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Detection *of Turnip mosaic virus* resistance phenotypes in three different mustard species

M. Kehoe, R.A.C. Jones, M.G.K. Jones and B. Coutts
School of Biological Sciences and Biotechnology, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Murdoch 6150
Department of Agriculture and Food WA, 3 Baron-Hay Court, South Perth 6151
Email: makehoe@agric.wa.gov.au

Mustards are emerging as a potential source of oil for the production of biofuels, to this end forty four genotypes of *Brassica juncea* (Indian mustard), nine genotypes of *B. carinata* (Ethiopian mustard) and five genotypes of *B. nigra* (Black mustard) were sap inoculated with the WA-1 isolate of *Turnip mosaic virus* (TuMV). Five different resistance phenotypes were observed: necrotic spots in inoculated leaves with a necrotic streak on the stem without systemic infection; necrotic spots in inoculated leaves with systemic necrotic infection resulting in either severe systemic symptoms or plant death; necrotic spots in inoculated leaves with systemic infection; chlorotic spots in inoculated leaves with systemic infection and chlorotic spots in inoculated leaves without systemic infection. Twelve genotypes of *B. juncea* and two each of *B. carinata* and *B. nigra* were also inoculated with two new isolates (NSW-1 and NSW-2) to determine if any of the resistance phenotypes were specific to the WA-1 isolate, and none were. The resistance phenotype seen in *B. carinata* was confirmed by the use of graft inoculation (no systemic infection). These three *Brassica* species all have the *Brassica* B genome in common, and to date very little research on resistances to TuMV within this genome have been carried out. We suggest that some of these resistance phenotypes from *B. juncea* (AABB genome) and *B. nigra* (BB genome) may originate from the B genome, and the resistance phenotypes seen in *B. carinata* (BBCC genome) are more likely to have originated in the C genome.

Demonstrating pest freedom – A quantitative approach

N.E.B. Hammond, P.A.J. Martin, D.C. Hardie, C. Hauser and S. Reid
CRC National Plant Biosecurity & School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch 6150
Email: N.Hammond@crcplantbiosecurity.com.au

Demonstration of pest freedom is an important part of the phytosanitary processes surrounding global trade, with many countries requiring declarations of pest free areas before accepting commodities from exporting countries. Claims of pest freedom are often not based on physical survey data, but are based on expert opinion, together with the fact that the pest has not been recorded in the area of interest. These claims, although they may be based on scientific evidence, are generally not transparent and are becoming less acceptable to trading partners. Surveillance data can provide substantial information for supporting claims of freedom. We have applied a quantitative approach to evaluating data from targeted grain surveillance for Karnal bunt (*Tilletia indica*) for the demonstration of pest freedom. This approach is based on scenario tree analysis of the surveillance systems and utilises Bayesian methods for incorporating prior knowledge of pest status in the analysis. It allows quantitative estimates of confidence in freedom to be derived from surveillance that is targeted for the pest in question and which therefore does not meet the criteria for a representative survey. This method provides transparency in the process of demonstrating pest freedom, providing a clear description of the surveillance system. The applications and limitations of this approach will be discussed.

A new *Phytophthora* species associated with *Eucalyptus gomphocephala* decline in Western Australia

P.M. Scott, B.L. Shearer, P.A. Barber, T. Jung, T. Burgess, I.J. Colquhoun and G.E.StJ. Hardy
Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Murdoch 6150
Email: P.Scott@murdoch.edu.au

Eucalyptus gomphocephala is a keystone tree species endemic to a narrow (5-10 km wide) coastal strip approximately 300 km in length in south-west Western Australia. *E. gomphocephala* is undergoing a significant decline that was first identified as a spot decline in 1994 and now occurs throughout large sections of its remnant distribution within Yalgorup National Park in the south-west of Western Australia, in some areas resulting in 100% mortality. Multiple factors, including soil-borne plant pathogens, have been identified as possibly contributing to the decline syndrome. Less fine roots are associated with trees on declining sites compared to those on healthy sites. Foliar analysis indicates that declining trees have lower concentrations of some micronutrients, including zinc, the uptake of which is typically impaired by fine feeder root loss. A range of Pythiaceous microorganisms have been isolated from declining roots, including an isolation of a yet to be described *Phytophthora* species. The *Phytophthora* isolates appear morphologically similar to the *Phytophthora citricola* holotype although they are distinct from this based on molecular analysis of the internal transcribe region. The exact phylogeny of the new *Phytophthora* species is being determined using additional gene regions. These isolates appear to be contributing to the loss of fine roots. Glasshouse trials are currently underway to determine whether these isolates are indeed pathogenic.

Canker disease of *Corymbia calophylla* (marri) in the southwest of Western Australia

T. Paap, T.I. Burgess, J.A. McComb, B.L. Shearer and G.E.StJ. Hardy
Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Perth, 6150
Email: t.paap@.murdoch.edu.au

The impact of a canker disease of *Corymbia calophylla* (marri) in the southwest of Western Australia (WA) has been increasing since it was first observed causing decline and death of this species in the 1970s. Despite increasing concern, there has been very little research into the disease. This study examined the range of fungal species associated with healthy and diseased *C. calophylla,* and the pathogenicity of isolates obtained from these surveys. DNA sequencing confirmed that *Quambalaria cyanescens* and *Q. pitereka* are present in southwest WA, with the latter associated with leaf and shoot disease. A third group isolated from cankers represented a new species of *Quambalaria.* Comparisons of disease symptoms and conidiogenesis indicate this species is synonymous with *Sporotrichum destructor,* a fungus historically implicated in the canker disease described in the 1920s on amenity planted *C. ficifolia,* and the species is formally described as *Q. coyrecup.* Pathogenicity trials show *Q. coyrecup* is capable of causing significant lesions similar to those observed in natural infections, confirming it is the fungus responsible for the current canker disease. *Endothiella eucalypti* also caused significant lesions, though these were not typical of natural infections, which together with its frequent isolation from both healthy and diseased trees suggests it is an opportunistic pathogen. The current cause of cankers in *C. calophylla* is the same as the fungus historically implicated in the canker disease described in the 1920s on amenity planted *C. ficifolia.* At that time it was described as an endophyte doing little or no damage in *C. calophylla.* It is of immediate importance to determine the factors potentially driving this decline, and develop control and management options.

The role of pastures in hosting Root Lesion Nematode in Western Australian cereal cropping systems

A. Bhatti, V. Vanstone and Ming Pei You
Department of Agriculture and Food WA, 3 Baron-Hay Court, South Perth 6151
Email: ABhatti@agric.wa.gov.au

The aim of this study was to investigate the role of pastures in the cereal cropping system in regard to their effect on Root Lesion Nematode (RLN) levels. Ability of pastures to host *Pratylenchus neglectus* was measured to determine the possible influence on subsequent susceptible cereal crops in the rotation. Pastures (25 cultivars representing 17 species) were grown in the glasshouse, and 10 replicates of each inoculated with 2,000 *P. neglectus*. The total number of *P. neglectus* per plant was determined 10 weeks after inoculation. Pasture cultivars were classified as susceptible or resistant to *P. neglectus*, based on comparison with the susceptible control Machete wheat (*Triticum aestivum*) and resistant Tanjil lupin (*Lupinus angustifolius*). Results indicate that some of the new annual pastures (Sulla, Serradella, Rose Clover, Purple Clover) introduced to WA are resistant to *P. neglectus,* but Clover species vary, ranging from resistant to very susceptible. Depending on pasture species and cultivar, *P. neglectus* levels could increase during the pasture phase and become damaging to subsequent cereals.

An indigenous plant virus from the South West Australian Floristic Region

C.G. Webster, B.A. Coutts, R.A.C. Jones, M.G.K. Jones and S.J. Wylie
Plant Biotechnology Research Group, WA State Agricultural Biotechnology Centre, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Murdoch 6150
Email: C.Webster@murdoch.edu.au

Hardenbergia comptoniana (native wisteria) is a climbing legume that is indigenous to the South West Australian Floristic Region (SWAFR). It is grown around the world in gardens for its attractive climbing habit and racemes of purple flowers. Young leaves of wild *H. comptoniana* plants showed mosaic patterns, deformation and stunting symptoms typical of virus infection. Using degenerate primers for potyviruses, capsid protein (CP) gene sequences were obtained and compared to CP's of *Potyvirus* sequences in GenBank. The sequences were most closely related to those of two distinct viruses named *Passionfruit woodiness virus* (PWV), *Hibbertia virus Y, Clitoria virus Y, Siratro 1 virus Y* and *Siratro 2 virus Y,* all potyviruses identified from eastern Australia, all members of an Australian subgroup of the *Bean common mosaic virus* group. Our sequences diverged by 27.2-37.1% (nucleotides) and 20.2-26.1% (amino acids) from its closest known relative, PWV. Phylogenetic analysis showed HarMV CP genes clustered to form eight clades which had intra-clade divergence of 0-7.2% (nucleotides) and 0-2.7% (amino acids), and inter-clade divergence of 8.2-24.6% (nucleotides) and 5.1-11.8% (amino acids). Experimental host range studies indicated this virus has a narrow host range, in common with many other potyviruses. Both the limited geographical range of its natural host and the high genetic diversity of the virus strongly indicate that this virus has been confined to the SWAFR for a considerable period of time and has diversified there. We propose the name Hardenbergia mosaic virus (HarMV) for this, the first indigenous plant virus described from the region.

Resistance to subterranean clover mottle virus in *Medicago truncatula*, and genetic mapping of a disease resistance locus

M. Saqib, S.R. Elwood, R.A.C. Jones and M.G.K. Jones
WA State Agricultural Biotechnology Centre, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Murdoch 6150
Email: m.saqib@murdoch.edu.au

Grain and pasture legumes suffer economic losses from several viral diseases, but their molecular genetic improvement has been impeded by large genomes, a lack of genetic tools and inefficient transformation methods. The annual pasture species *Medicago truncatula* is recognised as a model legume and is susceptible to a number of viruses, for example alfalfa mosaic virus, cucumber mosaic virus and subterranean clover mottle virus. In this study, *M. truncatula* has been established as a model to study subterranean clover mottle virus (SCMoV)-host interactions. Two hundred and nine accessions of the *M. truncatula* core collection, predominantly from the South Australian Research and Development Institute (SARDI) were screened for their disease phenotype by challenging with SCMoV isolate P23 using infectious sap. Symptoms that developed were recorded and virus presence confirmed by ELISA. Forty two accessions were resistant to SCMoV, with the remainder susceptible. Among these genotypes, DZA315 showed a resistant hypersensitive reaction, while J6 was susceptible. One hundred and fifty-five (Jemalong-J6 x DZA315) F8 recombinant inbred lines were phenotyped for their responses to inoculation with SCMoV. Eighty-five were susceptible and 70 resistant. Initial genetic mapping concluded the resistance is governed by a single resistance gene located on the long arm of chromosome 6. This research provides a basis for fine mapping and potential for isolating the SCMoV resistance gene.

Development of molecular diagnostic tools for the detection of *Phytophthora cinnamomi* from cryptic soil samples in southern Australia

N.M. Williams, E. O'Gara, P.A. O'Brien and G.E.StJ. Hardy
Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Murdoch 6150
Email: N.Williams@murdoch.edu.au

Phytophthora cinnamomi is responsible for the widespread destruction of native forest and heathlands across southern Australia. In these ecosystems, *P. cinnamomi* is responsible for the death of a broad range of susceptible species including members of the Proteaceae, Epacridaceae, Papilionaceae and Myrtaceae, and results in significant changes in species composition, community structure and ecosystem function. Management of the pathogen in native ecosystems is centred on pathogen containment and relys on the identification of dieback boundaries and detection of the pathogen directly from soil. Once an infestation is defined, preventative measures may be taken to ensure infested soil and plant material are not spread during land management activities. Ongoing monitoring of the pathogen then forms an integral component of sustainable pathogen management. Efficient management of *P. cinnamomi* is impeded by the inability to consistently detect the pathogen from infested soil samples. This is especially the case for cryptic sites in which there is no apparent expression of plant symptoms. Such situations commonly occur where sites are excavated during mining activities or are disturbed by fire. In the absence of a better alternative, land managers still use baiting analysis of soil samples in formulating management plans for *P. cinnamomi* containment. This is despite the fact that recoveries are often low and there is a high risk of false negatives. This in turn limits the confidence placed on the results of baiting analysis. DNA based detection offers improved sensitivity and higher sample throughput for the detection of *P. cinnamomi* than baiting assays. Through our research, comparative analysis using PCR based methods in parallel to baiting assays have shown a significant increase in the detection of *P. cinnamomi* by nested PCR. However, although the benefits of DNA based diagnostic tools have good promise for future disease management, low and variable target populations mean that sampling strategy and confidence levels remain the key issues in delivering a reliable and consistent diagnostic service. This presentation will examine the challenges encountered during the development and validation of nested and real time PCR protocols for the detection of *P. cinnamomi* from soil collected from native ecosystems throughout southern Australia. The implications for using molecular diagnostic assays as management and research tools for *P. cinnamomi* will be discussed.