THE TAXONOMY AND ECOLOGY OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) IN AGROFORESTRY SYSTEMS IN MALAWI

BY

JOYCE MNYAZI JEFWA

SUBMITTED IN FULFILLMENT OF PART OF THE REQUIREMENTS FOR THE DEGREE OF

PHILOSOPHIAE DOCTOR

IN THE FACULTY OF NATURAL AND AGRICULTURAL SCIENCES

UNIVERSITY OF PRETORIA

2004

SUPERVISOR: DR. ROBERT SINCLAIR

CO-SUPERVISOR: DR. JOHANNES V VAN GREUNING

DECLARATION

I hereby declare that the thesis submitted for the degree of PhD (Botany) at the University of Pretoria is my work and has not previously been submitted by me for a degree at any other university or institution of higher education.

DEDICATION

I dedicate this work to my late father who was my source of inspiration and motivator in my life.

ACKNOWLEDGEMENTS

I owe my sincere gratitude to my supervisors Dr. Robert Sinclair and Dr. Johannes van Greuning for their support, advice and critical review of the work. I would like to extend my gratitude to the World Agroforestry Center, formerly International Centre for Research in Agroforestry (ICRAF), for the Ford Foundation fellowship award for women in eastern and southern Africa region and the Rockefeller scholarship that enabled me to undertake this study. I am particularly grateful to my ICRAF supervisor, Prof. Magembe. I am indebted to all ICRAF Malawi staff, Aubrey Semu, Ketty Mkandawire, Rose Bell, Susan Ikerra, Kapeya, Joseph and the late Jenniffer for their help.

I am particularly grateful to Prof. Temu and Janet Awimbo, Rita Mulinge and former ICRAF staff Milcah Mbindyo, for facilitating logistics and my work while at ICRAF. I am also grateful to Jimmy Kiio and former ICRAF staff Alice Aduol, Mary Memia, Marie Kimenyi. I am grateful to Richard Coe, Louis Verchot and Marie Rarieya for assistance and advice in statistical analysis, Thomas McOwiti, Alfred Mureithi, Josephine Ngugi, Dali Mwangore and Elizabeth Mwamunga of library and information services.

I am grateful to the Botany department, University of Pretoria. I would like to thank Prof. Eicker for continuous support in the department. I thank all staff of the mycology section: Maria Khoza, Magda Nel and Rene Swart and my fellow student collegues Yolisa Pakela, Quenton and Buks for helping me during my work in the department.

Thanks to Lidya Swart and Rina Owens of the faculty of economics and statistics, University of Pretoria.

I am grateful to my institution the National Museums of Kenya, my collegues Victor Raul Otieno, Ann Aduol, Ann Onyango, Daniel Odhiambo, Mathias Muindi, and Jack Muriuki for their assistance and my collegues the late Stephen Muoki, Deche Mwamuye, Rogers Wayaya and Jackson Muriuki.

I owe my deepest gratitude to members of my family, my children Esther Neema, James and Daisy, my mother Esther Mishi and family members Dorah Umazi, Lidya Masika, Linda Nazi, Winnie Safi, Kenneth Noor, Dr. David Gome and Rachel Nyaega. Thank you all. Most of all I thank the Almighty God for this great privilege.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABSTRACT

Decline in soil quality and removal of subsidies on fertilizers has led to use of biological based farming practices such as agroforestry. Soil management and conservation practices have not used a holistic approach and have often overlooked the fact that soil is a dynamic system. Agroforestry improves soil fertility, but the effect on soil organisms has not been established. Knowledge of soil organisms in Africa is also scarce, yet soil organisms are crucial in soil processes and are greatly affected by farming practices. The Arbuscular Mycorrhizal Fungi (AMF) (*Glomeromycota* C. Walker & Schuessler) which form an interface between plant and soil systems and are sensitive to changes in plant and soil conditions can be used as indicators of changes in fertility.

A study was conducted in southern Malawi in agroforestry and maize monocrop systems to evaluate AMF species composition, diversity and frequency of occurrence as measured by spore occurrence, and AMF species effectiveness. Southern Malawi is a region prone to drought conditions and has nutrient deficient soils. The mycorrhizal symbiosis could be harnessed to improve root functions. The effectiveness of mycorrhizal symbiosis is highly dependent on AMF composition and inoculum potential of soils. In drought prone areas, spore propagules that persist longer under dry conditions are the best sources of inoculum. Two experiments were investigated for the AMF symbiosis, *Gliricidia sepium* (Jacq.) Walp/maize intercrop and maize monocrop system and *Sesbania sesban* (L) Merr./maize intercrop and *Sesbania macrantha* E Phillips & Hutch/maize intercrop and maize monocrop systems. A bioassay experiment was conducted under greenhouse conditions to evaluate colonization and effectiveness of selected AMF species from this site in plants.

The objective of the study was to determine AMF species using their spore morphological characters. A further aim of studying the agroforestry system with *Gliricidia sepium* was to determine the effect of agroforestry system on the diversity of AMF species and their frequency of occurrence. The *Sesbania* species experiment was used to establish whether two agroforestry systems would differ in their effects. In both agroforestry systems studies, it was determined whether the effect of the farming systems would be modified by application of inorganic nitrogen and phosphorus fertilizer and seasonal changes. Three AMF species extracted from the study site were evaluated for colonisation and effectiveness on growth of four host plants.

Soils were sampled and the spores extracted, fully described and taxon determined. The spore abundance of each individual species was quantified. Species relative diversity indices were computed from Shannon-Weinner and Simpson dominance diversity indices and the data was subjected to ANOVA. The Shannon-Weinner diversity Index was used to discuss the results. The species frequency of occurrence data measured by spore occurrence was analysed using logistic regression. The greenhouse bioassay experiment was subjected to ANOVA followed by comparisons of means by Least Square Means (LSM).

Twelve AMF species were extracted from the agroforestry sites, with four positively determined and three species affiliated to specific taxon. Out of the twelve AMF species only nine were successfully pot cultured. *Glomus etunicatum* Becker & Gerdemann and *Gigaspora margarita* Becker & Hall have been recorded from other parts of Africa. *Acaulospora rehmii* Sieverding & Toro, and *Scutellaspora cerradensis* Spain & Miranda were recorded for the first time in Africa. AMF species, namely *Archaeospora* sp. lacked

sufficient materials to diagonise it to species level while *Acaulospora* sp. 1, *Acaulospora* sp. 2, *Glomus* sp. and *Gigaspora* sp. had morphological features that strongly overlapped with closely related species. The study shows that 50 % of the species described from the agroforestry site in Malawi exist in other biogeographical regions of the world outside Africa. Two species, *A. rehmii* and *S. cerradensis* have been described only from South America. The study showed the mature stage of *A. rehmii, G. etunicatum, G. margarita* and *S. cerradensis* to have consistently similar morphological features over wider biogeographical regions. Additional morphological characters were observed in young spores of *A. rehmii* while some features observed in *Glomus* sp., which closely resembles *Glomus globiferum* Koske & Walker in most aspects, were not described in the type specimen description of *G. globiferum*, but were described in the type specimen description of *Glomus tortuosum* Schenck & Smith and *Glomus claroideum* Schenck & Smith. The possibly in young spores of *A. rehmii*, mature spores of *Acaulospora* sp 1, *Acaulospora* sp. 2, *Glomus* sp. and *Gigaspora* sp. described from spore cultures could not be distinguished easily from closely related allies.

In the *Gliricidia*/maize intercrop and maize monocrop systems, species diversity tended to be significantly ($p \leq 0.05$) higher in the wet season than the dry season in all treatments except for NP in agroforestry system. There was significant ($p \leq 0.05$) interaction between season, farming systems and fertility regimes. AMF species diversity was higher in agroforestry plots under conditions of low fertility and no addition of inorganic fertilizer than monocropped plots, although this was not significant ($p \leq 0.05$). Application of inorganic fertilizer modified the effects of farming systems, with inorganic fertilizer reducing species diversity in agroforestry plots. The fertilizer effect in agroforestry systems was more evident with N in the dry season and combination of N and P in the wet

season. Fertilizer effect showed AMF species diversity to be consistently low in all plots with a combination of inorganic N and P. Variable fertilizer effects were observed in maize monocrop in the two seasons. The effect of fertilizer in maize monocrop system was evident only in the dry season with species diversity higher in plots with P than plots with both N and P. The fertilizer effects in maize monocrop were more evident with P. There was no trend observed in species occurrence by members of the same family or genera. AMF species frequency of occurrence varied with season, farming systems and fertility regimes. The spores of five species occurred most in the maize monocrop system and the remaining seven were not affected. Spores of five species occurred most in the wet, three in dry season and four were not affected. The sporulation of five out of the twelve species decreased in plots with inorganic fertilizer, three species were enhanced, three species were not affected and spores of one species were variably affected.

In the two *Sesbania* spp./maize and maize monocrop systems, analysis of variance data showed farming systems to significantly ($p \leq 0.05$) affect AMF species diversity, species diversity being reduced by agroforestry systems. Plots with *Sesbania macrantha* intercropped with maize had significantly ($p \leq 0.05$) lower species diversity than maize monocrop but were not significantly different from plots with *S. sesban* intercropped with maize. Inorganic nitrogen fertilizer had the greatest effects on AMF species diversity measured by spore occurrence, species diversity being significantly ($p \leq 0.05$) enhanced by inorganic nitrogen fertilizer. Studies on frequency of occurrence of spores of AMF species showed season to have the most significant ($p \leq 0.05$) effect, nine species being prevalent in the dry season and three in the wet season. Farming systems exerted selective pressure on the occurrence of spores of four AMF species; *Gigaspora* sp. and *Scutellospora dipurpurascens* Morton & Koske were significantly ($p \leq 0.05$) affected and *Acaulospora* sp.

and *Glomus* sp. were slightly ($p \leq 0.1$) affected. Inorganic fertilizer also showed spores of *A. rehmii*, *Archaeospora* sp. and *S. cerradensis* to be significantly ($p \leq 0.05$) affected and *G. etunicatum* and *S. dipurpurascens* slightly ($p \leq 0.1$) affected.

The bioassay experiment showed AMF species from similar habitats to differ in their effects on plant growth. The closely related *Glomus aggregatum* Schenck & Smith and *G. etunicatum* showed preference for *G. sepium* while *A. delicata* showed preference for the closely related *S. sesban* and *S. macrantha*. There was no correlation between root colonisation levels and the three growth parameters in the three tree species, although colonisation levels were negatively correlated with the growth of maize. This study shows AMF species from the same site to differ in their effects on plant growth while AMF species effective on tree growth not necessarily improving the growth of maize.

Observations made in this study show little divergence of AMF species, some species to have a wide biogeographical range of distribution and the morphological characters inadequate diagnostic tools for separating closely related species. The effects of agroforestry systems are site specific and highly dependent on the fertility regimes and AMF species diversity seemed to respond most to the manipulation of the most deficient nutrient N. Root hairs seemed to play a vital role in determining host response to AMF association and the association of host plants with AMF species seemed to be a hereditary trait. The spores of AMF species from the same site differed in their adaptations with few species showing consistency and most changing with changes in environmental conditions. These results have great implication on management of AMF symbiosis and inoculation programs.