AUSTRALASIAN PLANT PATHOLOGY SOCIETY, WA
STUDENT SYMPOSIUM ABSTRACTS
29 OCTOBER 2010, DAFWA, SOUTH PERTH

1. The role of *Phytophthora* species in the decline of *Eucalyptus rudis* (flooded gum) in south-west Western Australia

Katherine Edwards, G.E.St J. Hardy, W. Dunstan and T. Jung
Centre for Phytophthora Sciences and Management, School of Biological Sciences and Biotechnology, Murdoch University, 90 South Street, Murdoch 6150
Email: 30423568@student.murdoch.edu.au

The *Eucalyptus rudis* (flooded gum) is in decline across its range in the south-west of Western Australia. It is a keystone species in riparian ecosystems where it provides important ecosystem functions such as habitat provision and water quality maintenance. Symptoms of decline resemble dieback symptoms caused by pathogens from the genus *Phytophthora*. The rhizosphere soil of *E. rudis*, and water, were sampled along several watercourses in the south-west and species of *Phytophthora* isolated. Several species were recovered from soils and water, including *P. multivora* and *P. elongata*, two species which are believed to be involved in Eucalypt decline. None of the species recovered acted as primary pathogens on *E. rudis* in pathogenicity tests, but they may be acting in conjunction with other biotic and abiotic factors in the field. Several different treatments, including phosphite, insecticide and complete (macro- and micro-) nutrients, were tested on mature *E. rudis* in a Perth wetland to compare their effectiveness in restoring health. The health of these *E. rudis* were also assessed using aerial remote sensing imagery. The results of this study will have significant implications for the diagnosis and treatment of *Eucalyptus rudis* decline.

2. Managing fungicide resistance: Protecting Western Australia's barley crops against *Blumeria graminis* f. sp. *hordei*

Madeline Tucker [1,2], R. Oliver [1] and J. Lichtenzveig [1]
[1] *Australian Centre for Nectrotrophic Fungal Pathogens (ACNFP), Curtin University of Technology, Kent Street, Bentley*
[2] *State Agricultural Biotechnology Centre (SABC), Murdoch University, South Street, Murdoch 6150*
Email: m.tucker@murdoch.edu.au

Powdery mildew is a common disease of many monocotyledonous and dicotyledonous plant species. In barley the disease is caused by the obligate Biotrophic fungus *Blumeria graminis* f. sp. *hordei (Bgh).* In Western Australia powdery mildew is estimated to be the most economically damaging disease of barley, causing $33m in loses annually. Currently in WA the majority of fungicides registered for control of *Bgh* belong to the triazole class and numerous reports of resistance towards this chemical class have been reported globally. Instances of reduced efficacy of the in furrow application of fungicides has been noted recently in southern WA. In this study the level of fungicide sensitivity of current WA *Bgh* isolates was assessed by examining colony inhibition in the presence of both registered and novel fungicides. One strain with reduced sensitivity was genotypically characterized in the target site for triazole fungicides, the *Cyp51* gene, and was found to harbor a mutation common in triazole resistant *Bgh* isolates. This is the first confirmed case of fungicide resistance in Australia.

3. *Stemphylium* grey leaf spot disease of lupins in Western Australia

Arbab Ahmad [1], G.J. Thomas [2], S.J. Barker [1] and B. MacLeod [2]
[1] *School of Plant Biology, The University of Western Australia, Crawley 6009*
[2] *Department of Agriculture and Food Western Australia, South Perth 6151*
Email: arbab.ahmad@uwa.edu.au

Stemphylium grey leaf spot disease has been among the major constraints to lupin production in Western Australia in the past. During the early-mid 1970's the disease caused heavy yield losses. Wild accessions of *Lupinus angustifolius* were found resistant to the disease in the USA. This provided a source of resistance that was later incorporated into Western Australian varieties. Though it has remained continuously associated with the crop, over the last 20 years the disease has been inconspicuous in Western Australian lupins due to the continued breeding of resistant cultivars. Recently however, *Stemphylium* grey leaf spot was discovered at damaging levels in lupin breeding experiments Perth, WA. Screening of breeding lines indicates that a proportion of the existing breeding material no longer contains resistance to this disease. During spring of 2009 and 2010 disease surveys were carried out across lupin growing regions of WA to assess the qualitative spatial distribution of the grey leaf spot pathogen. Symptoms of infection were not common, however *Stemphylium botrysum* was associated with leaf lesions in all the sampled areas. Virulence of survey isolates on susceptible genotypes was less than those from Perth. Physiological growth studies were carried out *in vitro* for representative isolates to investigate the difference in their nutritional and environmental conditions requirement in an attempt to correlate these with those existing in the field. Maximum *in vitro* colony growth and sporulation was recorded on V8 Agar at 20/25°C night/day temperature combination, with 75% Relative Humidity and 12 hour day/night cycles favouring sporulation.

4. Application of gene silencing for nematode control

Jo-Anne C.H. Tan, J. Fosu-Nyarko and M.G.K. Jones
Plant Biotechnology Research Group, School of Biological Sciences and Biotechnology, WA State Agricultural Biotechnology Centre, Murdoch University, South Street, Murdoch 6150
Email: joanne.tan@murdoch.edu.au

Root lesion nematodes (*Pratylenchus* spp., RLNs) are major pests of most crops, and reduce yields of wheat in Western Australia by up to 15%, with Australia-wide losses of more than $36 million per annum. The aim of this project is to investigate the use of RNA interference (RNAi) as an approach to confer resistance to RLNs. RNAi is a well established technology that can be used to silence specific genes in animals and plants. Exposure to artificially introduced double-stranded RNA (dsRNA) leads to the silencing of endogenous genes with homologous sequence. RNAi can silence genes in *Caenorhabditis elegans*, and some success has been reported in root-knot nematodes. There is no evidence yet that RNAi works for RLNs. RLNs are migratory endoparasitic nematodes, and so mobility is an important aspect of parasitism. In this study, we are investigating genes involved in locomotion in RLNs via RNAi. We have shown that *P.thornei* and *P.zeae* are indeed amenable to RNAi. Exposure to dsRNA for locomotion specific genes by 14 hours soaking in medium containing M9 buffer with 50 mM octopamine, 3 mM spermidine and 0.05% gelatin led to locomotion impairment in both of these species. In addition, dsRNA originating from *P.thornei* also led to abnormalities in the closely related species *P.zeae* and vice versa, indicating that inter-species gene knockdown is possible. The outcome of this study is economically significant as no reported natural resistance genes have broad effectiveness against RLNs. Bioengineered crops expressing dsRNA that silence essential target genes to interrupt the parasitic process represents a potential approach to develop novel, broadly applicable and durable RLN-resistance in crop plants.

5. Weapons of cell destruction; necrotrophic fungal effectors of *Dydimella pinodes*

Kessie Francis [1], J. Lichtenzveig [2] and R. Oliver [2]
1) SABC, Murdoch University, South Street, Murdoch 6150
2) Dept of Environment and Agriculture, Curtin University of Technology, Kent Street, Bentley

Ascochyta blight, one of the most important diseases affecting field peas is caused by a complex of four fungi species with *Didymella pinodes* (syn; Mycosphaerella pinodes, anamorph: *Ascochyta pinodes)* being the most important. All the species are members of the *Didymellaceae* family of the order *Pleosporales.* The concept that pathogenic fungi within this order produce multiple necrotrophic effectors (NE) to modulate their host defence is gaining grounds following the discovery of other necrotrophic effectors in other species e.g., *Phaeosphaeria nodorum*, and *Pyrenophora tritici-repentis*. These NE act in the inverse gene for gene manner and play a dominant role in pathogen virulence mostly by conditioning host susceptibility. The *D. pinodes* field pea interaction is a complex system for which no total immunity to the disease has been discovered in any commercially grown cultivar. Disease resistance is recessive and is controlled by multiple QTLs. Identification and characterization of NE in this pathosystem will be essential not only for a comprehensive understanding of the plant host–pathogen interactions but also for providing insights into the evolution of the ability of the pathogen to cause disease. We have employed a combination of biochemical techniques coupled with genome sequencing and analysis to evaluate for possible NE produced by *D. pinodes*. This presentation will explore interesting discoveries from this fungus and the potential roles of NE in the host pathogen interaction.

6. Calcium enhances the control of *Phytophthora* Dieback by phosphite

Patsy Stasikowski, J. McComb, P. O'Brien and G.E.St J. Hardy
School of Biological Sciences and Biotechnology, Murdoch University, South Street, Murdoch 6150

Over the years several hypotheses have been put forward to account for the "fungistatic" action of phosphite against *Phytophthora*, but biochemical detail is scarce. The complexity of the interaction between host and pathogen is such that no single aspect of phosphite chemistry is able to explain all the various effects of phosphite that have been observed. For instance, why does the temporal efficacy of phosphite vary up to ten-fold between horticultural and native plants? And how can this observation be reconciled with the fact that pathogen growth *in vitro* is not inhibited by the concentration of phosphite that gives disease control *in planta*? This talk will outline a biochemical model that explains how and why phosphite has the effects on *Phytophthora* that it has. However, when considering models of phosphite action it should be noted that "Essentially, all models are wrong, but some are useful" (George Box), and within this context "usefulness" can be defined as increased disease control and better management of *Phytophthora* dieback. The model suggested that the efficacy of phosphite in controlling disease caused by *Phytophthora* may be enhanced by maintaining high levels of extracellular calcium. Results from a glasshouse trial confirmed this, and showed that although phosphite and calcium are not lethal to the pathogen, combined treatments of soil calcium supplementation and foliar phosphite application were synergistic in controlling infection caused by *Phytophthora cinnamomi* in *Banksia leptophilia.* Rigorous biochemical testing is needed to confirm that inhibition of the calcium-dependent ATPase by phosphite does in fact cause the synergistic effect. However, in the absence of evidence to the contrary, the suggested model of phosphite action has proved useful.

7. Novel molecular diagnostic frame work for plant parasitic nematode pest of plant biosecurity concern-better and faster than classical taxonomy?

Matthew N.H. Tan [1, 2], *V.A. Vanstone* [3] *and M.G.K. Jones* [1, 2]
[1] *Plant Nematology Section, Plant Biotechnology Research Group, School of Biological Sciences and Biotechnology, WA State Agricultural Biotechnology Centre, Murdoch University, South Street, Murdoch 6150*
[2] *Cooperative Research Centre for National Plant Biosecurity*
[3] *Department of Agriculture and Food Western Australia, South Perth 6151*

Phyto-nematodes are one of the four most important agricultural pests worldwide. The annual losses in agricultural production from nematode infection is about USD$120 billion. Although phyto-nematodes cannot move more than a metre per year, human activities have lead to the dispersal of these major pests around the world, and the transportation of infected plant materials from one continent to another can put Australian agriculture at risk. “Plant Biosecurity” requires cutting edge molecular techniques to identify biosecurity pathogens. The aim of this project is to develop new protocols for early detection of exotic phyto-nematodes: this will benefit Australia and help safeguard its borders. A requirement is to reduce the time taken for nematode identification so that pest incursions can be detected and controlled early. Most nematode identification has been done by classical taxonomy. However, this requires trained taxonomists familiar with nematode morphology, and identification is time consuming for an unknown nematode. More recent molecular techniques of nematode diagnostics have shown promising results for nematode identification. Molecular diagnostics has proved to be reliable and rapid compared to classical approaches. In this project, work is being undertaken to compare DNA-based, protein-based and novel methods of nematode identification. So far, DNA and protein diagnostic methods have been developed for a range of root lesion and cyst nematodes. Characteristic sequences of ITS regions have been generated and phylogenetic relations of these nematodes studied. Similarly protein biomarkers have been established that can be used both to identify species and genera of these nematodes. Although plant nematodes can only move short distances on their own, they have been transported around the world effectively by human activities. The outcome of this project will help reduce entry of exotic nematodes into Australia, and so help reduce potential crop losses.

8. Australian native plant susceptibility to *Phytophthora ramorum*

Kylie Ireland [1,2], *D. Hüberli* [2,3], *B. Dell* [2], *I. Smith* [4], *D. Rizzo* [5], *D. Kriticos* [1,6] *and G.E.St J. Hardy* [1,2]
[1] *Cooperative Research Centre for National Plant Biosecurity, PO Box 5012, Bruce, 2617, ACT*
[2] *Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, South St, Murdoch 6150*
[3] *Plant Pathology, Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court, South Perth 6151*
[4] *Department of Forest and Ecosystem Science, University of Melbourne, 500 Yarra Boulevard, Richmond 3121, Vic.*
[5] *Department of Plant Pathology, University of California, 1 Shields Avenue, Davis 95616, CA, USA*
[6] *CSIRO Entomology and CRC for National Plant Biosecurity, GPO Box 1700, Canberra 2601, ACT*
Presenting author: k.ireland@murdoch.edu.au

Phytophthora ramorum causes considerable and widespread damage in nurseries, gardens and natural woodland ecosystems of the USA and Europe, and is classified as a Category 1 plant pest in Australia. It is of particular interest to Australian plant biosecurity as, like *P. cinnamomi*, it has the potential to become a major economic and ecological threat in areas with susceptible hosts and conducive climates. Research was undertaken in California to assess the pathogenicity of *P. ramorum* on Australian native plants. Sixty-nine plant species within 24 families were sourced from established gardens and arboretums, and selected based upon provenance from areas of climatic suitability for *P. ramorum* as well as ecological and economical importance. Foliar, branch and log susceptibility were tested using detached leaf, branch and log inoculations. Sporulation potential and chlamydospore production was also tested on detached foliage of a select mid to upper storey species. Potentially highly susceptible foliar Australian host species included *Eucalyptus regnans*, *Isopogon cuneatus*, *I. formosus*, *Leptospermum scoparium*, *L. lanigerum*, and *Melaleuca squamea*, while potentially resistant hosts included *Hedycarya angustifolia*, *Olearia argophylla*, *Phyllocladus aspleniifolius*, *Pittosporum undulatum*, and *Podocarpus lawrencei*. Disease incidence and severity were greater during the summer, and when the leaves were wounded. Putative sporulating hosts included *Agonis flexuosa*, *C. ficifolia*, *Eucalyptus delegatensis*, and *E. viminalis*. Highly susceptible branch hosts included *E. nitens*, *E. sideroxylon*, *E. viminalis*, *Hardenbergia violaceae*, *I. formosus* and *N. cunninghamii*, and potential bole canker hosts included *E. delegatensis* and *E. regnans*. A simulation model developed using CLIMEX suggests a high likelihood of potential distribution in Australia along coastal NSW, and in cooler, wetter regions of Victoria and Tasmania. These results extend the known potential host range for *P. ramorum*, confirming it as a potential threat to Australian plant industries and ecosystems, and highlighting additional potential hosts prevalent in global horticultural trade. Results of the studies will be discussed in relation to their implications for disease entry, spread and development of an epiphytotic within an Australian biosecurity framework.