

disease, harbours a cryptic 25.3 kbp plasmid, pXA3. A physical map of pXA3 has been constructed. Preliminary sequencing of pXA3 has revealed very high levels of homology to a gene (*pnp*) which codes for a cold shock protein, polynucleotide phosphorylase (PNPase). Cold shock involves a sudden chilling in the surrounding environment. Hence, studies have also been initiated to investigate the effects of a fall in temperature on the physiology of the cell. SDS-PAGE of the protein preparations from *X. albilineans* cultures, grown at different temperatures, were analysed. Higher levels of protein of approximately 65 kDa were observed at lower temperatures. The present evidence points to PNPase production in KEN6 cells. However, further tests need to be performed to confirm this. Future work would include cloning of the putative *pnp* gene into an expression vector.

Evaluating the efficacy of a PCR-derived DNA probe for the detection of *Clavibacter xyli* subsp. *xyli*

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Ratoon stunting disease (RSD) is one of the most widely distributed and economically important diseases of sugarcane. Diagnosis of RSD and rapid assessment of disease severity in the field have been difficult as similar symptoms may be due to various biotic and abiotic factors. The objective of this study was to evaluate the efficacy of a DNA-based probe for the detection of *Clavibacter xyli* subsp. *xyli* (*Cxx*), causal agent of RSD. Random amplified polymorphic DNA (RAPD) profiles were generated from genomic DNA of various strains of *Cxx*. A common 1 124 bp polymerase chain reaction (PCR) amplification product was produced for all strains analysed. This product was gel purified, digoxigenin labelled and used as a probe in Southern hybridisation. It was found that the probe hybridised to all *Cxx*-derived PCR products. Future work will include cloning and sequencing of this PCR-derived DNA probe and the designing of a PCR primer. This primer will be employed in detecting *Cxx* in pure culture and subsequently in sugarcane sap collected from inoculated but symptomless sugarcane. This research approach may contribute to the effective control of RSD by enhancing the sensitivity, accuracy and early detection of *Cxx*.

* Infection and colonisation pattern of *Amaranthus hybridus* by *Alternaria alternata*

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Amaranthus hybridus has been identified as a highly nutritious food crop with potential for increased cultivation throughout the world. Endophytic and latent-infecting fungi have been shown to play an important role in the health and vigour of many plants by affecting various ecological and physiological processes. The increased utilisation of *A. hybridus* as a vegetable crop necessitates the understanding of these relationships. The objectives of this study were to determine how endophytic fungi infect this host and their colonisation pattern within leaf tissues. Five-month-old asymptomatic *A. hybridus* leaves were collected from Tempe, Free State, in 1997. For surface observations (infection patterns), leaf sections were examined using scanning electron microscopy. Tissues were fixed, critical-point dried in liquid CO₂, and sputter-coated with gold. The presence of fungal mycelium within host tissues (colonisation pattern) was confirmed using light microscopy. Leaves were fixed, cleared, and stained with acid fuchsin-malachite green. The most common fungus isolated from surface-sterilised leaf tissue was *Alternaria alternata* (89% of the samples). Surface examinations revealed *A. alternata* spores germinating on the surface and only entering leaves through stomata. The leaves had an internal net of hyphae in the mesophyll tissue growing intercellularly with no observed cell penetration or host-cell response. These results confirm that *A. alternata* is able to infect and colonise *A. hybridus* leaf tissue in a manner consistent with other endophytic or latent-infecting fungi.

Differentiation of *Clavibacter xyli* subsp. *xyli* strains by ribotyping

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Intraspecific genomic relatedness of 12 *Clavibacter xyli* subsp. *xyli* strains from different geographic origins was evaluated based on their ribosomal RNA gene restriction patterns (ribotyping). Chromosomal DNA was prepared, digested with the restriction endonucleases *Ava*I, *Bgl*II and *Hind*III, blotted and hybridised with the digoxigenin-labelled 16 + 23S rRNA from *Escherichia coli*. The strains of *C. xyli* subsp. *xyli* investigated produced different ribotype patterns for each strain. Ribotype patterns showed certain *C. xyli* subsp. *xyli* strains to be more distinguishable than others. The various ribotypes obtained demonstrated the genetic divergence of the *C. xyli* subspecies. Some fragments appeared to be common to all strains, indicating relatedness. Ribotyping demonstrated good genetic markers for intraspecific differentiation of *C. xyli* subsp. *xyli* strains, suggesting that it may be a useful tool for the genotypic differentiation of closely related strains. Genotypic differentiation of *C. xyli* subsp. *xyli* by ribotyping confirms the trends observed in our laboratories using random amplified polymorphic DNA analysis.

Infection and colonisation of cowpea stems by *Colletotrichum dematium*

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Colletotrichum dematium was first recorded on cowpea stems in South Africa in 1996. Symptoms begin as small, light brown lesions which darken to a purplish-brown colour and enlarge, often girdling the stem. The aim of this research was to study the pre-penetration structures and the infection process of *C. dematium*. Stems of four-week-old cowpea seedlings were painted with a spore suspension (10⁵ conidia per ml) and covered with plastic bags to maintain high humidity. Stems were cut at various time intervals before preparation for examination under light and scanning electron microscopes. Conidia began germinating 6 h post-inoculation (hpi), forming appressoria directly or at the ends of germ tubes. Ungerminated conidia were aseptate, but two to three septa became visible upon germination. Almost all appressoria were melanised and direct penetration of host tissue had begun by 14 hpi. At 20 hpi, appressoria had formed infection vesicles in epidermal cells. Thick, knotted primary hyphae formed from these infection vesicles, entered and filled adjacent epidermal and cortical cells. The pathogen grew biotrophically during this symptomless stage of the infection process. At ~40 hpi, *C. dematium* produced thinner, highly branched secondary hyphae, which grew extensively intracellularly and intramurally. After approximately 48 hpi light brown lesions appeared on the stems due to the invasion by secondary hyphae, causing the degradation of cell walls and necrosis of epidermal cells. This represents the beginning of the necrotrophic phase of *C. dematium* infection. Acervuli with one or two melanised setae formed from the hyphal aggregates at ~65 hpi.

Registration of a fungal inoculant to prevent re-growth of cut wattle tree stumps

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Several of the introduced Australian *Acacia* species have become important invasive weeds in South Africa. There is currently a major thrust to clear mechanically some of these from water-courses and water catchment areas, where they contribute to a considerable loss of valuable water. Since the cut stumps of many of these trees sprout and re-grow into multi-stemmed trees, the stumps have to be treated, usually with a chemical herbicide. This is often not desirable, particularly along water-courses, and an alternative method was sought. In the damp forests near