The ecology, distribution and population structure of *Phytophthora cinnamomi* associated with root rots and trunk cankers of macadamia in Kenya

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ABSTRACT

In Kenya, macadamia (*Macadamia integrifolia* Maiden and Betche and *Macadamia tetraphylla* L.A.S. Johnson) is grown mainly for export by over 100,000 small scale and 500 large-scale growers. However, slow decline is a major production constraint. While decline may be caused by other factors such as soil and water management in the orchard, root rots and trunk cankers have been associated with macadamia decline in Kenya and in other countries such as Australia, California and Hawaii. The two diseases are caused by the soil borne pathogen *Phytophthora cinnamomi* Rands. The objective of this study was to determine the ecology, distribution and population structure of *P. cinnamomi* in macadamia growing areas of Kenya.

Surveys were carried out in macadamia growing areas of Kenya between December 2005 and April 2006. To capture data on macadamia production practices, questionnaires were administered by face-to-face interviews with farmers. Field sampling of macadamia trees was done to assess incidence and severity of the two diseases. *Phytophthora cinnamomi* was recovered from soil samples by baiting and from plant tissues by plating on *Phytophthora* selective medium. Green apples (*Malus domestica* × *M. sylvestis*) cultivar Granny Smith were used to separate *Phytophthora* from other co-isolated species such as *Pythium*. Root rots and trunk cankers were recorded in 85 % of the farms sampled. There were significant (P=0.05) differences in disease incidence and severity between the sampled districts. *Phytophthora cinnamomi* had a wide distribution in all the macadamia growing areas. Three *P. cinnamomi* populations, the A1, A2 and homothallic types were recovered from macadamia tree rhizospheres, stems and roots. This led to the conclusion that *P.*

cinnamomi has a sexual reproduction in macadamia disease situations in Kenya. Sexual oospores are formed when compatible gametangia of different mating types of a heterothallic species cross, however, self crossing gametangia form oospores in homothallic species. Oospores survive for long in soil as dormant propagules and depending on their ability to germinate in the presence of a susceptible host, can have an implication on use of crop rotation as a disease management strategy.

The three *P. cinnamomi* populations were examined for their phenotypic variation by studying their macro and micro-morphological phenotypes. The macro-morphological phenotypes studied were, colony morphology and growth rate at 20, 24 and 28 °C on PDA, pathogenicity, virulence and capacity to kill macadamia seedlings. The micro-morphological phenotypes examined were sporangia formation, morphology and dimensions. Isolates differed significantly (P=0.05) in growth rate and colony morphology that differed at different temperatures. The homothallic isolates were the fastest growing. There was no significant (P=0.05) difference between isolate sub-population and pathogenicity but there was significant difference in virulence. The homothallic isolates were the most virulent. More than 50 % of the test isolates killed macadamia seedlings 53 days after inoculation. There were no significant (P=0.05) differences in sporangia dimensions among the isolates. These findings of large phenotypic variations among isolates have important disease management implications.

Six fungicide formulations and a bio-control product recommended for management of *Phytophthor*a diseases were evaluated for their *in vitro* growth inhibition of seven virulent *P. cinnamomi* isolates. All the isolates were sensitive to the fungicides and

the bio- control agent with *in vitro* growth inhibition ranging from 40.3 to 98.2 %. It was recommended that depending on the mode of action, these fungicides and the bio-control agent should further be evaluated for their use in integrated management of root rots and stem canker of macadamia as root dip, soil drench, trunk injections, foliar spray or wound treatments.

Macadamia cultivars were evaluated for their response to *P. cinnamomi* by use of the leaf and stem inoculation techniques. Results of leaf infection percentage and lesion extension were similar through out the experiments. Using the two parameters, variability of macadamia varieties in their susceptibility to *P. cinnamomi* was apparently clear. The stem inoculation results had a similar trend. Results showed that *M. tetraphylla* and MRG-20 were the least susceptible to *P. cinnamomi* infections. These two should be evaluated for use as rootstocks in macadamia propagation as an effective way of managing root rots.

To confirm the accuracy of conventional methods for isolating and identification of *P. cinnamomi*, DNA based methods were used. Pathogenic *P. cinnamomi* isolates had the region of the ribosomal repeat from the 3' end of 18S gene (through ITS1, the 5.8S gene and ITS2) to the 5' end of the 28S gene defined by oligonucleotide primers. Isolates were characterized by conducting the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis. Amplicons were subjected to three different restriction enzymes, *Msp1*, *Rsa1*, and *Taq1* to obtain diagnostic deoxyribonucleic acid (DNA) fingerprints. The DNA sequence data was aligned for species identification on GenBank® database. This study identified for the first time, presence of *Pythium vexans* in diseased macadamia trees in Kenya. The role of *P.*

vexans as a primary pathogen, predisposing organism, opportunistic pathogen or non-pathogen in macadamia is unknown. There is need to establish this considering that presence of *P. vexans* and other *Pythium* spp in macadamia nursery growth media could have a big impact on spread of root rots and trunk cankers of macadamia.