



**AUSTRALASIAN PLANT PATHOLOGY SOCIETY, WA
STUDENT SYMPOSIUM ABSTRACTS
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1. Factors affecting dispersal of fungal pathogens: Chickpea ascochyta blight as a model

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Ascochyta blight of chickpea, caused by *Ascochyta rabiei*, is a significant disease world-wide including southern Australia. To explore its incidence and pattern of spread, a weather-based spatio-temporal model was developed. The major parameters of the model 'Spread *Ascochyta rabiei* in chickpea' (SArC) were either derived from laboratory or field experimental data, or estimated through calibration with 2007 field data. The model was then subjected to qualitative and quantitative validation using 2008 field data. In qualitative validation, observation and prediction for each m² unit were recorded on the lay-out of the plot; then each unit (m²) was coloured according to five arbitrary categories: Low (0-30% of diseased plants, green); Medium (31-60%, yellow); High (61-80%, purple); and, Very high (81-100%, red) incidence. For quantitative validation, the performance of the model was analysed statistically using a deviation-based approach (MSD). MSD statistics indicated that the lack of positive correlation (LCS) was the major cause of deviation between the observation and prediction, perhaps because of unusual disease patterns observed and/or an over simplification of the infection process. SArC; however, largely simulated the spread of incidence in fields sown with two chickpea cultivars (cvs) of different resistance to ascochyta. Given the strength of the SArC model, both in respect to parameter estimation, calibration and validation, it has potential to be used in managing ascochyta blight in South Australian farming systems and/or in plant biosecurity in modelling exotic plant pathogens with similar dispersal characteristics.

2. Differential development and colonisation of *Fusarium oxysporum* in roots of the resistant and susceptible cultivars of strawberry

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Strawberry (*Fragaria x ananassa*) is a high-value export crop grown in Western Australia. *Fusarium oxysporum* is the most dominant pathogen on strawberry, which causes the rapid wilting and death of plants. Until now, no study has been conducted to determine the development and colonisation of *F. oxysporum* in susceptible strawberry plants, not to mention the comparison of the differential development and colonisation of *F. oxysporum* in strawberry plants with different susceptibility. Therefore, this study was conducted for the first time to determine the differential development and colonisation of *F. oxysporum* in roots of two strawberry cultivars, one resistant 'Festival' and one susceptible 'Camarosa', by light and scanning electron microscopy from 4 h to 7 d post inoculation (pi). Results showed that the resistant 'Festival' impeded the spore germination and penetration since the early stage from 4 to 12 hpi, and the fungal development and colonisation until 7 dpi. At 7 dpi, in resistant 'Festival', fungal colonisation was mainly confined to the epidermal layer of the cortex tissue, while in susceptible 'Camarosa', the hyphae continued growing in the intra and inter-cellular spaces of the cortex tissue and had colonized the vascular tissue. This study demonstrates for the first time that the resistance of strawberry plants to *F. oxysporum* is a result of retardation of pathogen development and colonisation both on the plant surface and within host tissues. The resistance mechanisms identified in this study will be useful for developing markers for screening of resistance against this pathogen and developing more advanced disease control strategies.



3. Fluorescent *in situ* hybridization (FISH) assay to view *Phytophthora cinnamomi* growth within plants; a new tool for *in situ* studies of oomycete plant pathogens

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Viewing of *Phytophthora cinnamomi* by microscopic examination *in planta* is often difficult as structures such as hyphae, chlamydospores and oospores are often indistinguishable from those of other fungi with histological stains not enabling species differentiation. This lack of staining specificity makes the localisation of *P. cinnamomi* hyphae and reproductive structures within plant tissue difficult, especially in woody tissues. This study demonstrates that utilising a species-specific fluorescently labelled DNA probe allowed *P. cinnamomi* to be specifically detected and visualised using fluorescent *in situ* hybridisation (FISH) without damage to plant or pathogen cell integrity. This approach provides a new application of FISH with potential use in the study of plant-pathogen interactions *in planta*.

4. Using anti-primer technology for nematode diagnostics

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With increased global trade, accurate identification of biosecurity pests considered to be significant risks to agriculture is critical. At present, there is a need for molecular aids to improve nematode diagnostics. In classical nematode diagnostics, a trained person who has acquired many years experience and knowledge of nematode morphology is required. To train a qualified nematode taxonomist takes time, and few experienced personnel are available in Australia. This research is part of a project to develop new strategies to identify biosecurity nematode pests that will contribute to safeguarding Australian agriculture from incursion of nematode pests.

The aim of this part of the project is to develop 'anti-primer' technology as a potential new technique for diagnostic identification of endemic and exotic plant parasitic nematodes. 'Anti-primer' technology was first used as a diagnostic method in a clinical setting, for example in cancer research. An anti-primer is a labeled probe which includes a quencher of fluorescence, similar to the quencher component of a Taqman qPCR probe, and this is used in addition to a forward primer which is fluorescently labeled, together with a standard reverse primer as used in normal PCR reactions. In this form of PCR, in which target DNA is detected using qPCR, the 'anti-primer' with quencher attached is added to the reaction mixture to quench available free unincorporated fluorescently labeled forward primer. The sample with target DNA will show an increase of fluorescence, whereas the anti-primer will quench fluorescence of unincorporated free forward primer.

With some modification to this technology, a multiplex system can be customized using different fluorescence labels such as FAM or TET. Using a molecular approach such as 'anti-primer' technology, data can be generated within two hours without further need for analysis (such as sequencing the PCR products), and this technology can be specific and achieve robust results. With the multiplex system under development, preliminary data has shown that this technology can be used to differentiate species of *Pratylenchus* (*P. neglectus*, *P. penetrans* and *P. thornei*) in a single diagnostic reaction.



5. *Phytophthora* for sale: A survey of pathogens in Western Australian nursery plants

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Many species of the *Phytophthora* genus are pathogenic to a broad range of plants. Recent outbreaks of pathogenic *Phytophthora* sp. in the United States and European Union have been linked to internationally-traded nursery plants. A targeted survey was conducted at two wholesale plant nurseries in Western Australia over the course of one year, to identify *Phytophthora* sp. present in plant stock being sold to retail nurseries. One nursery was accredited with the Nursery and Garden Industry Australia (NGIA) whilst the other was not. Multiple species of *Phytophthora* were detected at the non-accredited nursery, but none at the accredited nursery. Species included *P. cinnamomi* and *P. multivora*. The results suggest that the best management practices suggested by the NGIA are important for maintaining the disease free status of plants sold through nurseries, and preventing the spread of plant pathogens.

6. Niche construction by plants in soils modified by bauxite mining in the jarrah forest, south-western Australia

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Niche construction theory predicts that plants not only respond to their environment but modify it. We sought for evidence of niche construction among jarrah forest plants and tested if the modification of soil by bauxite mining and subsequent restoration influences their niche construction capacity. *Eucalyptus marginata*, *Acacia pulchella*, and *Bossiaea ornata* were grown in pots of unmodified and modified soils under glasshouse conditions. Plant growth, chemical and biological changes in soils were assessed after 4.5 months and compared to those of unplanted controls. In unmodified soils, niche construction by plants was detected as a change in the chemical characteristics of soils, only *A. pulchella* showed differences in its nematode-trophic structure. In modified soils, only *E. marginata* and *B. ornata* changed the soil chemistry. All the plants changed the microbial biomass-C content in this soil. Higher root:shoot ratios of *B. ornata* and *A. pulchella* in unmodified compared with modified soils suggested a greater potential for niche construction in this soil (i.e., larger rhizosphere) but this did not translate into differences in plant biomass. Niche construction by plants was observed in unmodified soils. Modification of J-F soil by bauxite mining and subsequent restoration appears to influence how plants construct their niche.

7. Trouble in the truffière: Investigating the cause of truffle rot in Western Australia

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The recent successful establishment of a truffle (*Tuber melanosporum*) industry in Australia has been significantly hampered by the occurrence of a disorder known as truffle rot. Truffle rot occurs at varying levels of severity in all truffle growing regions of the country and losses are believed to amount to over 35% of the annual national harvest. The incidence of rot is strongly related to the depth of truffle formation. Truffles forming within the top 5 cm of the soil profile are over 3 times more likely to become rotten than those forming below 5 cm. The involvement of a particular fungal rot organism is uncertain. Pathogenicity studies using bacteria isolated from within rotten truffles as well as macerated tissue from affected fruiting bodies has failed to demonstrate the induction of symptoms in healthy tissue under both field and laboratory conditions. The use of cultural treatments to encourage truffles to form deeper in the soil profile has shown some promise. Soil tillage using a rotary hoe between the tree rows resulted in a 50% reduction in the amount of truffle affected by rot, although the precise mechanism for this effect requires further investigation. Reducing the rate and frequency of irrigation over the truffle development period only had a minor impact on the proportion of truffles forming at the soil surface. Further research is required to understand the etiology of truffle rot and aspects of truffière management that can be used to ameliorate truffle rot.