

**DETECTION OF VARIABILITY OF
RIGIDIPORUS MICROPORUS,
THE CAUSATIVE FUNGUS OF THE
WHITE ROOT DISEASE OF RUBBER**

**A DISSERTATION SUBMITTED
IN PARTIAL FULFILMENT OF THE
MASTERS DEGREE IN APPLIED MICROBIOLOGY
TO THE DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF KELANIYA**

SRI LANKA

BY

CHANDRIKA MALKANTHI HEWAWASAM NANAYAKKARA

RP/M.Sc./Micro/ 97/05

Department of Botany
University of Colombo
Colombo
Sri Lanka

31 July, 2000

ABSTRACT

White root disease caused by the fungus *Rigidiporus microporus* (Fr.) Overeem is the most destructive disease of rubber in Sri Lanka. An elevated severity of the disease was reported in 1977 and this increase was attributed to the presence of a biologically variable population of the fungus (Liyanage *et al.*). In the present study, attempts were made to characterize the genetic variability between the isolates of *Rigidiporus microporus*.

Preliminary studies on variability in pathogen biology was carried out by making observations on cultural characteristics, growth rates, pathogenicity and the ability to produce rhizomorphs in four isolates of *Rigidiporus microporus* collected from four different rubber growing area, viz, Galle, Kalutara, Kuruwita and Kegalle. Fungal colonies of all isolates grown on MEA were white in colour with prominent concentric rings making it impossible to identify separate groups. Growth rates of isolates on three solid media, CDA, MEA and PDA and two liquid media, viz. CDB and PDB, showed a differential reaction. Three different groups of the fungus can be identified by analyzing growth on CDA with isolates AK and KE falling to the same group and KW and NKD in two other groups. Growth on MEA and PDA of the isolates showed that the isolates were divisible in to four different groups, each isolate in a different category. Growth in MEB of all isolates were the same where as growth in PDB and CDB demonstrated the existence of three different groups of the fungus with isolates AK and NKD in the same category while KE and KW formed another two separate groups.

Studies on pathogenicity using autoclaved root pieces of PB 28/59 clone showed a differential reaction where all isolates formed four different groups with respect to their ability to colonize roots internally. All isolates produced rhizomorphs in soil despite their differences in pathogenicity.

Analysis of RAPD-PCR data revealed that *R. microporus* is genetically variable and this is in accordance with the observations made in the preliminary studies. All forty primers from two primer kits, OPA and OPC, except OPA3, OPC 3 and OPC 6 resulted in the amplification of 9 and 10 fragments in the range of 3000 - 200bp. The total number of strong polymorphic bands obtained with OPA primers were 117 whereas OPC primers resulted in 39 polymorphic bands making the primer kit OPA more suitable to detect polymorphism in *R. microporus*. A dendogram for the four isolates was constructed using genetic distance matrix obtained from RAPD data. According to the dendogram, isolate AK has a genetic distance of 0.52 with the other three isolates and the isolates KE and NKD showed the least difference having only a genetic distance of 0.22. Isolate KW differs from isolates KE and NKD in a genetic distance of 0.30. Therefore, it is evident that the four isolates vary with respect to their genotypes and that variation is reflected in the biology of the organisms.