

**MOLECULAR CHARACTERISATION OF *Verticillium dahliae* Kleb
ISOLATES AND COMPARISON OF THEIR VIRULENCE ON
SELECTED COTTON VARIETIES IN ZIMBABWE**

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**A thesis submitted in Partial fulfilment of the requirements for the
degree of Master of Science in Crop Protection**

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University of Zimbabwe
May 2005**

UNIVERSITY OF ZIMBABWE

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The undersigned certify that they have read and recommend to the Department of Crop Science for the acceptance of the thesis titled: **Molecular characterisation of Verticillium dahliae Kleb isolates and comparison of their virulence on selected cotton varieties in Zimbabwe.**

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ABSTRACT

A study to determine the virulence of five *Verticillium dahliae* isolates on five varieties of cotton (SZ 9314, G 501, FQ902, BC 853 and 563-97-12) was carried out under both field (at five sites Cotton Research Institute (CRI), Henderson, Rafingora, Chisumbanje and Chinhoyi) and greenhouse conditions. The molecular differences of the five isolates were also compared using the Polymerase Chain Reaction (PCR) at Tobacco Research Board's Molecular Biology Laboratory. In the field experiments to determine virulence, the five varieties named above were planted at each site in a Completely Randomised Block Design with four replications. Monthly infections and fortnight scoring were done from January to the end of May. Infection percentages were calculated at the end of the season. They were arc sine transformed and then subjected to analysis of variance using Gestat 3.2. Fortnightly scores were used to calculate the area under the disease progress curves (AUDPC) using Sigma Plot 8. Significant differences ($p < 0.05$) in infection percentages (arc sine transformed) were obtained at CRI, Chisumbanje and Chinhoyi but not at Rafingora and Henderson. There were no significant varietal differences for AUDPC at four sites (CRI, Chisumbanje, Rafingora and Henderson) except Chinhoyi, which had significant AUDPC differences between the varieties. From the five sites, soil samples were taken to isolate *Verticillium dahliae*. The isolates were tested for their virulence on five varieties used in the field. The trial was laid in a split plot design with *Verticillium dahliae* isolates as main plots and varieties as subplots. A single pot with one plant constituted a plot. Inoculum was prepared from the isolates and plants were stem pricked at the base at six weeks after planting. Severity scores were done a week after infection for the next four weeks. All the isolates were virulent, with isolates from CRI and Chinhoyi producing significantly higher AUDPC on all varieties than other isolates from other sites. Significant AUDPC were observed with G501 and BC853 having the lowest AUDPC while SZ9314 and F902 had the highest AUDPCs and variety 563-97-12 had intermediate AUDPC. PCR was run to compare five *Verticillium dahliae* isolates against the standard fungus from imported from South Africa at the Tobacco Research Board. Two primers were used (primer 19/22 and 42/70). Primer 22/70 managed to produce more bands (eleven bands) than primer 19/22 which produced five bands. Bands were scored and a cluster analysis was done to compare the differences of the banding patterns. Results show that there were differences at molecular level between the isolates. Differences were evident between the Rafingora isolate and the other isolates. Relationship between virulence and molecular differences was not established. However our results are preliminary and further research still need to be carried out on the isolates.

ACKNOWLEDGEMENTS

I would like to thank the Government of Zimbabwe that granted me a two-year study leave and the Belgian government that offered me a two-year scholarship to study at the University of Zimbabwe. The Director of Agricultural Research and Extension (AREX) and the Head of Institute, Cotton Research Institute who allowed me access to facilities, varieties, sites and other resources that made this study a success. I am indebted to my supervisors Mrs. J. Sibiya and Mr. Manyagarirwa for their guidance. I am also grateful to Dr. Garwe of the Tobacco Research Board and her staff who provided the expertise on the Molecular work. Staff at the Cotton Research Institute especially Dr. L.T. Gono, Mr. A. R. Chimoga, Mr. E. Mupanehari, Ms C. Chabvongodze and the Research Hands in the Pathology section of the Institute were very helpful especially Mr. J. Kamidza and Mr. Simoyi.

I would like to express my sincere thanks to Dr. P. Jowah who encouraged me throughout the MSc course and my course mates who were very critical of the work.

I am also greatly indebted to son Branton, my Mother and my wife Juliana for encouragement and support.

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