



Variation in susceptibility among macadamia genotypes and species to *Phytophthora* root decay caused by *Phytophthora cinnamomi*



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ABSTRACT

Phytophthora cinnamomi is a major pathogen of cultivated macadamia (*Macadamia integrifolia*, *Macadamia tetraphylla* and their hybrids) worldwide. The susceptibility of the two non-edible *Macadamia* species (*Macadamia ternifolia* and *Macadamia janseni*) to *P. cinnamomi* is not well-understood. Commercial macadamia trees are established on grafted seedling (seed propagation) or own-rooted cutting (vegetative propagation) rootstocks of hybrids of the cultivated species. There is little information to support the preferential use of rootstock propagated by either seedling or own-rooted cutting methods in macadamia. In this study we assessed roots of macadamia plants of the four species and their hybrids, derived from the two methods of propagation, for their susceptibility to *P. cinnamomi* infection. The roots of inoculated plant from which *P. cinnamomi* was recovered showed blackening symptoms. The non-cultivated species, *M. ternifolia* and *M. janseni* and their hybrids were the most susceptible germplasm compared with *M. tetraphylla* and *M. integrifolia*. Of these two species, *M. tetraphylla* was less susceptible than *M. integrifolia*. Significant differences were observed among the accessions of their hybrids. A strong association ($R^2 > 0.75$) was recorded between symptomatic roots and disease severity. Root density reduced with increasing disease severity rating in both own-rooted cuttings ($R^2 = 0.65$) and germinated seedlings ($R^2 = 0.55$). *P. cinnamomi* severity data were not significantly ($P > 0.05$) different between the two methods of plant propagation. The significance of this study to macadamia breeding and selection of disease resistant rootstocks is discussed.

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1. Introduction

Four species of macadamia (*Macadamia integrifolia*, *Macadamia tetraphylla*, *Macadamia ternifolia* and *Macadamia janseni*) originate in Eastern Australia and represent a significant resource for the macadamia industry, as potential sources of resistance to biotic and abiotic stress, and for improving yield and quality. The wild germplasm is vulnerable to extinction due to habitat loss and fragmentation (Pisanu et al., 2009; Neal et al., 2010; Powell et al., 2010). Under the Australian Environment Protection and Biodiversity Conservation Act, 1999, three species (*M. integrifolia*, *M.*

tetraphylla and *M. ternifolia*) are listed as vulnerable while *M. janseni* is listed as endangered (Shapcott and Powell, 2011). *M. ternifolia* and *M. janseni* do not produce edible nuts and mostly exist in the wild ecosystem (Hardner et al., 2009). *M. integrifolia* and hybrids with *M. tetraphylla* constitute the current commercial macadamia production worldwide (Hardner et al., 2009). Genetic diversity that exists in the wild populations of *Macadamia* has not been explored for resistance to pathogens and pests, yield, quality or tolerance to abiotic stresses.

Macadamia trees in commercial orchards are propagated on grafted rootstocks derived from either vegetatively propagated clonal cuttings or germinated open-pollinated seeds (Hardner et al., 2009). Most rootstocks are selected based on ease of germination, propagation and grafting rather than their resistance to biotic or abiotic stress. In Australia, nearly all the grafted trees in commercial

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macadamia orchards are established on seedlings rootstocks of the cultivar 'H2', a *M. integrifolia* & *M. tetraphylla* hybrid. In South Africa, clonal cuttings of cultivar 'Beaumont' (HAES 695) a *M. tetraphylla* & *M. integrifolia* hybrid are preferred as rootstocks (Trochoulis, 1992; Huett, 2004; Hardner et al., 2009). Phenotypic characteristics of 'H2' are similar to the *M. integrifolia* characteristics, whereas, the 'Beaumont' characteristics are similar to *M. tetraphylla*. There is little information to support the choice of either genotype as rootstock in macadamia. The root systems of seedling populations are likely to have a high degree of variation in terms of root architecture and efficiencies of nutrient utilization (Stephenson and Cull, 1986), which may influence their performance with regard to impact on disease expression. The root systems of vegetatively propagated cutting-derived rootstocks of a particular variety are more likely to be very similar and equally susceptible to invading root pathogens since they are clonally propagated.

Phytophthora cinnamomi is an important soilborne pathogen in macadamia, and is considered a major constraint of macadamia production worldwide (Drenth et al., 2009; Akinsanmi and Drenth, 2013a). Since *P. cinnamomi* was introduced into Australia, it now occurs widely in all states and territories where it causes disease in a several plant species (Cahill et al., 2008; Hee et al., 2013). Information on the susceptibility of the Australian native *Macadamia* species is scanty. The diseases caused by *P. cinnamomi* are of significant economic importance in many horticultural crops including macadamia therefore research, focused on its control, continues to be a priority in many Australian horticultural and forestry industries.

P. cinnamomi can cause a range of symptoms in macadamias. At the early stage of *P. cinnamomi* infection, disease symptom expression is often misdiagnosed, or appears as general tree decline due to poor nutrition. Tree decline associated with *P. cinnamomi* is usually expressed as pale or yellow green leaves instead of dark green. Under conditions of moisture stress, in the advanced stages of infection, the leaves of infected trees rapidly wilt and readily abscise from the tree, giving rise to a sparse canopy appearance. The later stages of symptoms are more evident in macadamia following prolonged water-logging or drought conditions (Akinsanmi and Drenth, 2013b). Most times, fresh leaf flushes and shoot growth are absent or sparse and branches die back from the tip (Pegg, 1973). *P. cinnamomi* may directly infect macadamia stems or branches causing numerous small stem or trunk cankers. Infections of the stem are first characterized as gummosis or bleeding of the trunk and cracking of the bark which, in more advanced stages of the disease may develop in irregular areas of dead bark that extend from the soil line to several feet high. This often results in furrowed deep cankers on the trunk.

Diseases caused by *P. cinnamomi* from root infections are difficult to control (Ali et al., 2000). In macadamia, chemical applications with phosphonates and metalaxyl have been reported to be effective against diseases caused by *P. cinnamomi* (Akinsanmi and Drenth, 2013b). Selection for resistance to *Phytophthora* in macadamia may offer additional advantages and may be an effective means of controlling *Phytophthora*-incited diseases compared with chemical control in macadamia. The resistance system in macadamia genotypes to *P. cinnamomi* is not well understood.

Although early reports suggested that macadamia roots are resistant to *P. cinnamomi* (Zentmyer, 1960), subsequent studies have reported that *P. cinnamomi* is able to infect macadamia roots. These studies suggested that symptoms of root infection may be expressed as root necrosis and soft root rot (Ko and Kunimoto, 1976; Serfontein, 2008; Mbaka et al., 2009). Black to dark necrotic lesions observed on macadamia fine roots are associated with *P. cinnamomi* infection, but there has been no clear or consistent re-isolation of *P. cinnamomi* from roots showing black root symptoms. Although

several varieties are affected by *P. cinnamomi* under orchard conditions, there is still confusion as to whether *P. cinnamomi* causes soft root rot or necrosis. Although a preliminary study by Zentmyer and Storey (1961) suggested differences between *M. integrifolia* and *M. tetraphylla* to *P. cinnamomi* infection and variation is observed among varieties of grafted trees in orchard conditions, there is little information on the relative susceptibility of different *Macadamia* species and selections to *P. cinnamomi*.

Selection of *P. cinnamomi* resistant rootstocks depends on the presence and variability in susceptibility to *P. cinnamomi* among different macadamia selections and species. Material with genetic resistance to *P. cinnamomi* may be used directly as rootstocks or as parents in breeding programmes. This will provide a more long-term and economical approach to managing diseases caused by *P. cinnamomi* in macadamia than the current application of agrochemicals as the main control method (Akinsanmi and Drenth, 2013b). In this study, we hypothesize that the non-cultivated species (*M. ternifolia* and *M. janseni*) are more tolerant to *P. cinnamomi* than the two species (*M. integrifolia* and *M. tetraphylla*) or their hybrids that are cultivated in commercial production systems. We also evaluated the range of susceptibility among the four species of macadamia and their interspecific hybrids to root infections by *P. cinnamomi*. This study provides a more definitive description of symptoms of root infection by *P. cinnamomi* in macadamia. For the purpose of this study, we defined root necrotic lesions as any localized area of root tissue with extended spot, canker or scab, while root rot refers to the softness or decay of the root tissue that compromise its structural integrity.

2. Materials and methods

2.1. Plant materials and inoculation

Plants of four *Macadamia* species (*M. integrifolia*, *M. tetraphylla*, *M. ternifolia* and *M. janseni*), two accessions of reverse crosses of *M. ternifolia* × *M. janseni*, three accessions (ITH-1425, ITH-4323, ITH-679) of *M. integrifolia* & *M. tetraphylla* hybrids and one accession (ITH-Beau) of *M. tetraphylla* & *M. integrifolia* hybrid that represent a range of genotypes were selected. A total of 132 plants were established in the glasshouse, propagated as own-rooted cuttings (clonal) or seedlings as described by Topp and Neal (2015) were used in this study. Three cultures of *P. cinnamomi* (UQ7100, UQ7097 and UQ7098) were obtained from the University of Queensland *Phytophthora* culture collection in Brisbane. These three isolates were originally obtained from macadamia trunks expressing symptoms of stem canker and the ability of the three *P. cinnamomi* isolates to cause stem canker in macadamia had been confirmed in a previous study (Akinsanmi and Drenth, 2012). The isolates were grown on V8 juice (Campbell's soups Australia) agar amended with 10 ug/ml Pimaricin, 50 ug/ml Penicillin and 50 ug/ml Polymixin B final concentration (Tsao, 1960; Eckert and Tsao, 1962). The cultures were incubated in the dark at 25 °C for 10 days and used to inoculate sterilized wheat grain culture. The wheat grain culture was prepared by soaking 150 g grain in 40 ml of water in 500 ml conical flasks for 24 h. Thereafter, the flasks were autoclaved twice at 121 °C for 30 min on two consecutive days, before inoculating with three pieces of mycelial agar plugs (10 mm diameter) of each *P. cinnamomi* isolate in separate flasks. Flasks that were inoculated with V8 juice agar plugs without *P. cinnamomi* served as the control. The inoculated flasks were incubated at 25 °C for three weeks, routinely agitated once a week, to prevent clogging of the grains, before grinding the content to produce the inoculum. Approximately 1 g of inoculum per litre of potting mix was placed in the potted macadamia plants. Inoculum for each isolate was placed in a 120 mm deep hole at approximately 10 cm from the stem of each

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