Armillaria root rot

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Armillaria luteobubalina is a fungal phytopathogen endemic to Australia. First described by Podger *et al* [1], this species affects a wide range of plants in horticultural and native environments of temperate regions within Australia, colonising root and trunk tissue. This colonisation causes tissue necrosis and ultimately death of the host, giving it the disease name of Armillaria root rot.

This disease has brought about considerable economic loss to horticultural, forestry and amenity plantings. To date, control options are limited, with removal of the infected material as the only proven successful management procedure.

Armillaria sp. endemic to Australia

Prior to the 1970s, all *Armillaria* species were identified as *A. mellea,* the major species found throughout Europe. During 1970-80 Kile and colleagues [1-3] identified five species based on morphological and biological species concepts: *A. luteobubalina, A.. hinnulea, A. fumosa, A. novae-zelandiae* and *A. pallidula*.

Only *A. luteobubalina,* the most abundant species found within Australia, is considered to be a pathogen of significant concern. This species can be generally characterised by its yellow/brown fruiting body, thick yellow annulus and ivory white spores. Rhizomorph production is sparse under most field environments [4] but abundant in culture. Specimens have been found in a wide range of environments, such as coastal dune plains, dry schlerophyll forests and blue gum high forests, but are generally restricted to temperate regions of the Australian coastline and ranges [5].

Sites of high disease prevalence are usually composed of more than one clone (genetically different individuals) with individual clones relatively small compared to those found in some of the northern hemisphere species [2].

Although the majority of disease spread is thought to be through root contact, studies that have investigated mating allele combinations [2, 6] [authors' unpublished data] have shown that approximately half of all clones at a given site will be related through the process of sexual recombination. This suggests that basidiospore dispersal and sexual recombination have an impact on the epidemiology of *A. luteobubalina.*

Disease development and host response

The major disease symptoms include rotten water-soaked roots, leaf wilting, dieback, trunk-splitting and collar rot. The development of these symptoms is highly dependent upon environmental conditions and the host species. Some plants show no or few symptoms for many years until the combination of extensive infection and significant stress causes them to die within a number of days.

There are three forms of disease transmission exhibited by *Armillaria*: the production of rhizomorphs; contact between infected and non-infected roots; and the dispersal of basidiospores. As rhizomorphs are not produced to any significant degree by *A. luteobubalina*, this is not a significant form of transmission and so contact between infected and non-infected roots is the main method of clonal expansion. In comparison, the dispersal of basidiospores as a form of disease transmission is considered to occur at a very low rate but has been shown to occur regularly enough to initiate new clonal infections.

Once penetration of the host has been achieved, *Armillaria* characteristically releases a group of cell degrading enzymes that include hemicellulases and cellulases. These enzymes breakdown the cellulase fibrils and hemicellulase matrix of the primary and secondary plant cell walls. It is this process that causes the majority of damage to the host.

Colonisation was thought to be restricted to the base of the plants; however, recent findings by the authors have shown that colonisation as high as 5 metres up the stems of certain species can occur [authors' unpublished data].

Identification

For many years the identification of Armillaria root rot and *Armillaria* species relied upon macro- and micro-scopic variations in basidiome morphology, characteristics in culture and mating studies.

Armillaria fruiting bodies are generally produced annually from the end of Autumn to early Winter. This can be a simple and time efficient technique for identification when the fruiting bodies are produced. However, not all field isolates of *Armillaria* produce fruiting bodies every year and mis-diagnosis can be made.

Identification to species level from the root material is extremely difficult and unreliable due to the similarity across all species. Isolation of the fungus can be extremely difficult and may require incubation for extensive periods of time. In recent years, however, identification techniques for *Armillaria* have broadened to encompass molecular characteristics. A molecular technique for the identification of *A. luteobubalina* has been developed recently based on a PCR-RFLP technique [7].

Control

The are no effective means of control of this disease other than by removing all the

infected material from soil. This may involve extensive excavation of root systems and may involve large machinery and considerable expense. No chemical control is currently available; however, biological control options using other fungi are being investigated. Host resistance is poorly understood but may offer some hope for control of the disease.

References

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Mycorrhizas and revegetation

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Much of Australia has extremely impoverished soil. Phosphate is particularly deficient. The major difficulty in revegetating these soils after severe disturbance is that plant survival and growth is unpredictable [1].

Mycorrhizas are associations between soil-borne fungi and the roots of plants. Of particular interest are the arbuscular mycorrhiza (AM) whose fungi form an internal colony in the roots of some 70% of all plant species. In AM, the fungi function as extensions of the root system, enabling the plant to increase uptake of non-labile minerals, especially phosphorus (P), from soil. The fungus gains its organic energy from the plant, and can only be maintained in the presence of living roots.

Experiments in the laboratory over many years have demonstrated the importance of mycorrhizas to plant growth [2]. However, as most Australian plants are adapted to impoverished soils, their growth response to mycorrhizas is difficult to detect in the laboratory.

Use of fertiliser improves survival and growth of most seedlings. If seedlings of Australian plants store minerals in their tissues, then we may be able to transplant fertilised seedlings in revegetation programmes, and expect them to thrive, albeit at slow rates of growth. However, much of Australia is also subject to regular fire. Seedlings are burnt back to the ground, releasing minerals to smoke and ash. Thus fertiliser may simply enable survival to the first fire unless the seedlings first become colonised by appropriate mycorrhizal fungi.

We set out to determine whether AM may play a role in revegetation of highly disturbed sites. We were particularly interested in whether inoculation with mycorrhizal fungi was necessary, whether the inoculated fungi would establish in disturbed soils, and whether we could provide a long-term benefit by inoculation.

The experimental site was a waste disposal area south of Sydney. The region is characterised by open vegetation typical of Hawkesbury Sandstone. In broad terms, the operators at the site move the bedrock and soil to one side, the waste is deposited and compacted, and then the crushed sandstone mixed with soil is placed over the waste (Figure 1). In addition, the local community expect the site to be returned to a state resembling the original vegetation when the site is returned. Results from initial plantings from seed by the operators indicate that a very limited number of plant species would grow. None of these formed AM. A much more effective approach to revegetation was required.

We germinated seed of four plant species collected from the site. Seedlings were either inoculated with AM fungi obtained from a similar soil, or fertilised to a similar size. We transplanted the seedlings to the site and followed survival and growth of the plants, and survival and spread of the AM fungi over 20 months.

Only fertilised or mycorrhizal seedlings survived to the transplanting stage, indicating that, unless fertiliser was continually applied to the soils, mycorrhizal fungi were essential for the long-term maintenance of vegetation at the site. At the field site, fertilised and mycorrhizal seedlings had a similar rate of

Figure 1. View of the experimental site following capping of the waste.