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# STUDENT SYMPOSIUM ABSTRACTS
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## Genomic sequence characterisation of wheat streak mosaic virus (WSMV) in Australia

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*Wheat streak mosaic virus* (WSMV) is a devastating disease of wheat. WSMV is known to exist in many parts of North America, Europe and the Middle East and was first reported in Australia in 2002. Since 2002 it has been found widely dispersed throughout all wheat growing states in Australia. Between 2002 and 2006 14 WSMV isolates were collected from wheat grown in different Australian states including Western Australia (3 isolates), New South Wales (3 isolates), South Australia (3 isolates), Victoria (3 isolates), Australian Capital Territory (1 isolate) and Tasmania (1 isolate).

The complete nucleotide sequences of these isolates were determined based on complement DNA sequences derived from the 9,384-nucleotide (nt) RNA of the virus. The genome of WSMV has a 130-nt 5' leader and 149-nt 3'-untranslated region and is polyadenylated at the 3' end. *WSMV* RNA encodes a single polyprotein of 3,035 amino acid (aa) residues and has a deduced genome organization typical for a member of the family Potyviridae (5'-P1/HC. -Pro/P3/6K1/CI/6K2/VPg -NIa/NIb/CP -3'). It was shown that the 14 WSMV isolates shared very high identities at both the nt (99 - 100%) and aa (99.7 – 100%) levels. When compared with overseas isolates, Australian isolates shared identities of between 72.9 – 98.2% at nt level and identities between 81.8 to 98.2% at aa level. The highest identities at the nt and aa levels were with the USA Type isolate and the USA Sidney isolate respectively.

The Phylogenetic study demonstrated that Australian WSMV isolates are most closely related to the USA isolates. However, no solid conclusion can be made to the origin of the Australian WSMV isolates as the sequences between Australian and USA isolates are uniquely different.

## Screening *Brassica napus* genotypes against *Sclerotinia sclerotiorum* using a novel cotyledon assay

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*Sclerotinia sclerotiorum* is a major pathogen of many crops, including oilseed rape (*Brassica napus*), and there is keen interest worldwide to identify *Brassica* genotypes with resistance. However, field testing to identify resistance in *B. napus* germplasm is expensive, time consuming and at times unreliable due to variability in field environmental conditions and plant architecture. To address this, we set out to examine the feasibility of utilizing for *B. napus*, a cotyledon test already developed for Sclerotinia disease on legumes. Initially, cotyledons of 32 *B. napus* genotypes were drop-inoculated using macerated mycelium ($1 \times 10^4$ mycelial fragments $mL^{-1}$) under controlled environmental conditions. Significant differences were recorded between *B. napus* genotypes, and the experiment was repeated twice using genotypes selected from the first experiment. Responses of genotypes between the three screening experiments were significantly and positively correlated. . Results obtained in the first experiment were compared with those from our earlier field screening for stem rot that utilized the same strain of *S. sclerotiorum* and the same *B. napus* genotypes. In particular, there was significant positive correlation ($r = 0.62$, $P < 0.01$) between published field data for stem rot with our cotyledon test results across genotypes in common. This indicates the usefulness of this cotyledon method to provide a relatively reliable indication of field performance of genotypes. We believe that this is the first report demonstrating that a cotyledon assay can be successfully applied to rapidly differentiate the reactions of *B. napus* genotypes against *S. sclerotiorum.*

## Phylogenetic analysis of *Olpidium virulentus*, *Lettuce Big-Vein associated Virus* and *Mirafiori Lettuce Big-Vein Virus* isolates from Western Australia

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Lettuce Big-vein Disease (LBVD) causes significant losses in the quality of produce. Although lettuce plants with this disease normally contain two viruses; *Lettuce Big-Vein associated Virus* (LBVaV) and *Mirafiori Lettuce Big-Vein Virus* (MLBVV), the latter is considered responsible for the disease symptoms. Both viruses are vectored by a chytrid (*Olpidium virulentus*), an obligate parasite of roots that is soil and water-borne. During the winter growing seasons of 2007 and 2008, lettuce leaves with symptoms and roots from affected plants were collected from seven different lettuce growing properties in Western Australia. Leaf tissue was tested by PCR. Both viruses were found and amplicons for the coat protein gene generated by PCR were sequenced and compared to those previously sequenced from elsewhere. The seven lettuce root samples tested positive by PCR for *O. virulentus*. The ITS region of this vector was sequenced to examine relationships with previously sequenced *O. virulentus* strains from overseas. The virus and *O. virulentis* sequences obtained and those already available on Genbank were aligned by ClaustalW and then phylogenetic trees were constructed by Bootstrap Neighbour Joining analysis. This is the first time such a comparison of these viruses and the chytrid vector has been performed on lettuce isolates infected with LBVD. With MLBVV, all Western Australian isolates grouped together forming a sub-clade containing only Australian isolates. Similarly, all Western Australian isolates of LBVaV grouped within a sub-clade consisting only of Australian isolates. *O. virulentus* isolates fell into 2-3 distinct clades indicating multiple introductions.

## World's worst pest of stored products, the Khapra beetle

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The genus *Trogoderma* contains some of the world's most economically serious pest species of wheat and other stored grain products. *Trogoderma* have cryptic behaviour, are capable of causing extensive damage, and may result in product exclusion from Australia's export markets. Currently, Australia harbours the less serious Warehouse beetle, *Trogoderma variabile* Ballion, which is morphologically similar to one of the most serious pests of stored grain in the world, the Khapra beetle, *Trogoderma granarium* Everts (Lowe, Browne *et al*. 2000). In addition, two other exotic *Trogoderma* species (*T. glabrum* and *T. inclusum*) are biosecurity threats to Australia. Furthermore, Australia has 52 described and at least 100 undescribed native *Trogoderma* species, which share similar morphological characteristics to the exotic species. The Australian native species of *Trogoderma* are not known pests, but may cause concern to quarantine authorities in countries that import Australian grain. Misidentification of exotic or of native *Trogoderma* has the potential of causing marketing and biosecurity concerns with Australia's trading partners. Therefore, the development of rapid molecular based diagnostics and acquiring a phylogenetic understanding of the family Dermestidae is paramount to protecting Australia from such a devastating pest.

## Fluorescent In-Situ Hybridisation (FISH) investigations as a new tool for determining dissemination of *Phytophthora cinnamomi* by feral pigs

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Feral pigs have been widely implicated in the spread of dieback disease, caused by *Phytophthora cinnamomi*, via the transport of infected soil material. *P. cinnamomi* is an important introduced plant pathogen with a wide host range which is widespread throughout much of Australia. This pathogen currently threatens many unique and diverse natural ecosystems throughout the south west corner of Western Australia and in areas where feral pigs are both present and absent. The disruption of native ecosystems caused by the rooting and wallowing activities of feral pigs are believed to increase their susceptibility to dieback infections. As such, feral pigs may play an important role in the spread of *P. cinnamomi* as well as the re-introduction of new infections to previously exposed areas. This study aims to determine the role of feral pigs in the spread of *Phytophthora* dieback through the transport of infected soil as well as investigating the potential for disseminating the pathogen via passage of infected plant material through their gastrointestinal tract. A new detection technique, fluorescent *in situ* hybridisation (FISH) targeting conserved 16S rRNA allows for easier sample processing and visualisation of the pathogen *in situ* within root fragments.

## Severity of root rot in mature subterranean clover and associated fungal pathogens in the wheatbelt of Western Australia

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Pasture decline is considered to be a serious challenge to agricultural productivity of subterranean clover across southern Australia. Root disease is a significant contributing factor to pasture decline. However root disease assessments are generally carried out in the early part of the growing season and in areas predominantly sown to permanent pastures. For this reason, in spring 2004, a survey was undertaken to determine the severity of root disease in mature subterranean clover plants in pastures located in the wheatbelt of Western Australia. DNA-based soil assays were used to estimate pathogen population density in the soil of a variety of soil-borne pathogens known to commonly occur in the Mediterranean-type environments of southern Australia. The relationships between severity of disease on tap and lateral roots and root diameter, root length, nodulation and total rainfall were determined. The survey showed, for the first time, that severe root disease is widespread in spring across the wheatbelt of Western Australia. There was a positive correlation between rainfall and tap root disease, and between tap root disease and average root diameter of the entire root system. Despite the high levels of root disease present across the sites the DNA of most root disease pathogens assayed was present in trace concentrations. Only *Pythium* Clade F showed high DNA concentrations in the soil. DNA concentrations in the soil, in particular for *P. clandestina* and *R. solani* AG 2.1 and AG 2.2, were higher in the smaller autumn sampling in 2006. This study suggests the productivity of subterranean clover based pastures is severely compromised by root rot diseases throughout the growing season in the wheatbelt of Western Australia.

## The impact of Phytophthora dieback on fauna habitat

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The introduced soil-borne plant pathogen *Phytophthora cinnamomi* (Phytophthora dieback) kills a broad range of native plants from the northern jarrah (*Eucalyptus marginata*) forest of Western Australia. Many common and structurally important jarrah forest plants are sensitive to *P. cinnamomi*, therefore the impact is often be severe. However, few studies have assessed how this influences the habitat of native mammals. Mark, release and recapture data show that mardos (*Antechinus flavipes*) favour areas with thick leaf litter, dense understorey with plentiful large logs and grasstrees (*Xanthorrhoea preissii*). These habitat characteristics provide the mardo with cover, shelter, nesting opportunities, food resource and protection from predators. Yet, all of these habitat characteristics are altered by *P. cinnamomi*. This strongly suggests that *P. cinnamomi* is a subsequent threat to the conservation and management of the mardo.