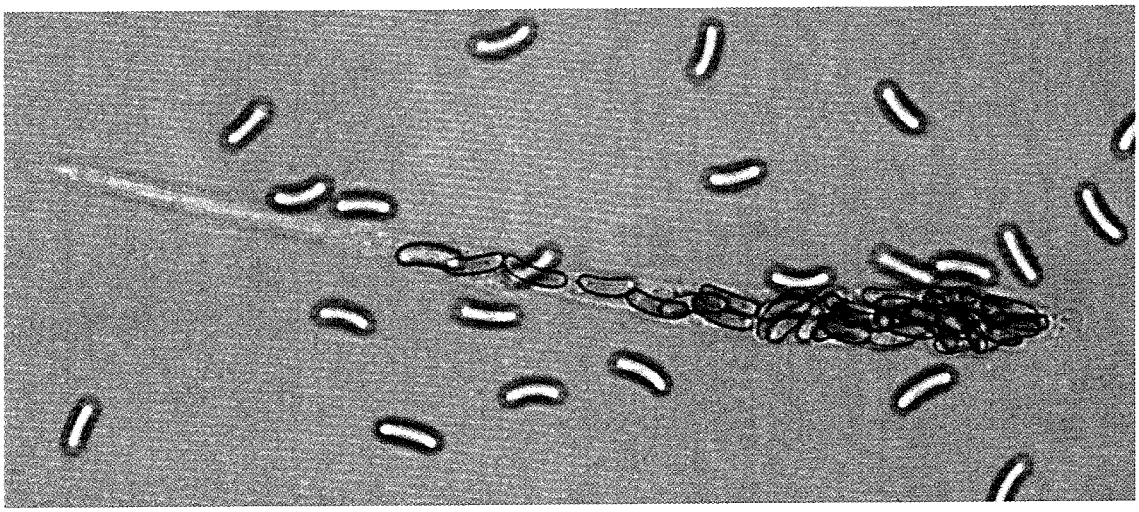
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NATIONAL Wine & Grape INDUSTRY CENTRE

CHARLES STURT UNIVERSITY
CENTRE FOR RESEARCH AND GRADUATE TRAINING
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GWRDC GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

Taxonomy and Biology of Diatrypaceous Fungi Associated with Grapevine Trunk Disease in New South Wales and South Australia



FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **GWT 09/05**

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Research Organisation:
National Wine and Grape Industry Centre

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Project Title: Taxonomy and biology of Diatrypaceous fungi associated with grapevine trunk disease in New South Wales and South Australia

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Abstract

The objectives of the project were to; (i) investigate the occurrence, taxonomy and biology of diatrypaceous fungi in vineyards and other hosts in NSW, (ii) study the pathogenicity of these fungi toward *Vitis vinifera* and, (iii) to disseminate the research findings to the industry and other researchers. Vineyards, hardwood trees, ornamentals and native plant species were surveyed for the presence of these fungi and morphological and molecular techniques were used to characterise them. Pathogenicity trials were established in NSW and SA on Cabernet Sauvignon and Chardonnay. Workshops were conducted to disseminate information on grapevine trunk diseases to growers and industry personnel.

Executive summary

Grapevine trunk diseases such as Eutypa dieback, Petri disease and Botryosphaeria canker have been well documented in Australian vineyards. However, the role of other fungi often isolated during surveys and belonging to the family Diatrypaceae remains unknown. In 2008, Dr Florent Trouillas (University of California, Davis) visited researchers at SARDI and the NWGIC to conduct a preliminary assessment of the Diatrypaceae in Australia. To continue the study further a collaborative project was established between Dr Florent Trouillas and Dr Doug Gubler (University of California, Davis) and researchers at the NWGIC, SARDI and The University of Adelaide.

A number of vineyard surveys were conducted throughout NSW and SA and previously established pathogenicity trials were assessed from SA. Over 100 specimens were analysed resulting in the discovery of many Diatrypaceae fungi from grapevines with canker diseases. Morphological and molecular techniques are being used to identify each organism to species level. In addition, Diatrypaceae were also isolated from hosts (*Citrus*, *Fraxinus*, *Populus* and *Ficus* etc.) other than grapevine and in the vicinity of vineyards suggesting that these fungi have the potential to spread from other hosts into grapevines. Results from the pathogenicity trials in SA revealed that *Cryptovalsa ampelina*, *Eutypa leptoplaca* and *Eutypella* spp. were capable of colonising glasshouse-grown Cabernet Sauvignon grapevines and causing vascular discolorations similar to those observed in the field. These results show that the Diatrypaceae may also be contributing to the grapevine trunk disease syndrome and that these should be considered when adopting management strategies. Consideration should also be given to other host plants in the vicinity of vineyards and their potential role in harbouring these pathogens. To our knowledge, this is the first study to characterise species of Diatrypaceae associated with *Vitis vinifera* and other host plants in Australia.

To increase the awareness of grapevine trunk diseases in Australia, a number of workshops were held throughout NSW, VIC and SA in November and December, 2009. Workshops were also held in WA in conjunction with GWRDC project RT 08_02-1. The workshops focused on the identification and management of Eutypa dieback, Botryosphaeria canker and Diatrypaceae. In NSW and VIC oral presentations were delivered to growers and industry personnel followed by a hands-on identification of trunk diseases in the vineyard. A total of 156 growers and industry personnel attended the workshops; 20 in NSW and VIC, 91 in SA, 45 in WA. Workshops were delivered by Drs Florent Trouillas, Sandra Savocchia, Mark Sosnowski, Wayne Pitt and Mr Anthony Somers. In addition, the results

of this study were presented at the 7th International Workshop on Grapevine Trunk Diseases, Chile in January 2010 and in various industry publications. The contributors are currently preparing manuscripts to appear in peer-reviewed journals.

Background

Grapevine trunk diseases are the primary cause of plant mortality and economic loss to the Australian wine industry. Eutypa dieback caused by the fungus Eutypa lata (Diatrypaceae) is regarded as the most important canker disease of grapevines in Australia (Carter, 1991). While recent surveys have highlighted the role of Botryosphaeriaceae fungi in the death and decline of grapevines (Pitt *et al.*, 2010a), an abundance of diatrypaceous species including Eutypa leptoplaca and Cryptovalsa ampelina have recently been detected both in vineyards and on nearby vegetation throughout New South Wales and South Australia (Pitt *et al.*, 2010b). In order for management of trunk diseases to be successful, the role of these fungi in the decline and dieback of grapevines in Australia requires further investigation.

In California, recent studies have shown that E. leptoplaca (Trouillas and Gubler, 2004), Diatrype stigma (Rolshausen *et al.*, 2005), C. ampelina as well as various other species of Eutypella and Diatrype are pathogenic to grapevines (Trouillas *et al.*, 2001; Trouillas et al., in press) causing canker and dieback diseases. To date, little work has been conducted on diatrypaceous species occurring in Australian vineyards. Mostert *et al.* (2004) reported the occurrence on grapevine of C. ampelina in Coonawarra and Eden Valley, and several other species were collected and described by Rappaz (1987). But, because species in this family are indistinguishable in culture from E. lata, it is likely that many fungi in this family were overlooked by previous studies.

Consequently, the objectives of this project were to identify pathogenic diatrypaceous fungi occurring in Australian vineyards as well as within natural ecosystems and ornamental plantings adjacent to vineyards. The distribution and pathogenicity of these fungi on grapevines in New South Wales and South Australia is currently unknown. Hence, much work remains not only in the collection and identification of the various species, but also in the determination of their pathogenicity to grapevines, and their role in grapevine decline.

In 2008, Dr Trouillas (University of California, Davis) visited researchers at SARDI and the NWGIC and began identifying these fungi to species level, however time and resources did not allow for the work to be completed. This travel funding allowed Dr Trouillas to undertake a three month research project in Australia to investigate the fungal species further and to assess their pathogenicity in grapevines. Along with researchers at SARDI and the NWGIC, he visited vineyards and presented workshops to growers and industry personnel in wine grape growing regions of NSW, SA and WA (in collaboration with GWRDC project RT 08_02-1). The results of the workshops and project were presented at the 7th International Workshop on Grapevine Trunk Diseases, Chile in January 2010 and will appear in peer-reviewed journals.

Project aims and performance targets

The outputs and performance targets were as follows;

Output	Performance Targets	Date
1. Occurrence and distribution of diatrypaceous fungi in NSW and SA winegrowing regions.	Vineyards in major grapegrowing regions of NSW (eg. Cowra, Griffith, Hunter Valley) will be surveyed for the presence of diatrypaceous fungi. Hardwood trees, ornamental and native plant species in the vicinity of vineyards will also be examined for the presence of these fungi. A collection of isolates will be established.	October 09
2. Taxonomy of diatrypaceous fungi associated with grapevine canker diseases in NSW and SA.	Morphological and molecular techniques will be used to characterise fungi isolated from NSW vineyards. A collection of diatrypaceous fungi collected from SA in 2008 by Dr Trouillas and maintained at SARDI will also be characterised. A phylogenetic analysis of all isolates will be performed.	December 09
3. Pathogenicity of Diatrypaceae species in grapevines determined.	The pathogenicity of selected isolates of Diatrypaceae will be conducted in SA and NSW on glasshouse-grown Cabernet Sauvignon and Chardonnay.	December 09
4. Presentation of results and knowledge transfer from international expert to grapegrowers and industry personnel.	Results and additional presentations relating to the management of grapevine trunk diseases will be presented at a minimum of three grapegrowing regions in each of NSW, SA and WA.	September – December 09
5. Presentation of results in peer-reviewed and industry publications.	Drafts of at least two peer-reviewed and one industry manuscript and will be prepared.	March 2010

Method

Output 1: Investigating the occurrence and distribution of diatrypaceous fungi in NSW and SA winegrowing regions.

During 2008, surveys were conducted in NSW and SA grape growing regions to collect perithecia of Diatrypaceae fungi. Grapevine plants, as well as hardwood trees, including ornamental and native plant species, found in the vicinity of vineyards were examined for the presence of fruiting bodies or stromata characteristic of Diatrypaceae. Additional isolates were obtained from infected grapevine spurs and wedge shaped cankers in cordons and trunks. Specimens were brought to the laboratory to be examined using morphological and phylogenetic analyses.

Output 2: Investigating the taxonomy of diatrypaceous fungi associated with grapevine canker diseases in NSW and SA.

Cultivation of isolates

Diatrypaceae isolates were grown from ascospores or from hyphae from infected grapevines as described by Trouillas *et al.* (2010). Ascospores were obtained from perithecia that were sliced opened with a blade and wetted with sterile water. Masses of ascospores were collected using a sterilized needle and were placed directly onto Petri plates containing potato dextrose agar (PDA) amended with 100 ppm tetracycline (PDA-tet). Pure cultures of each isolates were obtained by transferring single hyphal tips onto PDA-tet. Cultures were incubated at approximately 24°C under 12 h intermittent fluorescent lighting.

Identification

Representative samples of fruiting bodies were identified in accordance with the treatments of Glawe and Rogers (1984) and Rappaz (1987). Also, specimens from Australia were compared with reference specimens collected in California in their teleomorphic state (Trouillas *et al.*, 2010). Examinations were carried out with standard light microscopy. Conidial masses as well as perithecial contents were mounted in water and observed by brightfield microscopy. Digital images were recorded and measurements for at least 30 conidia, 50 ascospores and 20 asci were made. Representative isolates of Diatrypaceae from Australia were deposited at the Australian Scientific Collections Unit, NSW Industry & Investment, Orange, NSW, Australia (DAR).

DNA extraction, Polymerase chain reaction, Phylogenetic analyses

Fungal mycelia were obtained from liquid cultures of potato dextrose broth and prepared for DNA extraction as described by Pitt *et al.* (2010b). Total genomic DNA was extracted from pure cultures using the Qiagen Plant Mini Kit according to the manufacturer's instructions. The Internal Transcribed Spacer regions (ITS1 and ITS2), including the 5.8S rDNA operon of the nuclear ribosomal DNA region were amplified by the polymerase chain reaction (PCR) using primers ITS1 and ITS4 (White *et al.*, 1990). PCR reactions were performed as described by Trouillas *et al.* (2010). For sequencing, PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Australia). Both strands of the ITS and β-tubulin regions were sequenced by the Australian Genome Research Facility (University of Queensland, St Lucia, Qld, Australia). Sequencing results were edited and assembled using Sequencher™ version 3.1.1. Sequences were aligned using

ClustalW multiple alignment program (Thompson *et al.*, 1994) and were adjusted manually using BioEdit Sequence Alignment Editor Version 7.0.8 (Hall, 1999). Phylogenetic analyses were performed with PAUP version 4.0b10 (Swofford, 1999) using maximum parsimony with a heuristic search and 1000 random addition sequence replicates. Bootstrap support was evaluated using 1000 replicates to test branch strength.

Output 3: Investigating the pathogenicity of Diatrypaceae species in grapevine.

Pathogenicity tests were conducted to determine if these fungi were grapevine pathogens. Pathogenicity studies of *C. ampelina*, *E. leptoplaca* and *Eutypella* spp. collected in Australia were conducted as described by Sosnowski *et al.* (2007). The experiment also included known isolates of *E. lata* as well as newly collected isolates. Fresh wounds were made by drilling a 3-mm-diameter hole into the wood of potted Cabernet Sauvignon grapevines. Inoculations were made by inserting an agar plug with fresh mycelium obtained from the margin of 8-day-old colonies. Inoculated wounds were covered with Vaseline and wrapped with Parafilm. Plants were then maintained in a shade house for 10 months before being assessed for lesion development and fungal recovery. After 10 months, canes were surface-sterilised using 2.5% sodium hypochlorite for 5 min and air dried. Fragments of necrotic tissue developing outside the area of inoculation were cultured onto PDA amended with streptomycin (25µg/L). Fragments were collected at every 5 mm interval for 50 mm upward and 100 mm downward from the inoculation point. Diatrypaceae isolates obtained from re-isolation were identified based on colony shape, colour and growth rate. Statistical analyses were performed using an analysis of variance (ANOVA) in SAS version 9.1. Log transformations were performed to reduce heteroscedasticity and to stabilise variance. Dunnett's t-test was used to assess significant differences in the extent of vascular discoloration between the different treatment group means. Additional pathogenicity tests have been established at the NWGIC to assess the pathogenicity of Diatrypaceae isolated from vineyards in NSW. The results of these tests will be available in early 2011.

Output 4: Presentation of results and knowledge transfer from international expert to grapegrowers and industry personnel.

A series of eight grapevine trunk disease identification and management workshops were held in Griffith (NSW), Rutherglen (VIC); Limestone Coast, Adelaide Hills and McLaren Vale regions of SA; Swan Valley, Margaret River and Great Southern regions of WA (RT 08_02-1).

Results/Discussion

Output 1: Investigating the occurrence and distribution of diatrypaceous fungi in NSW and SA winegrowing regions.

Over 100 specimens were collected for this study (Table 1, Appendix 5). Preliminary results of surveys from infected grapevine wood were previously reported (Pitt *et al.*, 2010b). In many instances, fungal colonies displaying morphological characteristics typical of Diatrypaceae were isolated from grapevine cankers. For cankers sampled from vineyards in SA and NSW the incidence of *C. ampelina* varied between 5-10%. Isolates of *Diatrypella* sp. were recovered from 30% of cankers collected in a young vineyard in Tumbarumba whereas *Eutypella* spp. comprised 60% of all fungi isolated from cankers in the Hunter Valley. The majority of these fungi grow as white colonies in culture on PDA-tet resembling *E. lata*, which suggests they may have been misidentified and overlooked in previous studies. Fruiting bodies of Diatrypaceae were also found commonly on dead grapevine wood or ornamental trees surrounding vineyards. Major hosts among ornamental bearing fruiting body of Diatrypaceae included *Schinus molle var. areira, Populus nigra 'italica', Ulmus procera* and *Fraxinus angustifolia*. Species of *Eutypella* occurring on grape also appeared to be commonly distributed in abandoned Citrus orchards in NSW. These findings suggest that there is potential for the spread of these fungi from orchard and ornamentals into grapevine, which could result in the emergence of new diseases.

Output 2: Investigating the taxonomy of diatrypaceous fungi associated with grapevine canker diseases in NSW and SA.

Morphological studies and phylogenetic analyses of the complete sequence of the ITS region of the rDNA identified *C. ampelina*, *Diatrypella* sp., as well as two species of *Eutypella* from grapevine cankers in Australia, in addition to *E. lata* (Fig.1, Appendix 5). *Eutypella* spp. included *E. citricola*, as well as a putative new species of *Eutypella*. Similar species occurred on other agricultural host plants and ornamentals adjacent to vineyards. Surveys also documented *E. leptoplaca* on *Fraxinus*, *Populus* and *Schinus* spp., however it was not isolated from grapevine. Interestingly, species isolated from grapevine and surroundings trees in Australia were similar to species previously isolated from grapevine in California (Fig.1, Appendix 5). All species or species-groups (clades) found in the phylogenetic analyses also could be differentiated morphologically by at least one character of either the anamorphic or teleomorphic state. Accordingly, asci of *C. ampelina* and *Diatrypella* spp were polysporous, while eight-spored (octosporous) in *Eutypa* spp. and *Eutypella* spp. Asci of *C. ampelina* contained 32 ascospores, while asci of *Diatrypella* spp. contained numerous ascospores. Stromata of *Eutypella spp.* were usually elongated to form linear pustules on the surface of the grapevine wood or bark, with white entostroma. *Eutypella* spp. could be separated from one another by the pink pigmentation present in *Eutypella* sp. *nov.*

Output 3: Investigating the pathogenicity of Diatrypaceae species in grapevine.

Results of pathogenicity tests are illustrated in Fig. 2, Appendix 5. Lesions produced by diatrypaceous fungi consisted of vascular discolorations similar to those produced by *E. lata*. Pathogenicity test proved that all Diatrypaceae tested were capable of infecting grapevine wood. All fungal isolates tested were recovered at 100% rate, 10 months after

inoculations in grapevine cuttings. Average length of vascular discolorations varied among isolates. Length of vascular discolorations in general varied between 12 mm and approx. 45 mm. Averaged vascular discoloration produced in the control was only 5 mm. All fungal groups colonised approx. 20 to 60 mm of wood during the 10 month incubation period. Dunnett's t-test showed significant differences in vascular discolorations between treatments with isolates of *C. ampelina*, *E. lata*, *E. leptoplaca*, and *Eutypella* from control vines.

Output 4: Presentation of results and knowledge transfer from international expert to grapegrowers and industry personnel.

Industry personnel and growers were presented the latest research on the identification and management of grapevine trunk diseases from Australia and California (Appendices 1 & 5). A total of 156 personnel attended the workshops, see table below.

Workshop location	Attendees
Griffith, NSW	7
Rutherglen, Vic	13
Limestone Coast, SA	34
Adelaide Hills, SA	29
McLaren Vale, SA	28
Swan Valley, WA	10
Margaret River, WA	22
Great Southern, WA	13
Total	156

Output 5: Presentation of results in peer-reviewed and industry publications.

Results of the research were presented at one international conference and in industry journals and newsletters (Appendix 1). The final results are currently being analysed and it is anticipated that this will result in two peer-reviewed manuscripts.

Outcome/Conclusion

The occurrence and ease of isolation of Diatrypaceae fungi from the margin of active grapevine cankers suggests they may participate in the dieback of grapevine and thus impacting on vineyard longevity. The abundance of Diatrypaceae on ornamentals surrounding vineyards, together with the more sporadic occurrence of similar species in vineyards suggests these fungi spread from ornamentals to vineyards. All species isolated from grapevine in Australia are being identified and described using morphological and molecular analyses. Identifications as well as descriptions will provide more accurate tools for diagnosing organisms responsible for grapevine canker diseases. Results of pathogenicity tests showed that *C. ampelina*, *E. leptoplaca* and *Eutypella* spp. were capable of colonising dormant canes and causing vascular discolorations. These fungi were re-isolated from the margin of developing lesions, with generally 100% frequency of re-isolation, suggesting they are pathogenic toward grapevine. This work constitutes the first

comprehensive effort to characterise species of Diatrypaceae associated with *V. vinifera* and other host plants in Australia.

Recommendations

Further research is required to document the organisms responsible for grapevine canker diseases and to ascertain their pathogenicity in the field. Perhaps efforts should be centred towards studying the organisms as complexes rather than individual contributors to the canker disease syndromes. It is also important that management strategies developed for Eutypa dieback and Bot canker are effective for all pathogenic fungi that inhabit grapevines in Australian vineyards.

Appendix 1: Communication

Conference Presentations

Trouillas FP, Pitt WM, Huang R, Sosnowski M, Loschiavo A, Savocchia S, Steel C, Scott E, Gubler WD (2010) Diatrypaceae associated with *Vitis vinifera* in New South Wales and South Australia. Oral presentation at the 7th International Grapevine Trunk Disease Workshop, Santa Cruz, Chile, 17-21 Jan.

Somers A, Pitt W, Savocchia S, Blake A, Weckert M (2010) Communication and application of grapevine trunk disease research through extension and education. Oral presentation at the 7th International Grapevine Trunk Disease Workshop, Santa Cruz, Chile, 17-21 Jan.

Industry Journal and Newsletters

Focus on Trunk Disease, GrapeGrower & Vignerons, December 2009.

Trunk Disease Workshops in WA. Wine Industry Newsletter, Newsletter of the Department of Agriculture and Food Government of Western Australia, No. 93, December, 2009, p. 11-12.

Group delivers expert advice on grapevine trunk diseases at WA workshops. The Australian & New Zealand Grapegrower & Winemaker 552: 24-25.

Workshop Presentations

Trouillas FP (2009) Grapevine canker diseases in California. Western Australia Trunk Disease Workshop, Jane Brook Estate, Swan Valley, WA, 2 Nov.

Trouillas FP (2009) Grapevine canker diseases in California. Western Australia Trunk Disease Workshop, Vasse Felix Winery, Margaret River, WA, 4 Nov.

Trouillas FP (2009) Grapevine canker diseases in California. Western Australia Trunk Disease Workshop, West Cape Howe Winery, Mt Barker, WA, 6 Nov.

Trouillas FP (2009) Grapevine canker diseases in California. Griffith Trunk Disease Workshop, DPI Agriculture Centre, Hanwood, NSW, 24 Nov.

Trouillas FP (2009) Grapevine canker diseases in California. Victorian Trunk Disease Workshop, Rutherglen Research Institute, Rutherglen, NSW, 25 Nov.

Trouillas FP (2009) Grapevine canker diseases in California. Limestone Coast Trunk Disease Workshop, Katnook Estates Winery, Coonawarra, SA, 3 Dec.

Trouillas FP (2009) Grapevine canker diseases in California. Adelaide Hills Wine Region Trunk Disease Workshop, Lenswood Research Centre, SA, 8 Dec.

Trouillas FP (2009) Grapevine canker diseases in California. McLaren Vale Grape, Wine and Tourism Association Trunk Disease Workshop, McLaren Vale Tourist Centre, SA, 10 Dec.

Appendix 2: Intellectual Property

Not applicable

Appendix 3: References

Carter M.V. (1991) The status of *Eutypa lata* as a pathogen. Monograph, Phytopathological Paper No 32. Commonwealth Agricultural Bureau, International Mycological Institute: U.K.

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Pitt W.M., Huang R., Trouillas F.P., Savocchia S., Steel C.C. (2010b) Evidence that *Eutypa lata* and other diatrypaceous species occur in New South Wales vineyards. *Australasian Plant Pathology* 39: 97-106.

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Rolshausen P.E., Mahoney N.E., Molyneux R.J., Gubler W.D. (2005) A reassessment of the species concept in *Eutypa lata*, the causal agent of Eutypa dieback of grapevine. *Phytopathology* 96: 369–377.

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Trouillas F.P., Rolshausen PE., Gubler W.D. (2001) Importance of *Eutypa lata* and occurrence of other diatrypaceous fungi in Northern Californian vineyards. *Phytopathology* 91: S89.

Trouillas F.P., Gubler W.D. (2004) Identification and characterization of *Eutypa leptoplaca*, a new pathogen of grapevine in Northern California. *Mycological Research* 108: 1195–1204.

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White T.J., Bruns T., Lee S., Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols, a Guide to Methods and Applications (eds. Innis MA, Gelfand DH, Sninsky JJ and White J) 315–322. Academic Press: San Diego, Ca, USA.

Appendix 4: Staff

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Appendix 5: Other relevant material

Table 1. List of specimens collected for this study and used in morphological, phylogenetic and pathogenicity studies

Collection number	Species	Host	Origin
B10-15A	*Cryptovalsa*	*Vitis vinifera*	South Australia (SA)
B10-16[a]	*C. ampelina*	*Vitis vinifera*	SA
ADSC200	*C. ampelina*	*Schinus molle var. areira*	Adelaide, SA
ADSC201	*C. ampelina*	*Schinus molle var. areira*	Adelaide, SA
AD100	*C. ampelina*	*Vitis vinifera*	SA
AD101	*C. ampelina*	*Vitis vinifera*	SA
C14A	*C. ampelina*	*Vitis vinifera*	SA
C17A	*C. ampelina*	*Vitis vinifera*	SA
1RH07	*C. ampelina*	*Vitis vinifera*	SA
40RE01	*C. ampelina*	*Vitis vinifera*	SA
R40V205	*C. ampelina*	*Vitis vinifera*	SA
B2-15[a]	*C. ampelina*	*Vitis vinifera*	SA
B5-17[a]	*C. ampelina*	*Vitis vinifera*	SA
RGA05	*C. ampelina*	*Fraxinus angustifolia*	Adelaide Hills, SA
ABA100	*C. ampelina*	*Fraxinus angustifolia*	Barossa Valley, SA
AH01	*C. ampelina*	*Acer macrophyllum*	Adelaide Hills, SA
SAPN03	*C. ampelina*	*Populus nigra 'italica'*	McLaren Flat, SA
TUVN01	*C. ampelina*	*Vitis vinifera*	Tumbarumba, New South Wales (NSW)
TUUP01	*C. ampelina*	*Ulmus procera*	Tumbarumba, NSW
HVVIT04	*C. ampelina*	*Vitis vinifera*	Hunter Valley, NSW
HVFIG01	*C. ampelina*	*Ficus carica*	Hunter Valley, NSW
CSU01	*C. ampelina*	*Pistachio*	Wagga Wagga, NSW
D02	*C. ampelina*	*Vitis vinifera*	Murrambateman, NSW
D04	*C. ampelina*	*Vitis vinifera*	Murrambateman, NSW
D06	*C. ampelina*	*Vitis vinifera*	Murrambateman, NSW
KC6	*C. ampelina*	*Vitis vinifera*	Book Book, NSW
SH20	*C. ampelina*	*Vitis vinifera*	Murrumbateman, NSW
VR4	*C. ampelina*	*Vitis vinifera*	Canowindra, NSW
CV9	*C. ampelina*	*Vitis vinifera*	Orange, NSW
HVPT02	*Diatrype* sp.	*Schinus molle var. areira*	Hunter Valley, NSW
HVGRF03	*Diatrypella*	*Citrus paradisi*	Hunter Valley, NSW
HVFRA02	*Diatrypella*	*Fraxinus angustifolia*	Hunter Valley, NSW
HVFRA03	*Diatrypella*	*Fraxinus angustifolia*	Hunter Valley, NSW
HVFRA04	*Diatrypella*	*Fraxinus angustifolia*	Hunter Valley, NSW
HVPT01	*Diatrypella*	*Schinus molle var. areira*	Hunter Valley, NSW
ADSC300	*Eutypa lata*	*Schinus molle var. areira*	Adelaide, SA
ADSC400	*E. lata*	*Schinus molle var. areira*	Adelaide, SA
SACEA01	*E. lata*	*Ceanothus* sp.	Adelaide, SA
RGA01	*E. lata*	*Fraxinus angustifolia*	Adelaide Hills, SA
RGA03	*E. lata*	*Fraxinus angustifolia*	Adelaide Hills, SA
SAPN01	*E. lata*	*Populus nigra 'italica'*	McLaren Flat, SA
POP1	*E. lata*	*Populus nigra 'italica'*	

Table 1 cont.

Collection number	Species	Host	Origin
ADFIC100	*Eutypa leptoplaca*	*Ficus macrophylla*	Adelaide, SA
ADFIC101	*E. leptoplaca*	*Ficus macrophylla*	Adelaide, SA
RGA02	*E. leptoplaca*	*Fraxinus angustifolia*	Adelaide Hills, SA
RGA04	*E. leptoplaca*	*Fraxinus angustifolia*	Adelaide Hills, SA
ABA200	*E. leptoplaca*	*Fraxinus angustifolia*	Barossa Valley, SA
ABA300	*E. leptoplaca*	*Fraxinus angustifolia*	Barossa Valley, SA
SAPA01A	*E. leptoplaca*	*Populus alba*	Adelaide, SA
ADSC500	*E. leptoplaca*	*Schinus molle var. areira*	Adelaide, SA
SAPN02	*E. leptoplaca*	*Populus nigra 'italica'*	McLaren Flat, SA
SAPN04	*E. leptoplaca*	*Populus nigra 'italica'*	McLaren Flat, SA
SACOO1	*E. leptoplaca*	*Populus nigra 'italica'*	Coonawarra, SA
SACOO2	*E. leptoplaca*	*Populus nigra 'italica'*	Coonawarra, SA
TUQU01	*E. leptoplaca*	*Quercus* sp.	Tumbarumba, NSW
TUPN01	*E. leptoplaca*	*Populus nigra 'italica'*	Tumbarumba, NSW
TUPN02	*E. leptoplaca*	*Populus nigra 'italica'*	Tumbarumba, NSW
ADEL100	*Eutypella citricola*	*Ulmus procera*	Adelaide, SA
ADEL101	*E. citricola*	*Ulmus procera*	Adelaide, SA
ADSC100	*E. citricola*	*Schinus molle var. areira*	Adelaide, SA
T10R4S7	*E. citricola*	*Vitis vinifera*	Hunter Valley, NSW
T10R2S6	*E. citricola*	*Vitis vinifera*	Hunter Valley, NSW
T2R3S3	*E. citricola*	*Vitis vinifera*	Hunter Valley, NSW
T3R2S2	*E. citricola*	*Vitis vinifera*	Hunter Valley, NSW
HVIT03	*E. citricola*	*Vitis vinifera*	Hunter Valley, NSW
HVIT07	*E. citricola*	*Vitis vinifera*	Hunter Valley, NSW
HVIT08	*E. citricola*	*Vitis vinifera*	Hunter Valley, NSW
HVOT01	*E. citricola*	*Citrusinensis or C. aurentium*	Hunter Valley, NSW
HVFIG04B	*Eutypella caricae*	*Ficus carica*	Hunter Valley, NSW
HVFIG05	*E. caricae*	*Ficus carica*	Hunter Valley, NSW
HVAV01	*Eutypella* sp.	*Avocado*	Hunter Valley, NSW
ADEL200	*Eutypella* sp.	*Ulmus procera*	Adelaide, SA
ADEL300	*Eutypella* sp.	*Ulmus procera*	Adelaide, SA
YC16	*Eutypella* sp.	*Vitis vinifera*	Hunter Valley, NSW
YC17	*Eutypella* sp.	*Vitis vinifera*	Hunter Valley, NSW
YC23	*Eutypella* sp.	*Vitis vinifera*	Hunter Valley, NSW
YC24	*Eutypella* sp.	*Vitis vinifera*	Hunter Valley, NSW
T2R2S7	*Eutypella* sp.	*Vitis vinifera*	Hunter Valley, NSW
T7R2S2	*Eutypella* sp.	*Vitis vinifera*	Hunter Valley, NSW
T10R3S9	*Eutypella* sp.	*Vitis vinifera*	Hunter Valley, NSW
T11R4S9	*Eutypella* sp.	*Vitis vinifera*	Hunter Valley, NSW
T20R4S2	*Eutypella* sp.	*Vitis vinifera*	Hunter Valley, NSW
HVGRF01	*Eutypella* sp.	*Citrus paradisi*	Hunter Valley, NSW
HVGRF02	*Eutypella* sp.	*Citrus paradisi*	Hunter Valley, NSW
HVVIT05	*Eutypella* sp.	*Vitis vinifera*	Hunter Valley, NSW
WA01SV	*Eutypella* sp.	*Vitis vinifera*	Swan Valley, Western Australia (WA)
WA02BO	*Eutypella* sp.	*Vitis vinifera*	WA
WA03LE	*Eutypella* sp.	*Citrus* sp.	Swan Valley, WA
WA04LE	*Eutypella* sp.	*Citrus* sp.	Swan Valley, WA

Table 1. cont.

Collection number	Species	Host	Origin
WA05SV	*Eutypella* sp.	*Vitis vinifera*	Swan Valley, WA
WA06FH	*Eutypella* sp.	*Vitis vinifera*	WA
WA07CO	*Cryptovalsa* sp.	*Vitis vinifera*	Cowaramup, WA
WA08CB	*Cryptovalsa* sp.	*Vitis vinifera*	WA
WA09LE	*Eutypella* sp.	*Citrus* sp.	Swan Valley, Waneroo, WA
NSW01PO	*Cryptosphaeria* sp.	*Populus alba*	Adelong, NSW
NSW02PO	*Cryptosphaeria* sp.	*Populus alba*	Adelong, NSW
NSW03PO	*Cryptosphaeria* sp.	*Populus balsamifera*	Khancoban, NSW
NSW04PO	*Cryptosphaeria* sp.	*Populus balsamifera*	Khancoban, NSW
NSW05PO	*Cryptosphaeria* sp.	*Populus balsamifera* (canker)	Khancoban, NSW
WA10NO	*Botryosphaeria* sp.	*Vitis vinifera*	Noack Road, WA
WA20JB	*Phomopsis* sp.	*Vitis vinifera*	Swan Valley, WA

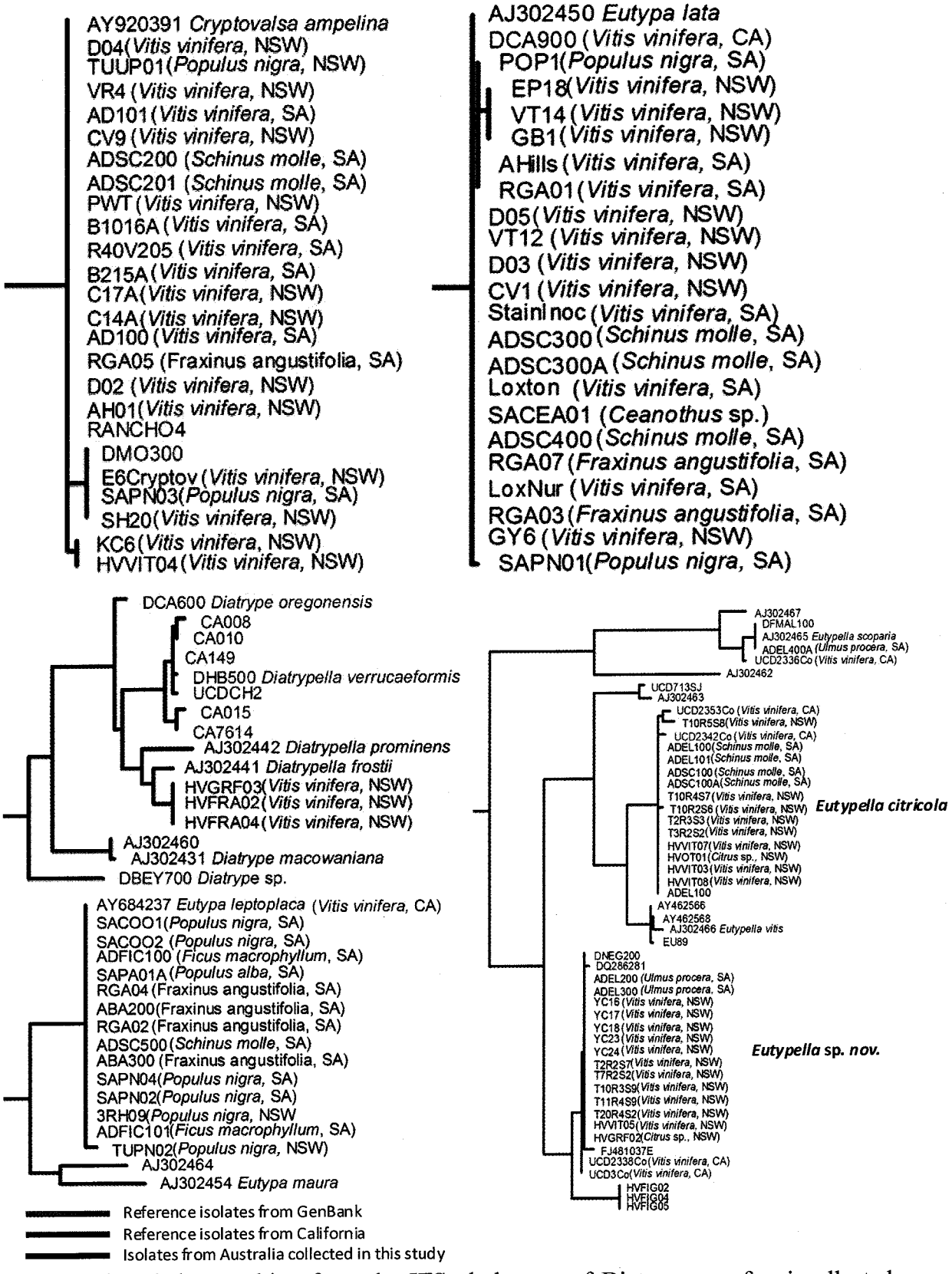


Fig. 1. Major clades resulting from the ITS phylogeny of Diatrypaceae fungi collected from grapevines and ornamental trees in Australia. Isolates were compared with referenced isolates from California and GenBank.

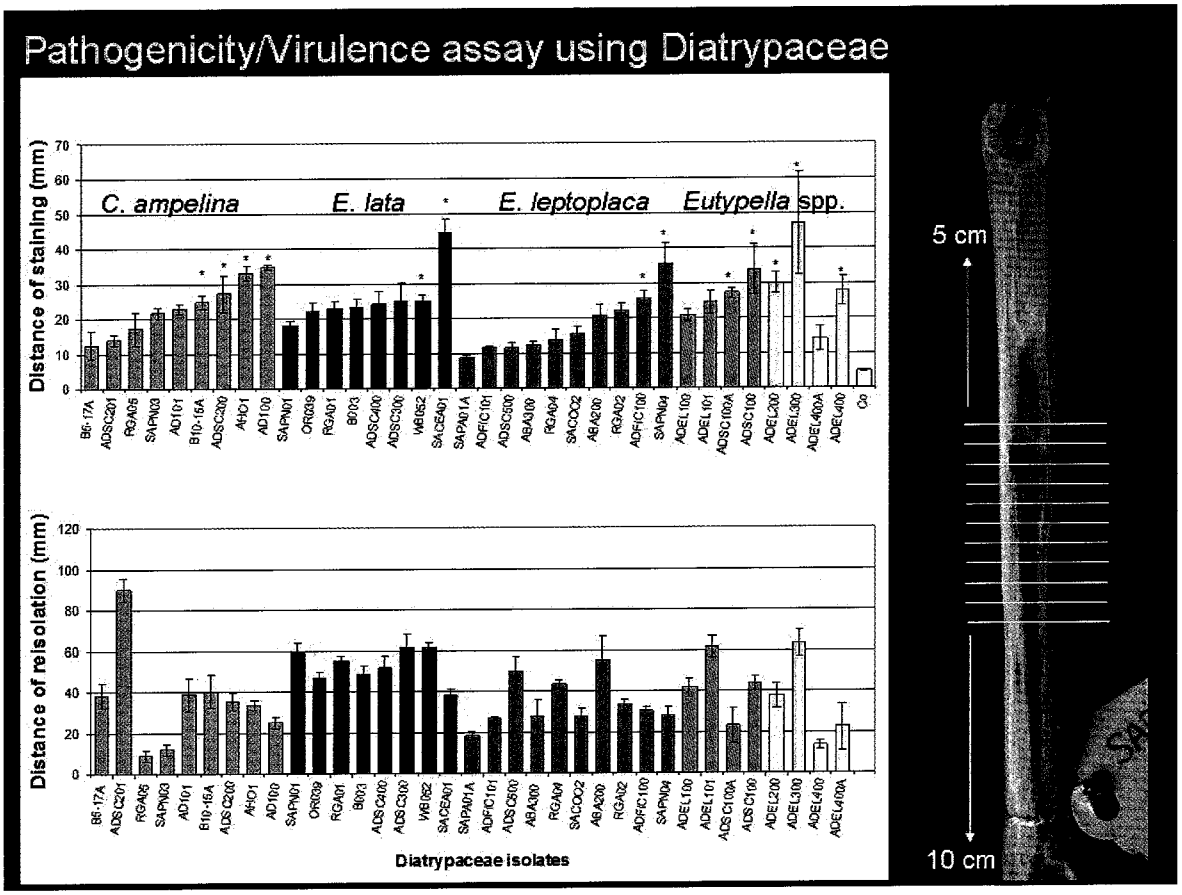


Fig. 2. Staining and reisolation distance (mm) in stems of potted Cabernet Sauvignon grapevines 10 months after wounding and inoculation with multiple isolates and species within the Diatrypaceae.

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NATIONAL Wine & Grape INDUSTRY CENTRE

Australian Government
Grape and Wine Research and Development Corporation

Winegrowing Futures
Vine Health Workshop
ManagingTrunk Diseases in Vineyards
DPI Agriculture Centre
Farm 217, Murray Road, Hanwood.
24TH November 2009 From 8.30 am to 2.00

About the workshop.

You will learn how to identify, manage and control wood rot diseases of grapevines.

Presenters:

- **Sandra Savocchia, National Wine and Grape Industry Centre. Wagga Wagga**
- **Florent Trouillas, University of California Davis.**
- **Tony Somers, National Wine and Grape Industry Centre. Paterson.**

Program:

8.30-9.00	Registration and Coffee
9.00-9.10	Welcome
9.10-9.40	What are trunk diseases, how to recognise them and wood rot diseases in Californian vineyards
9.40-10.30	Incidence, distribution and control of trunk diseases
10.30-11.00	Coffee break
11.00–11.45	Principals of pruning and retraining to rejuvenate vines with trunk diseases
11.45–12.00	General discussion
12.00–12.30	Light lunch
12.30–2.00	Footwear sanitation. Relocate to demonstration vineyard

Acknowledgement: Riverina Wine Grape Marketing Board
RSVP by 20TH November to KRISTY BARTROP at the WGMB office.
email kbartrop@wgmb.net.au or facsimile 02 6962 6103

UCDAVIS
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NATIONAL Wine & Grape INDUSTRY CENTRE

Australian Government
Grape and Wine Research and Development Corporation

Winegrowing Futures
Vine Health Workshop
Managing Trunk Diseases in Vineyards
Rutherglen Research Institute
25TH November 2009 From 8.30 am to 2.00

About the workshop.

You will learn how to identify, manage and control wood rot diseases of grapevines.

Presenters:

- **Sandra Savocchia, National Wine and Grape Industry Centre. Wagga Wagga**
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Acknowledgement: Victorian Wine Industry Association



Western Australia Grapevine Trunk Disease Workshops



This workshop series, funded by GWRDC, will provide members of the WA wine industry with the latest information on identification and management of eutypa dieback and botryosphaeria canker diseases of grapevine. It aims to better equip you to recognise symptoms of these diseases in your own vineyard and how to control them effectively.





Workshop program

9am	Welcome and Introduction	
9.15	Eutypa dieback - symptoms and identification	*Adrian Loschiavo - Scholefield Robinson*
9.45	Eutypa dieback - management	*Mark Sosnowski - SARDI*
10.15	Grapevine Trunk Diseases in California	*Florent Trouillas - Uni of California Davis*
10.45	Morning tea	
11.15	Botryosphaeria canker - WA situation	*Andrew Taylor - DAFWA*
11.45	Botryosphaeria canker - management	*Wayne Pitt - Charles Sturt Uni*
12.15	Vineyard demo, lunch & discussion	

Workshop locations

Swan Valley - Mon 2 Nov 2009
Jane Brook Estate, 229 Toodyay Road, Middle Swan

Margaret River - Wed 4 Nov 2009
Vasse Felix Winery, Cnr Caves Road & Harmans Road Sth, Cowaramup

Mt Barker – Fri 6 Nov 2009
West Cape Howe Winery, ex Goundry site 'The Arts House',
14923 Muirs Highway, Mt Barker

Registration

Bookings can be made by contacting

Diana Fisher - Ph: 9777 000 email: diana.fisher@agric.wa.gov.au

Andrew Taylor - andrew.taylor@agric.wa.gov.au

Keith Pekin - keith@winewa.asn.au

WINE INDUSTRY ASSOCIATION WESTERN AUSTRALIA

Scholefield Robinson HORTICULTURAL SERVICES

Department of Agriculture

GWRDC GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

UC DAVIS UNIVERSITY OF CALIFORNIA

SARDI SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

McLarenVale Grape, Wine & Tourism Association

Growers' Seminar
Research Update

The Grower Development Group is hosting a seminar to update Grape Grower Members on a number of ongoing research projects.

1. **Eutypa Dieback Research Update**
   Presenters: Dr Florent Trouillas, University of California Davis & Mark Sosnowski, SARDI

2. **Alternative Farming Systems**
   Presenter: Luke Johnston, PhD Student, Plant Research Centre, University of Adelaide

3. **Biodiversity Project & Incentives Program**
   Presenter: Claire Stephenson, Adelaide and Mt Lofty Ranges NRM

Date:	Thursday, 10th December 2009
Time:	9.00am
Location:	Theatre room, Visitors Centre, Main Road, McLaren Vale
RSVP:	Tuesday, 8th December 2009
	Alex Henkel on 8323 8999 or alex@mclarenvale.info
	Bookings Essential as places limited
Cost:	No charge

Additional Information

Dr Florent Trouillas from Dr Doug Gubler's Laboratory (University of California Davis) has extensive experience in the identification and taxonomy of diatrypaceous fungi associated with Eutypa dieback as well as those associated with other diseases of grapevines such as Botryosphaeria canker, Petri disease and Esca. His PhD studies involved identification of inoculum sources for *Eutypa lata*, but rapidly grew to encompass research on the incidence, distribution and pathogenicity of *E. lata* and other diatrypaceous species from grapevines and surrounding vegetation in California.

Dr Trouillas spent 3 months at SARDI in 2008 with researchers from SARDI and isolated a number of fungi other than *E. lata* from symptomatic grapevines and alternative hosts sampled from around South Australia. These fungi have been identified as belonging to the family Diatrypaceae. In order for management of trunk diseases to be successful, the role of these fungi in the decline and dieback of grapevines in Australia is being further investigated. Dr Trouillas will return to Adelaide in December 2009 to assess the pathogenicity of these other fungi in grapevines.

Whilst in Adelaide Dr Trouillas would be pleased to speak to growers about his findings in South Australia as well as the latest information of trunk disease research in California being undertaken by the Plant Pathology Department at UC Davis.

Have you had trouble in the past seasons with Eutypa or Phomopsis this Season?

Yes?

Eutypa and Phomopsis Workshop and Field Walk

Katnook Estates Stables - Coonawarra
December 3rd
8:30am to 12 noon

8:30am - 10:30 Presentations and Panel Discussion
11:00am to 12 noon Field Walk and In Field Assessment

Hear from Researchers with Local, National and International Experience

Eutypa

Dr Florent Trouillas - University of California Davis
Dr Mark Sosnowski - Research Scientist, Horticulture Pathology

Phomopsis

Dr Trevor Wicks - Senior Research Scientist, Horticulture Pathology
(Looking at the local trails and results)

Dr Florent Trouillas from Dr Doug Gubler's Laboratory (University of California Davis) has extensive experience in the identification and taxonomy of diatrypaceous fungi associated with Eutypa dieback. Dr Trouillas spent 3 months at SARDI in 2008 with researchers from SARDI and isolated a number of fungi other than *E. lata* from symptomatic grapevines and alternative hosts sampled from around South Australia. Dr Trouillas will to speak to growers in the Limestone Coast about his findings in South Australia as well as the latest information of trunk disease research in California being undertaken by the Plant Pathology Department at UC Davis

Adelaide Hills Wine Region Viticulture Technical Group
(AHWRVTG)
Presents
Ewe Got Eutypa!

AHWRVTG is a committee of professional grape growers, who give up their time freely to meet monthly to plan, discuss and disseminate information to Adelaide Hills Wine Region grape growers. The aim is to inform and educate its members by providing updates in the latest research that come to hand, presenting case studies based on grower experiences of putting research into practice and workshops.

One of the most keenly awaited 'events' is the Seminar series, where attendees not only get information straight from the source, but are also able to network with other growers.

The upcoming seminar presents an in depth look at Dead Arm Disease (Eutypa), a problem that afflicts every vineyard in the Hills, and Organic weed control in Vineyards.

Surviving the GFC is one thing but Eutypa left unchecked will kill a vineyard. We are very fortunate to be able to present the latest research on pruning wound protection to growers from SARDI and also the research findings of a visiting researcher from UC-Davis who is an expert in this field and has been studying the problem in the Hills Region with SARDI researchers. This may be the last chance to pick the brains of these people for some time because research funding is drying up on this insidious disease.

Organic grape growing is easy, but weed management is not. Hear a first hand account of the trials and tribulations of Henschke Wines in a 'warts and all' expose of what they've been up to on the road to Organic certification.

If you are thinking about going Organic, waging war on Eutypa or just plain curious, then we look forward to seeing you at the Lenswood PIRSA Research Station, Swamp Road on December 8th, 9.30 registration for 10am sharp and concluding at noon. Members are free, $5 at the door for non members, All welcome. To book your seat, contact Denise Manson on
P: 1300 305 577, F: +61 8 8391 1382, or E: wine@adelaidehills.com.au