. Travis[1], N. Wenner[1] and B. Hed[2]. [1]*The Pennsylvania State University, Department of Plant Pathology, Buckhout Laboratory, University Park, PA 16802, USA.* [2]*Lake Erie Research and Extension Center, North East, PA 1642, USA*

Grape growers in Pennsylvania and New York states have become increasingly aware of grapevine decline. In response to grower concern, a survey of vineyards was initiated in March 2001 to determine the status and causes of grapevine decline in our region. Individual vines within cultivar blocks were visually accessed as healthy, declining, dead/missing, healthy replant, or declining replant. Additionally, plant and soil samples were taken to ascertain the presence of fungi, and levels of Tomato Ringspot virus (TRV), nematodes, and soil fertility. Data were also taken on the incidence of *Agrobacterium tumefaciens* (crown gall), herbicide injury, broad leaf weeds, and phylloxera. Cross sections of declining vines revealed flecking typical of esca, pockets of white rotting fungi, and wedge-shaped discoloration symptomatic of *Eutypa lata*. Tissue explants from symptomatic plants were transferred onto 2% acidified malt agar. *Phaeomoniella chlamydospora* (*Pch*), *Phaeoacremonium* sp., *Phialophora* sp., *Verticillium* sp., *Fusarium* sp., *Trametes hirsutum* and an undetermined basidiomycete were identified from Chardonnay, Riesling, Pinot Noir, Chambourcin, Dutchess, Seyval, Vidal, Vignoles and Concord. Six isolates of *Pch* from New York and Pennsylvania vineyards were inoculated onto cuttings of rootstock 3309C and Concord to complete Koch's postulates and as pathogenicity tests. Single-node cuttings were inoculated below the bud with 10^6 ml^{-1} *Pch* conidia. *Pch* was re-isolated from all *Pch*-inoculated treatments. Cuttings inoculated with *Pch* exhibited dark brown-black vascular streaking. Water-inoculated controls exhibited no *Pch* symptoms. Among the *Pch* treatments, shoot initiation was reduced by 0–20% on 3309C and by 50–100% on Concord as compared to 0% for the control. Root initiation among the uninoculated checks was 70–90%. Root initiation was 100% on 3309C and was reduced by 20–60% on Concord.

First report of esca of grapevine in Iran. M.R. Karimi[1], B. Mahmoodi[2], M. Kazemiyan[3]. [1]*Agricultural Research Center of Khorassan, P.O.Box 488-91735, Iran,* [2]*Sugar Beet Seed Institute P.O. Box 31585-4114, Karadj, Iran,* [3]*Bojnourd Plant Protection Management, Iran.*

Grape is an important fruit crop in Iran and covers an area of 250,000 ha from which 21,250,000 tons of grapes are harvested every year. A survey of vineyards in the north of Khorassan province (Bojnourd) in Iran during 1998 and 1999 revealed the occurrence of a disease on grape similar to esca. Primary symptoms included foliage deterioration with gradual development of small chlorotic areas between the primary veins or along the leaf margin. In the later stage, light-green rounded or irregular shape chlorotic spots develop, which later became necrotic. The pattern of necrosis and the yellow brown or red brown stripes on the leaves is characteristic. The necrotic areas dry out and the leaves fall prematurely. Secondary symptoms include a white rot in the trunk, which in cross-section appears as a soft, friable, spongy mass of rotted yellowish or whitish tissue. The symptom of disease can also be observed as small dark brown or black spots which are sparsely distributed or arranged in groups around annual growth rings. The white rot is often surrounded by a thick black or dark brown line separating the rotted from the non-rotted parts. In longitudinal section the presence of deep brown or black streaks is another indication of disease. The rot is mostly restricted to the older parts of the wood. As a result of the disease, berries become dark brown and violet spots develop on the skin. In the affected plants, berries either do not mature, or they dry out before reaching maturity. Efforts made to isolate fungi revealed the association of *Fomitiporia punctata* with the white decay and *Phaeomoniella chlamydospora* with the brown red border. Identification of both fungi was confirmed by Laura Mugnai (University of Florence, Italy). This report should be considered the first on the presence of esca in Iran.

Role of temperature on fruiting body production and sporulation of *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp.
S.N. Rooney and W.D. Gubler. *Department of Plant Pathology, University of California, Davis, CA 95616, USA.*

Water-agar plates inoculated with plugs of *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. were incubated at 23ºC for two weeks for complete colonization of the plates. Autoclaved 5C rootstock wood shavings were then placed on the agar surfaces. Plates were placed in incubators with continuous cool white (15W) light at temperatures ranging from 5 to 35ºC. The wood pieces were examined weekly and observations were recorded. After twenty-one days, pycnidia formied on wood pieces inoculated with *P. chlamydospora* incubated at 10, 15, 20 and 25ºC. After twenty-eight days pycnidia were very abundant on the wood at these temperatures, but most abundant at 25°C. Pycnidia were dark, superficial to slightly embedded, subglobose to globose in shape and ranged in size from 110 to 190 μm. A cloudy gray conidial mass could be seen oozing from the ostiole after 21 days. Conidia contained in pycnidia were hyaline, subglobose to oblong and ranged in size from 2.0–3.0 μm$\times$1.0–1.5 μm. Conidia were viable and germinated after 48 hours on water agar. After twenty-one days, wood pieces inoculated with *Phaeoacremonium inflatipes* incubated at 10, 15, 20, and 25°C had small microsclerotia-like structures on their surfaces as well as on the agar surfaces near and around the wood pieces. Structures appeared as dark compacted masses of hyphae, as no conidia or other spore type were found inside them. They were globose, superficial to slightly embedded and ranged in size from 65 to 120 μm. Conidia occurred on conidiophores extending from mycelium around and attached to the structures. Conidia were hyaline, oblong to ellipsoidal and measured 2.5–5.0 μm $\times$1.25–2.0 μm. Neither pycnidia nor microsclerotia could be found on wood pieces or media inoculated with *P. aleophilum*. These findings are important for our understanding of the biology of these elusive fungi. The finding that *P. inflatipes* produces a kind of unidentified fungal structure on grapevine wood and artificial media, along with concurrent research showing that it is recovered from the soil, indicates that this fungus is a soilborne pathogen. In addition, this is the first reported study on the effect of temperature on pycnidia production of *P. chlamydospora* under laboratory conditions.

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

Susceptibility of grapevine pruning wounds to *Phaeomoniella chlamydospora*, and *Phaeoacremonium* spp.
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Phaeoacremonium inflatipes, Pm. aleophilum and Phaeomoniella chlamydospora have all been shown to be aerially dispersed in California vineyards. Dispersal was correlated to rain events and for the most part took place during the winter pruning season. Because of the presence of the pathogens

during pruning this study was conducted to examine the susceptibility of grapevine pruning wounds to pathogen infection. Studies were conducted on Thompson Seedless and Cabernet Sauvignon grapevines. Pruning wounds became less susceptible over time and reached the lowest success rate some four months after pruning. Vascular streaking was observed in pruning wounds inoculated from February to June 2001, indicating that grapevine tissue was susceptible from dormancy to green actively growing tissue. Distance of vascular streaking in Thompson seedless grapevines ranged from 1.4 cm in the control to 6.0, 7.0, and 9.1 cm for *P. chlamydospora, Pm. inflatipes,* and *Pm. aleophilum* respectively. The length of vascular streaking in Cabernet Sauvignon was 1.2 cm in the control and 4.5, 6.7, and 10.1 in the *P. chlamydospora, Pm. inflatipes* and *Pm. aleophilum* inoculated spurs respectively. Vascular discoloration and the presence of all three pathogens were documented at both bud positions of a 2-bud spur. In a related study, spurs of Thompson Seedless and Cabernet Sauvignon grapevines were inoculated in February 2001. Shoot growth from inoculated and control spurs was measured in June 2001. Growth of non-inoculated control shoots reached an average of 140 cm for Cabernet Sauvignon while shoot length was only 69.7, 76.1,and 89.9 cm for *P. chlamdospora, Pm. inflatipes* and *Pm. aleophilum* inoculated spurs respectively. Growth on Thompson Seedless control spurs reached an average length of 172.3 cm while shoot length was only 54.0, 86.0, and 87 cm for *P. chlamydospora, Pm. inflatipes,* and *Pm. aleophilum* inoculated spursrespectively. All three pathogens were capable of infecting pruning wounds and significantly reduced growth in shoots emerging from diseased spurs.

Can *Eutypa lata* infect grapevine through spring wounds? (Preliminary data under sheltered conditions). P. Lecomte, M. Clerjeau, P. Larignon, N. Bastien, S. Cardon, J. Lafitte and B. Dubos. *UMR en Santé Végétale (INRA-ENITA) Centre de Recherches de Bordeaux-Aquitaine, Avenue Edouard Bourleaux, BP 81, 33883 - Villenave d'Ornon Cedex, France.*

The ascomycete *Eutypa lata* is one of the main wood infecting fungi involved in grapevine trunk decline. It is the wound pathogen responsible for eutypa dieback and can also act as a pioneering agent in the esca syndrome. Several experiments based on artificial inoculations (100 and 1000 spores per wound), were performed between 1999 and 2001 in order to improve our knowledge of the role, including the epidemiological role, of pruning wounds made on the vines in spring by cultural practices (desuckering and disbudding). All trials were carried out with young plantlets of the cv. Cabernet-Sauvignon under favourable sheltered conditions (no rain, low competition with wood microflora). Wounds were analysed by classical isolation methods following incubation for two weeks to verify the presence of the fungus. *E. lata* was diagnosed by morphological assessment of whitish mycelia compared with a reference strain and was completed by subculturing or PCR testing with species-specific primers when necessary (doubtful cases). Results were relatively consistent over the three years. Artificial inoculations were generally highly successful under these favourable conditions, indicating that spring pruning wounds may serve as candidate infection courts for *E. lata* ascospores. We are currently experimenting in vineyards by checking the presence of *E. lata* after incubation periods lasting either 2 weeks or several months incubation periods. Complete results will be published in another report. These preliminary observations may encourage winegrowers to take measures avoiding such infection courts due to pruning.

Yearly variation in symptom expression of Eutypa dieback and the relationship to grapevine yield. M.L. Creaser [1,2] and T.J. Wicks [1,2]. *[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.*

Eutypa dieback, caused by the fungus *Eutypa lata*, is an insidious disease that contributes to the decline of vineyards by reducing growth and yield, and eventually killing vines. The disease occurs in all grape-growing areas and on all varieties in South Australia. Studies have demonstrated a significant linear relationship between the severity of symptoms of Eutypa dieback and grapevine yield, in that as disease severity increases, the yield of affected vines decreases. However, as the same vines were

not monitored for more than one year, no account was taken of yearly variation in symptom expression. Our aim was to evaluate the variation in symptom expression of Eutypa dieback and its relationship to yield. In the spring of 1999, 125 Shiraz vines each in two vineyards in the Coonawarra district of South Australia were selected so that about 25 vines fell into each of five disease severity categories. All vines were harvested by hand and the total yield per vine was recorded at maturity. In the spring of 2000, vines were re-assessed for disease severity and yield data collected at harvest. Symptoms of Eutypa dieback in spring 1999 differed significantly from those on the same vines in spring 2000, with symptoms less severe in many vines in 2000. There was no significant difference between vineyards. Regression analyses demonstrated significant linear relationships between disease severity and yield per vine, in both vineyards, in both years. To facilitate evaluation of the effects of disease, site and year, the yield data were transformed to standardise the intercepts to a value of 100%. When reanalysed, a single line described the relationship between disease severity and yield for both vineyards and both years. This study has shown that the severity of foliar symptoms of Eutypa dieback varies from year to year. However, the linear relationship between disease severity and yield appeared constant and unaffected by the yearly variation in symptom expression. Further work is now required to determine the underlying cause of the yearly symptom variation.

***Botryosphaeria* species associated with grapevine trunk diseases.** ALAN J.L. PHILLIPS. *SABT, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal.*

Several fungi have been associated with trunk diseases of grapevines; they include various species of *Botryosphaeria*. Although *Botryosphaeria* species are known to cause cankers and dieback in many woody hosts, their importance in grapevine diseases has been largely ignored. Indeed, they are more often regarded as saprophytes, or at most as weak pathogens. For these reasons a reassesment of the role played in grapevine diseases by fungi in this genus was considered necessary. In this work the species of *Botryosphaeria* associated with wood and trunk diseases of grapevines were determined and their pathogenicity was tested. Four species of *Botryosphaeria*, namely, *B. obtusa*, *B. parva*, *B. dothidea* and *B. lutea*, were regularly associated with trunk dieback, wood necrosis, brown wood streaking and incomplete grafts. Of these five species, only *B. parva* caused disease in experimental inoculations. The other species caused either minor symptoms, or no visible disease. *B. parva* was also the most common and widely distributed species in Portugal. The data indicate that *B. parva* can cause many of the symptoms normally associated with other fungi in grapevine decline.

Reaction of different grapevine cultivars to infection with root-rot fungi and its control. HUSSEIN A.H. MAHROUS. *Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.*

During the last several years, a decline of grapevines characterized by delayed and weak growth has been seen in several vineyards in Egypt. The decline was always associated with typical root rot symptoms, namely, abundant necrosis on roots and a reduction of feeder roots. The disease also occurs on cuttings in the nurseries and on the rooted transplants in the fields. The main pathogens involved are *Fusarium solani*, *Fusarium moniliforme*, *Rhizoctonia solani* and *Botryodiplodia theobromae*. Due to the increased import of new grapevine cultivars, and because of the seriousness of root rot on cuttings in the nurseries, it was thought important to screen local and imported cultivars for their tolerance to the disease under Egyptian environmental conditions. Furthermore, some fungicides and application methods were tested under greenhouse and field conditions for their ability to control the disease. Grapevine cultivars Thompson seedless, Early Superior, Superior, Flame seedless, Fiesta, Perlette, Ruby seedless, Cramson, Red Globe and Romi Red were tested under greeenhouse and field conditions. Thompson seedless (Banati), Flame seedless, Early Superior, Fiesta and Superior were the most susceptible to root rot, while Ruby seedless, Cramson, Perlette, Red Globe and Romi Red were the least susceptible. Rizolex / T, Vitavax / T, Topsin M_{70} and Moncerin were evaluated and applied as pre-planting root dips, or as soil drenches. All the fungicides tested gave good control root rot both in the greenhous experiments and in the field trials.

Origin of the infections of esca disease: histological studies in wounded reaction zones of *Vitis vinifera* cv. Sangiovese. A. Panattoni, A. Materazzi and E. Triolo. *Dipartimento di Coltivazione e Difesa delle Specie Legnose 'G. Scaramuzzi', Sezione Patologia Vegetale, Università, via del Borghetto 80, Pisa, Italy.*

The importance of wounds as a means of penetration, the type of agent causing esca and the low ability of grapevine to differentiate its histological 'apparatus' capable of limiting esca damage, suggested a study to examine non-specific plant responses to mechanical wounding and to observe regeneration processes during recovery and repair of lignified artificially infected tissue. Young Sangiovese rooted cuttings (clones CHCL 2000/1, CHCL 2000/2, CHCL 2000/3, CHCL 2000/4) grown in pots were used. Wounds were induced on shoots previously pruned to 50 cm and restricted to the first six distal internodes. Small longitudinal wounds were made on each shoot, including the removal of the periderm. The wounds were made 5 mm from each node and had a roughly elliptical perimeter 3–4 mm long. Unwounded lignified shoots served as control sand as a basis for comparison. The wounds were periodically assessed by standard procedures of embedded resin and by microscopic investigation of thin sections (20 μm). Small wounds initially caused the cambial tissue exposed by the wound to produce abundant callosium and lignin, which presumably had a waterproofing and antimycotic function. Subsequently, new tissue to restore functionality originated from the margins of the cambial zone. Observations on pruning cuts enabled repair processes in reaction to mild traumas to be excluded. For this reason cellular degeneration leading to plant death was constantly observed. The experiments suggest that the extent to which histological damage to vines is reduced depends on the severity of the damage. No difference was noticed in repair processes between the 4 Sangiovese clones. The results confirmed that the ability of vines to repair damaged tissue is weak when wounds are extensive and that wounds are an important venue for the entry of airborne fungi causing esca.

Investigating *Phaeoacremonium* spp. and *Fomitiporia punctata* for secondary metabolites implicated in esca disease. R. Tabacchi, E. Abou-Mansour and C. Poliart. *Institute of Chemistry, University of Neuchâtel, Av. de Bellevaux 51, 2007 Neuchâtel, Switzerland.*

Grapevine trunk diseases threaten the growth and sustainability of grape production by limiting the establishment of young vines and causing decline of established vines. In this report, we concentrate mainly on two fungi associated with black goo and esca diseases: first, *Phaeoacremonium aleophilum* which is responsible, with *P. chlamydosporum,* for black goo decline and can be transmitted in propagation, and *Fomitiporia punctata*, which has most often been associated with wood decay symptoms of esca. Even though *F. punctata* fruiting bodies and mycelium are associated with approximately 50% of vines showing esca symptoms, no toxins have been isolated or described till now. In a previous study, we isolated and identified several compounds from cultures of *P. chlamydosporum* and *P. aleophilum*, including scytalone. Scytalone (3,6,8-trihydroxytetralone) is known to be a precursor in the pathway of the fungal DHN melanin biosynthesis, and is isolated from several fungi involved in rice and wood diseases. In a recent work, we isolated several other compounds occuring in the DHN melanin pathway. We treated *P. aleophilum* with fungicides blocking melanin synthesis at two different sites, thus increasing concentration of intermediate compounds, such as scytalone and vermelone. We report here on the culture conditions for *P. aleophilum* and *F. punctata*, chemical analyses, and *in vivo* assays carried out to assign the bioactivity of the isolated compounds.

Evaluation of *Trichoderma* as bio-control for protection against wood-invading fungi implicated in grapevine trunk diseases. J.S. Hunt[1], D.S.J. Gale[1] and I.C. Harvey[2]. [1]*Agrimm Technologies Ltd, P.O. Box 13-245, Christchurch, New Zealand.* [2]*PLANTwise, P.O. Box 8915, Christchurch, New Zealand.*

Trichoderma spp. have been demonstrated to have considerable activity as bio-control agents against a number of soil-borne plant pathogens. They have also been reported to be able to protect woody

plants from infection by wood-invading fungal pathogens. Microscopic evaluation of cultures employing dual inoculation of various strains of *T. harzianum* together with isolates of the grapevine pathogens *Eutypa lata, Botryosphaeria stevensii, Phaeomoniella chlamydospora* and *Cylindrocarpon destructans* showed varying degrees of lytic activity. In other *in vitro* studies, three *Trichoderma* strains were shown to produce both volatile and non-volatile metabolites with toxic activity towards isolates of *E. lata*. Formulations containing a number of strains of *T. harzianum* were evaluated for their ability to protect grapevine pruning wounds from the ingress of wood-inhabiting fungal pathogens. *Trichoderma* spp. applied to freshly made pruning wounds had penetrated up to 16 mm from the wound site when sampled 30 days after application. Fungi growing from sections obtained from wood 4–5 cm below the pruning wound 8 months after *Trichoderma* treatment were identified and compared with those fungi growing from sections of wood from untreated control pruning wounds. A range of pathogens and saprophytes including *Fusicoccum, Phomopsis, Botryosphaeria, Pestalotiopsis* spp., *Fusarium* spp. and *Botrytis cinerea* were isolated from the sections made from control wounds. *Trichoderma* spp. were isolated from all sections made from the treated wounds with an 85% reduction in recoverable isolates of the pathogens. These data provide evidence for the belief that *Trichoderma* spp. are suitable bio-control agents against the wood-invading fungal pathogens implicated in grapevine trunk diseases and vine decline.

Implications of hot water treatment and other nursery practices in the production of quality grapevine planting material. H. Waite[1], J. Crocker[2], G. Fletcher[3], P. Wright[4] and A. de Laine[5]. *[1]Institute of Land and Food Resources, University of Melbourne, Dookie College, Victoria 3647, Australia. [2]South Australian research and Development Institute, Lenswood centre, Swamp Rd., Lenswood, South Australia 5001, Australia. [3]Agriculture Victoria, Sunraysia Horticulture Centre, Box 905, Mildura, Victoria 3502, Australia. [4]Orlando Wyndham Group, PO Box 943, Rowland Flat, South Australia 5352, Australia. [5]Australian Vine Improvement Association, PO Box 398, Nuriootpa, South Australia 5355, Australia.*

Early research to determine the cause of losses of grapevine propagating material subjected to hot water treatment (HWT) indicated no single cause, but a number of contributing factors including variety, collection date and position of cutting on original cane. A collaborative Grape and Wine Research and Development Corporation project investigating these factors, and including pre- and post-HWT practices, began in 1999. Of the 3 cv. investigated, Pinot Noir was most sensitive, with obviously retarded early-season growth after HWT. Chardonnay and Cabernet Sauvignon were more robust. Investigations to determine dormancy levels found a significant increase in respiratory rates in apparently dormant Chardonnay cuttings 5 weeks before budburst, indicating that cuttings may cease to be endodormant before budburst possibly increasing sensitivity to HWT. Delayed handling, exposing freshly harvested material to dehydration and, conversely, hydration for periods longer than 1 hour, are not necessarily beneficial and may contribute to a cumulative reduction in quality. Post-HWT cold storage was also found to have an effect on the quality of the cutting. Slightly better results were obtained when material was subjected to HWT after storage rather than before. Storing cuttings in punctured bags reduces the build-up of volatile aromatic compounds. Such material has a longer storage life and is of better quality than material stored in sealed bags. Included in the project was the development of a prototype bar coding system suitable for research purposes and for commercial vine nurseries. This system facilitated record-keeping and enabled material to be tracked through every nursery operation