1 INTRODUCTION

Zimbabwe's population within the communal lands demonstrate a heavy dependence on the agriculture economy and utilisation of natural resources. Woodlands and tree resources play a central role in the household economies, particularly in the semi–arid areas (less than 500 mm annual rainfall) where people cannot wholly depend on agriculture for their livelihood without the subsidies provided by woodlands and tree resources. Some of the products realised from woodlands are wood for use in rural industries such as brick burning, beer brewing, tobacco curing, civil construction and artefact making (Clarke *et al*., 1996).

In addition, woodlands also yield other products such as mushrooms, honey and indigenous fruits, without which, dietary needs of the local people would be incomplete. The continued availability of these products is, however, coming under severe threat as woodlands are continuously being cleared for agriculture. Moyo *et al*. (1991) estimated that 70 000 – 100 000 ha of Zimbabwe's woodlands are cleared for agriculture annually, presumably under the resettlement programme and other illegal settlers. With the current trend of deforestation and the general poor rainfall pattern, the nutritional status of the rural people will most likely continue to decline. Importance of indigenous fruits has been recognised and acknowledged widely, particularly in countries with the miombo type of woodland, which boasts of rich diversity of edible indigenous fruits (FAO, 1983; Gumpo *et al*., 1990; Maghembe and Seyani, 1991; Mwamba, 1989). Nutritional value of indigenous fruits is well acknowledged in the study on non-timber forest products (FAO, 1990).

Uapaca kirkiana Muel. Arg. (Euphorbiaceae), which is one of the most important indigenous fruits of the miombo, has been analysed for its nutritional content and has been found to contain glucose (4.1%), fructose (2.7%), sucrose (1.5%), xylose (0.2%) and traces of ribose, raffinose and galactose (Sufi and Kaputo, 1977). Other than direct nutritional benefit, the fruit can be processed into products such as jam, wine, fresh drink, resin and furthermore, the fresh fruit is also important as an income earner. The ripe fruits are sold along roadside and at country markets (Tredgold, 1990).

As these fruits are generally collected from the wild, their continued availability is highly dependent on existence and good management of woodlands, which are facing ever

increasing expansion of land for cropping. In an effort to arrest this threat, domestication of *U. kirkiana* and various other indigenous fruit trees has been investigated by various workers (Maghembe *et al.*, 1994; Mwamba, 1988; Sambo, 1992) and their results have been variable. The long juvenile period, a characteristic of the tree when raised from seed, and the failure of planted seedlings to establish, are the two major constraints in the domestication of this popular fruit tree. The reported poor seedling establishment has been attributed to the depletion or reduction of soil microbial populations. Khalil *et al.* (1999) working on *Pinus patula* established that previously cultivated lands were deficient in ectomycorrhizal fungal colonisation. It has long been established that *U. kirkiana* relies on its mycorrhizal association for its establishment (Högberg, 1982; Högberg and Piearce, 1986). A total of sixteen ectomycorrhizal fungi species have been reported to grow in the *U. kirkiana* woodland ecosystem in Malawi (Shorter, 1989).

Whether all the fungi identified by Shorter (1989) have mycorrhizal association with *U. kirkiana* has not been determined. It is therefore the objective of this study to develop a technological package for domestication of *U. kirkiana* whereby the most favoured and prolific edible ectomycorrhizal macro-fungi in Zimbabwe, are identified, isolated and evaluated for use as nursery inoculum. The expected end benefit is that over and above the provision of fruits, the *U. kirkiana* plantations would also provide edible mushrooms.

Literature review

U. kirkiana is an indigenous fruit tree which occurs in the miombo ecozone of East and Southern Africa, reported occurring naturally in Angola, Burundi, Malawi, Mozambique, Tanzania, Zaire, Zambia and Zimbabwe (Palgrave, 1995; White, 1983). This tree species grows in association with other woody tree species such as *Albizia, Brachystegia, Burkea, Isoberlinia, Julbernardia, Parinari, Pterocarpus* and other *Uapaca* species of the miombo ecozone (Ngulube, 1996). *U. kirkiana* is one of the approximately sixty species belonging to the genus *Uapaca* (Ngulube *et al*., 1998). *Uapaca* is a Malagasy name for genus 'Voca-paca' and was used for the Madagascar species *Uapaca thouarsii*, described by Henri Baillon (1827-1895) in 1858 (Ngulube, 1996). *Uapaca kirkiana* is reported to have been named by Jean Mueller of Aargau (1864) after Sir John Kirk [1832-1922] (Radcliffe-Smith, 1988) who collected the type specimen of the species on 8 March 1862 in the Soche Hills of Southern Malawi (Kirk's n.-holotype, K). The botanical description of *U. kirkiana* is given by several

authors (Drummond, 1981; Palgrave, 1995; Radcliff-Smith, 1988). This is an evergreen or semi-deciduous tree species with spreading multiple branches that form a dense rounded crown. It is a small to medium sized tree, growing to heights of 5-6 metres producing widely sought after, delicious fruit. Fruit-set is reported to occur between January and April with the ripening taking place between September and December (Drummond, 1981; Palgrave, 1981). Maghembe and Seyani (1991) reported that the ripening period extends up to March depending on the locality and prevailing climatic conditions. Mature fruits are globose, yellow-brown, about 2-4 cm in diameter, with a tough skin enclosing 3-5 seeds. The fleshy pulp of the fruit is edible and very sweet. Popularity of this fruit has led farmers to leave the fruit trees uncut during woodland clearing prior to cultivation and there has also been extensive reports of communities expressing interest in its domestication (Grundy *et al*., 1993; Maghembe and Seyani, 1991).

Work on the domestication of *U. kirkiana* has included studies on seed treatment and germination tests (Mwabumba and Sitaubi, 1994). Furthermore, seed storage studies have also been undertaken (Ngulube and Mkandawire, 1997; Ramachela, 1996). Other investigations have been on vegetative propagation but results from these initiatives have been variable. For instance, grafting trials carried out by Akinnifesi *et al*. (2003) produced 98% graft take and *in-situ* grafting by Mkonda *et al*. (2002) gave 74% graft take. Results from vegetative propagation through stem cuttings were not encouraging (Ramachela, 1996; Sambo, 1992).

A critical area of research that has received minimal attention is the understanding of mycorrhizal association of the tree, both in natural ecosystems and in the domestication processes. It is reported that establishment and growth of *U. kirkiana* seedlings is strongly dependent on mycorrhizal fungi that enhance water and nutrient uptake (Högberg, 1982). Fungal species found to be associated with *Uapaca* species belong to the Boletaceae, Russulaceae and Amanitaceae families (Thoen and Ducousso, 1989).

Mycorrhizal colonisation of *U. kirkiana* helps improve uptake of phosphorus, particularly in acidic soils and other soils with low levels of available phosphate (Högberg and Proctor, 1989). It has further been suggested that ectomycorrhizal fungi in symbiosis with tree species enable the uptake of less available forms of nitrogen and phosphorus (Griffiths and

Caldwell, 1992; Marschner and Dell, 1994). This hypothesis then leads to the question of what effect inorganic fertilisers of nitrogen and phosphorus would have on the mycorrhizal colonisation and also on the general growth and development of *Uapaca* tree species. Another question that would be of interest is whether *U. kirkiana* can be grown under conditions of high soil fertility in the absence of mycorrhizal fungi. Work on sweetgum seedlings (*Liquidambar styraciflua L.*) which are endomycorrhizal, reveal that high fertility levels alone is not sufficient for good seedling production (Kormanik *et al.,* 1977). The indication in this work is that mycorrhizal fungi have positive effects even on well-fertilised soil. It has, however, generally been accepted that low soil fertility enhances mycorrhizae formation (Högberg and Proctor, 1989). It would therefore be justifiable to infer that mycorrhizal fungi would have an important role in the domestication process of *U. kirkiana* particularly when the tree is grown in soils with low fertility.

Bowen and Theodorou (1967) reported threefold differences in phosphate uptake from solution culture between mycorrhizae types with up to four and half times the uptake of uninfected roots. With the high macro-fungi diversity under the *Uapaca* woodland it would be of great interest to determine their competitiveness in the colonisation process and their efficiency in improving nutrient uptake.

Understanding of the mycorrhizal fungi colonisation of the *Uapaca* species is made complex by the dynamic soil biochemical processes that occur in nature. Other than influence of factors such as soil nutrients, soil pH, temperature, soil moisture and aeration, mycorrhizal colonisation processes are also influenced by the microbial flora that occur in the rhizosphere. For instance, mycorrhizal helper bacteria isolated and selected from *Pseudotsuga menziesii-Laccaria laccata* symbiotic association has been shown to promote ectomycorrhizal establishment of *Laccaria laccata* but inhibited mycorrhizal formation by other fungi such as *Hebeloma cylindrosporum* (Duponnois *et al*., 1993). Microbial flora in the mycorrhizosphere, i.e. the zone in the soil surrounding mycorrhizae roots, often differ from the rhizosphere of non-mycorrhizal roots (Bowen, 1972; Harley, 1969). Work by Tribunskaya, (1955) established that pine mycorrhizosphere contained 9-10 times larger populations of fungi than the rhizosphere of non-mycorrhizal roots. Microbial populations occurring in the rhizosphere also differ with those occurring on the surface of mycorrhizal fungi (Vosnyakovskaya, 1954, cited by Slankis, 1974). Foster and Marks (1967) established

that outer layers of *Pinus radiata* mycorrhizal mantle had a bacterial population sixteen times larger than that present in the outermost region of the mycorrhizosphere. The variation in the microbial population occurring in the different zones of the rhizosphere was reported to be caused by the differences in the root exudates that support their growth (Bowen, 1972; Rovira, 1969; Wallenda and Read, 1999). Horan and Chilvers (1990) indicated occurrence of an attraction of mycorrhizal fungi to substances diffusing from compatible host root apices, suggesting that this exudate triggers the ectomycorrhizal infection process. The mycorrhizal fungi colonisation process in the miombo ecosystem is further complicated by the high tree species' population diversity that could be inter-connected root to root through mycelia, thereby providing complex extracellular metabolites. There is, nevertheless, scant information on mycorrhizae development in *U. kirkiana* under nursery conditions. Conditions that may be detrimental to mycorrhizal fungi development could possibly include soil compaction occurring due to watering and therefore causing poor aeration. Other important factors that may affect mycorrhizal fungi development under nursery conditions could include: pH of the growing medium, excessive irrigation resulting in poor aeration, and other soil micro-organisms that compete with mycorrhizal fungi. There is therefore need to examine different mycorrhizal fungi colonisation processes under nursery conditions.

Other than the artificial nursery conditions and many other factors, it has also been established that the effect of nitrogen on mycorrhizae development of the different mycorrhizal fungi can be influenced by phosphate levels. For example, at low and high soil phosphate levels, increased nitrogen levels decreased infection whereas at moderate soil phosphate level, increased nitrogen levels increased infection (Safir and Duniway, 1991). Findings by Marx *et al*. (1977a), working on *Pinus taeda,* indicated that high levels of nitrogen and phosphorus in soil decreased sucrose content of rootlets and decreased their susceptibility to ectomycorrhizal infection by *Pisolithus tinctorius*. The report that there is evident benefit of mycorrhizal infection even in fertilised soils could imply that mycorrhizae provide benefit over and above enhanced nutrient uptake. There are reports that mycorrhizae also assist plants to survive under adverse conditions such as drought, pH extremes, and soil borne diseases (Marx, 1973; Moawad, 1980).

The ability of mycorrhizal fungi to protect tree roots against soil-borne pathogens has been investigated by several workers (Levisohn, 1954; Marx, 1970, 1973; Ross and Marx, 1972).

Levisohn (1954) reported that feeder roots of various *Pinus* spp. seedlings were resistant to infection by a *Rhizoctonia* sp. and that the pathogen readily infected non–mycorrhizal feeder roots. Powell *et al*. (1968) noted that fungicides applied to control root infections of *Carya illinoiensis* failed to reduce populations of *Pythium*, although foliar and root symptoms gradually disappeared and this recovery was attributed to colonisation by the mycorrhizal fungus G*lesoderma bovista,* which was observed growing profusely. Ross and Marx (1972) reported mycorrhizal protection of *Pinus clausa* from *Phytophthora cinnamomi*. Non-mycorrhizal seedlings exhibited massive feeder root necrosis.

U. kirkiana seedlings which have been reported to experience a die-back when planted out (Mwamba *et al*., 1992), have also been noted to have root discolouration indicating a possibility for root infection. It is therefore of major scientific interest to understand the ecology of mycorrhizal fungi infection processes. This means understanding of the factors that govern the roots of the host tree, the inoculum (mycorrhizal fungi) and the environment, which includes soil nutrient levels, pH levels and other soil microbes including soil-borne pathogens. The objectives of this study were as follows:

Overall goal

To determine factors that affect establishment and growth of *U. kirkiana* seedlings in the domestication initiative and to develop a package of recommendations for raising seedlings particularly looking at identification of appropriate nursery soil, fertilisation, and use of mycorrhizal fungi to enhance seedling growth.

Specific objectives

(i) Examine the ecology and patterns of distribution of ectomycorrhizal fungi that grow in association with *U. kirkiana.*

(ii) Analyse macro and micro nutrient dynamics in the *U. kirkiana* woodland.

(iii) Assess influence of mycorrhizal fungi on the growth of *U. kirkiana* seedlings.

(iv) Evaluate use of mycorrhizal fungi in the management of soilborne diseases.

2 ECOLOGY AND PATTERNS OF DISTRIBUTION OF MYCORRHIZAL FUNGI IN THE *U. KIRKIANA* DOMINATED MIOMBO WOODLAND ECOSYSTEM

2.1 Introduction

Mycorrhizal fungi reported to be found in association with *Uapaca kirkiana* in East and Central Africa are in the fungal families of Agaricaceae, Amanitaceae, Russulaceae and Boletaceae (Chipompha, 1985; Morris, 1987; Pergler, 1977; Piearce, 1981; Rammeloo and Walleyn, 1993; Williamson, 1975). These fungi are also recorded in southern Senegal and additional species belonging to orders Cantharellales, Sclerodermatales, Hymenagastrales, Gautieriales, and Aphyllophorales are also identified to be of ecological importance in both the dry and wet miombo ecozone of Central Africa (Thoen and Ducousso, 1989; Thoen and Ba, 1998). As *U. kirkiana* generally grows in association with various other miombo woodland tree species, particularly *Brachystegia*, *Julbernardia* and *Isoberlinia*, these fungal species possibly interconnect tree root systems through mycelia network. Mycorrhizal fungi in the miombo woodlands have been reported to have a wide host range (Janos, 1980; Simard *et al.*, 1997). *U. kirkiana* is reported to occur in similar association in several African countries stretching from Central Africa extending down to southern Africa covering Mozambique, Malawi, Zambia and Zimbabwe (Ngulube *et al.*, 1995). These countries vary in their climate, soils and forms of human activities indicating a wide range of conditions in which *U. kirkiana* is capable of growing. Records show populations growing in areas of between latitudes 2^0S and 21^0S with elevation as low as 500 m to areas with elevation as high as 2400 m. From its northern limit in the northern district of Tanzania (02^0 31' S, $32^0$43' E), the population extends southwards to Zimbabwe ($20^0$58' S, $31^0$56' E), as the most southern limit (Ngulube *et al.*, 1995). Palgrave (1995) discusses the wide range of soil types in which this tree has been noted to grow.

Distribution of *U. kirkiana* in Zimbabwe is limited to the highveld area with altitude range of 1220 m to 1800 m. The population stretches from the eastern part of the country cutting across to the western part lying between longitudes 31^0 00' E and 33^0 00' E and latitudes 17^0 00' S and 19^0 00' S. Southwards the species occurs in isolated pockets in the midlands area. These areas generally receive an annual rainfall exceeding 1000 mm, which is probably the most important factor in determining its habitat. There are, however, populations that occur

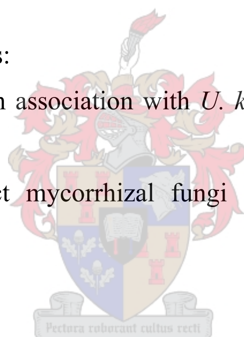
in areas that receive less rainfall. Ngulube *et al.* (1996) highlighted that occurrence of *U. kirkiana* is generally related to an unimodal rainfall regime with an annual rainfall range of 500-1400 mm received over a period of 5-7 months.

As soil and climatic factors are important for fungal growth and fruiting (Agerer *et al.*, 1998; Dahm *et al.,* 1997), mycorrhizal fungal species across the miombo ecozone belt would be expected to be highly influenced by the different conditions occurring in these areas. Fungal inocula occurring in these sites undergo an ecological and taxonomic selection process under the influence of factors occurring in respective areas. Furthermore, the tree populations and other biological factors are exposed to varying human disturbances such as fires and agricultural activities, creating distinct selection pressures. Naturally occurring veld fires do also influence the ecosystem (Khare *et al*., 1985). This study was therefore undertaken to examine the ecology and patterns of distribution of mycorrhizal fungi in Zimbabwe's varying climatic, soil and woodland conditions and to further determine the edible species, which could possibly be incorporated into *U. kirkiana* domestication initiatives. Incorporation of edible mushrooms in this programme would increase multi-productivity of the tree in an agroforestry system.

Specific objectives of the study were as follows:

(i) Identify mycorrhizal fungi that grow in association with *U. kirkiana* in the different areas of the country.

(ii) Analyse ecological factors that affect mycorrhizal fungi species distribution in different sites.



2.2 Methods and materials

2.2.1 Study Area

Ten sites were identified by use of vegetation map and field visits along the *U. kirkiana* dominated miombo woodland belt occurring in the highveld. To obtain accurate climatic data, areas close to meteorological stations were chosen and these are as follows: Henderson Meteorological Station to cover the three sites: Matiya (Mat), Tsikwa (Tsi) and Nzvera (Nzv); Rusape Meteorological Station to cover the three sites: Nemaire I (Nem I), Nemaire II (Nem II) and St Lukes (St L); Mutare Meteorological Station to cover the two sites: Chitakatira (Ch) and Rowa (Row), and Mvurwi Meteorological Station to cover the two sites:

Guyo (Guy) and Nyamuzihwa (Ny). Selection of sites was undertaken in such a way that a wide range of varying rainfall, soil and vegetation conditions were represented. Changes in the state of the vegetation in most cases were noted to have been highly influenced by human activities and this was also taken into consideration when these study sites were selected. Geographical Positioning System co-ordinates of the study sites are shown in Table 1.

Table 1.

Geographical Positioning System (G.P.S.) co-ordinates for the study sites

Site	Province	District	Altitude (m)	Latitude	Longitude
Nemaire I	Manicaland	Rusape	1769	18^0 17’ S	32^0 22’ E
Nemaire II	Manicaland	Rusape	1524	18^0 17’ S	32^0 23’ E
St. Lukes	Manicaland	Rusape	1569	18^0 32’ S	32^0 18’ E
Rowa	Manicaland	Mutare	1023	19^0 15’ S	32^0 33’ E
Chitakatira	Manicaland	Mutare	1240	19^0 10’ S	32^0 41’ E
Nzvera	Mashonaland central	Bindura	1355	17^0 33’ S	31^0 23’ E
Tsikwa	Mashonaland central	Bindura	1147	17^0 22’ S	31^0 20’ E
Matiya	Mashonaland central	Bindura	1368	17^0 32’ S	31^0 21’ E
Nyamuzihwa	Mashonaland central	Mazowe	1006	17^0 00’ S	31^0 04’ E
Guyo	Mashonaland central	Mazowe	1006	17^0 00’ S	31^0 05’ E

2.2.2 Sampling Procedure

On each site, one 20 m x 20 m plot was demarcated using permanent markings. Soil sampling at 0–10 cm depth was undertaken for all the respective ten study sites and analysed for their nutrient composition, including both macro and micro-elements. For detailed analysis of nutrient dynamics during the rainfall period, soil sampling was undertaken for the months of December, January, February, March and April for the four sites, Rowa, St Lukes, Tsikwa and Guyo. Sampling was restricted to a specific site within the plot to enable monthly assessment of nutrient changes during the season. These sites were chosen to represent the four meteorological regions, Mutare, Rusape, Henderson and Mvurwi. At the beginning of the rainy season, surveys of all the plots were undertaken to check occurrence of sporocarps as an indicator of mycorrhizal fungal status. This was done by recording the species of mycorrhizal fungi that were found in each plot at the time of collection. Due to the

difficulty and expense of identifying these fungi at the root colonisation site or as mycelium in soil, use of fruiting bodies was chosen as an indicator of presence of particular fungi species. Sampling was undertaken at an interval of two weeks beginning December when sporocarps were first observed until March when sporocarps cease to develop due to decline in rainfall levels. Collection of the samples was carried out by carefully uprooting the stipe including the base and these were put in paper khaki pockets, which were then labelled.

Care was taken to reduce possible damage to the cap and other parts, which are important for identification. Characteristics that are short-lived, such as surface texture, odour and colour, were recorded at the collection time. The other factors taken note of during collection were conditions under which the fungi were found growing, e.g. proximity to trees, shrubs and grasses. Collection of the sporocarps was carried out throughout the rainfall season and where possible the fungi were identified to genera or species level. Samples which were not easily identifiable, were taken to the laboratory and identification key and reference material (Kibby, 1979; van der Westhuizen and Eicker, 1996) were used to further examine the species. Examination was carried out in a sequential order, i. e. fruiting cap shape, texture, colour, and gill spacing, attachment to stipe, colour and presence or absence of volva. Where necessary, spore prints were made to verify confusing specimens. Spore prints were made by cutting the cap from the stipe and then placing the cap with lamella facing down on glass which was then air-tightly covered by a glass jar and left overnight. After identification of fungal species to either genera or species level, reference material was used to determine whether the respective fungi are known to be saprophytic, parasitic or have mycorrhizal association with trees or shrubs.

Rainfall and potential evapotranspiration data from the meteorological stations for the respective study sites were converted into graphs to determine periods of residual moisture. The residual moisture is the difference between precipitation and potential evapotranspiration (PET). This is the moisture not removed from the soil by drainage or evaporation and therefore remains available to the plant and mycorrhizal fungi.

2.2.3 Analysis

Soils from the ten experimental sites were analysed for all the nutrients using the Tropical Soil Biology and Fertility Programme methods (Anderson and Ingram, 1989), which are herewith briefly outlined. Soil samples were air dried and sieved through a 2 mm wire mesh. Extractable phosphorus was determined by the bicarbonate extraction method. Total nitrogen was measured colometrically by a modified indo-phenol blue method after digestion in sulphuric acid and hydrogen peroxide. Calcium, magnesium and potassium were extracted in ammonium acetate, with calcium and magnesium measured by atomic absorption spectrophotometer and potassium by emission spectro-photometry. Soil pH was determined in calcium chloride solution.

To understand the nutrient transport system from the soil across to the roots of host plants, sporocarps of selected species of the common edible mycorrhizal fungi species occurring in the study sites were analysed for their nutrient composition. Nutrient levels of the respective mycorrhizal fungi could be indicative of fungi being either a sequester or efficient nutrient transporter. P content was analysed using the method described by Koroleff (1976), N was analysed using the Kjeldahl method and the exchangeable bases were analysed by the method described by Hesse (1971).

Tree species composition in the trial plots was assessed by recording different tree species found in each plot and their frequency of occurrence. Biodiversity indices of each plot were calculated by use of Simpson's Diversity Index (Dv) formula. The biodiversity indices for the study sites were calculated and used to analyse the relationship of mycorrhizal fungi species with tree species populations. Comparative analysis of the number of mycorrhizal fungi species found in each research plot and the biodiversity index of the respective plot provides an indication of the influence of tree species composition on the fungal population or *vice versa*.

Comparison of fungal community populations among the ten sample sites was made by calculating Simpson's Similarity Coefficient using numbers of the fungal species collected over the sporocarp production period. Similarity of species between a pair of sample plots under comparison was determined by calculating Similarity Coefficient: $C = 2w/a+b$, where a = the number of species in plot 1, b = the number of species in plot 2, and w = the number of

species held in common. The result multiplied by 100 gives the percentage similarity. Fungal community comparisons with values greater than 50% are considered similar (States, 1981).

Regression analysis was conducted on the experimental data to examine the relationship between foliar nutrient levels (dependent variable), and soil and climatic factors. The analysis was based on the following model:

$$y_i = \beta_0 + \sum_{j=1}^{p-1} \beta_j X_{ij} + e_i, i = 1,2,\ldots,n$$

Where

y_i = i^{th} observation of the dependent variable (foliar nutrient level)

X_{ij} = the ith observation of the jth independent variable (soil and climatic factors)

β = parameters $\beta_1, \beta_2, \ldots, \beta_{p-1}$ (partial regression coefficients)

p = number of parameters

e_i = random error

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Analysis of variance (ANOVA) was carried out to test significance of this relationship at 5% level. To enable comparison of several pairs of data from different locations, a common Regression Coefficient had to be used (R-sq. adjusted). R-sq. adjusted value was obtained from the adjusted mean values. Adjusted sample mean values were calculated by adding the residuals/error of each sample to the population mean. Correlation analysis (Pearson's) of the data from the 1998/99 experimental season was carried out to determine the relationship between soil factors, rainfall and mycorrhizal fungi similarity coefficients.

2.3 Results

Soil nutrients for each site are shown in Table 2. Two values of N are presented, the initial-N being available N in the soil sample at time of collection and the Incu-N being nitrogen that is released when soil is incubated at 30^0C for a period of two weeks. This temperature was used to simulate conditions during the crop-growing season. The relatively high levels of N, P, K, Mg, and Ca in Guyo and Nyamuzihwa experimental sites could have been caused by a fire that occurred shortly before sampling.

Table 2.

Soil analytical data for the experimental sites

Site	Soil textural class[1]	pH ($CaCl_2$)	Initial N[2] (mg/kg)	Incu N[3] (mg/kg)	P (mg/kg)	K	Ca	Mg	Total cations (TC)
						Exchangeable cations ($cmol_c$/kg)			
Chitakatira	MG/S CL	4.7	3	37	6	0.43	2.57	0.96	4.0
Rowa	MG/S	5.6	5	22	15	0.28	1.61	0.48	2.4
Nemaire I	MG/S	4.8	7	19	4	0.28	0.85	0.32	1.5
Nemaire II	MG/S	5.4	3	60	13	0.68	4.14	1.99	6.8
St.Lukes	MG/S	4.7	7	40	11	0.58	1.54	0.73	2.8
Nzvera	MG/S	5.0	5	30	10	0.46	1.57	0.49	2.5
Tsikwa	MG/S	4.8	2	17	33	0.44	2.61	0.87	3.9
Matiya	MG/S	4.7	4	44	19	0.50	1.56	0.82	2.9
Guyo	MG/SL	5.1	50	102	47	0.74	7.32	2.40	10.5
Nyamuzih-wa	MG/SL	5.2	73	90	36	0.81	5.41	3.69	9.9
Mean		5.0	5.9	36.1	19.4	0.52	10.0	1.28	4.7

1Texture Key: C - Clay, L - Loam/loamy, MG - Medium grained, S - Sand/sandy, SL - Sandy loam, TC - Total cations
2 Initial N - available N at sampling
3 Incu N – incubated N

Soils from the study sites were slightly variable in texture ranging from medium grained sands to medium grained sandy clay loam soils. Of the ten sites, only Chitakatira had medium grained sandy clay loams, whereas Guyo and Nyamuzihwa had medium grained sandy loams, and the rest of the sites were noted to be medium grained sands. Nutrient composition of the soils from Guyo and Nyamuzihwa were remarkably different from the rest. Although fires have been noted to increase quantities of nutrients in the soil, this effect particularly on N, P, K seems to decline within a season as indicated in Figures 1 (a), (b), and (c). The soil levels of these nutrients show a decline for the assessed months.

Table 3 (a).

Regression analysis of soil factors and rainfall on similarity coefficeint indices of mycorrhizal population species between the pairs of the compared sample plots

Source	DF	SS	MS	F	P
Regression	6	1640.9	273.5	2.58	0.034*
Error	38	4032.0	106.1		
Total	44	5673.0			

* Statistically significant at 5% level.

Table 3(b).

Summary of statistics on the regression analysis of soil factors and rainfall on similarity coefficient indices of mycorrhizal population species between the pairs of the compared sample plots

Regression equation: Similarity Coeff. Index=311 - 46.0pH + 0.556N + 0.521P - 163K + 0.104Ca + 0.00115Rainfall

Predictor	Coeff.	St. dev	t-Ratio	Probability
Constant	311.2	103.9	3.00	0.005
PH	-46.02	20.12	-2.29	0.028*
Nitrogen	0.5564	0.5495	1.01	0.318
Phosphorus	0.5205	0.5320	0.98	0.334
Potassium	-162.51	79.97	-2.03	0.049*
Calcium	0.1036	0.1199	0.86	0.393
Rainfall	0.001151	0.002777	0.41	0.681

S = 10.30 R-sq = 28.9% R-sq (adj.) = 17.7%

Although elements such as N and P failed to demonstrate any statistically significant influence on the mycorrhizal fungi population, their levels in the soil showed periodic variation during the rainfall period in all four selected sites (Rowa, St. Lukes, Tsikwa, Guyo), in the 1998/99 rainfall season. This nutrient variation between rainfall months is shown in Figures 1 (a), (b), (c) and (d).

Table 3(c).

Correlation analysis of soil factors, rainfall and similarity coefficient indices (S.C.I) in the 1998/99 season*

	pH	Nitrogen	Phosphorus	Potassium	Calcium	Rainfall
Nitrogen	0.306					
Phosphorus	0.311	0.690				
Potassium	0.187	0.921	0.649			
Calcium	0.383	0.918	0.805	0.846		
Rainfall	0.142	0.482	0.760	0.512	0.470	
S.C.I	-0.328	-0.325	-0.165	-0.388	-0.268	-0.124

* All correlation coefficient values were not significant

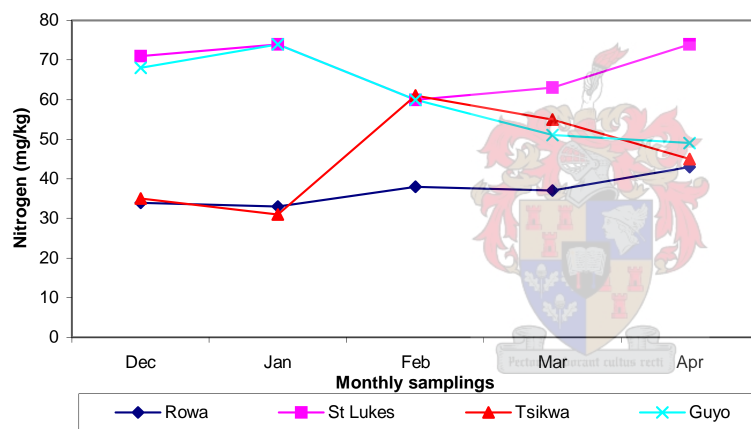

Figure 1 (a) Monthly assessment of soil Nitrogen levels

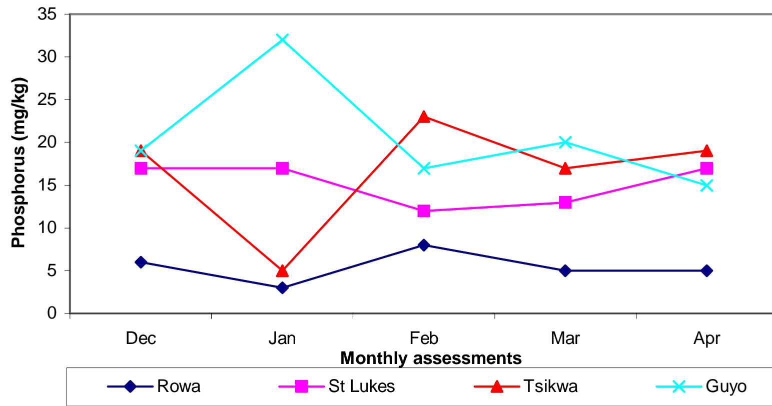

Figure 1 (b) Monthly assessment of available soil phosphorus levels

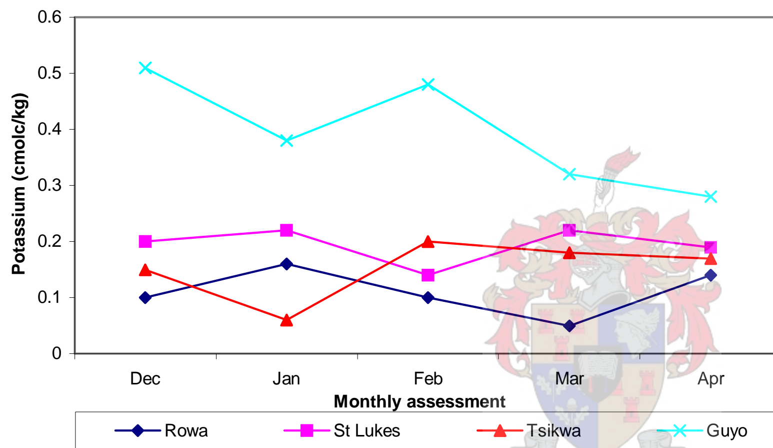

Figure 1 (c) Monthly assessment of soil potassium levels

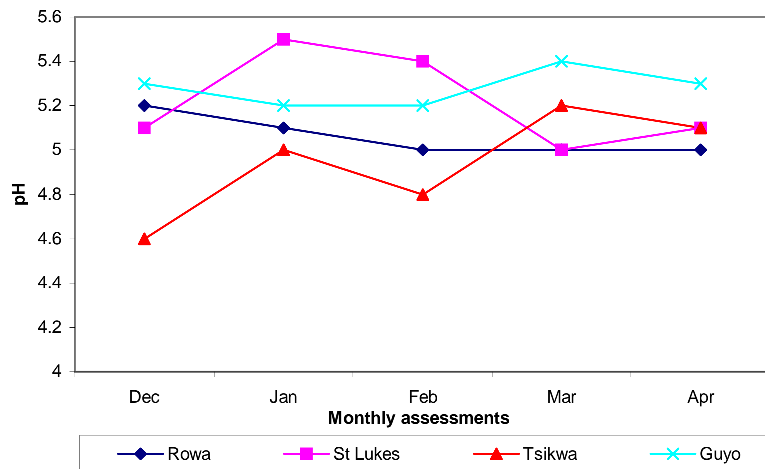

Figure 1 (d) Monthly assessment of soil pH levels

Sites that did not experience a fire did not show nutrient decline of a similar magnitude as observed with Guyo. Further analysis of soil nutrient dynamics, assessed by analysing sequestration of nutrients by mycorrhizal fungi, revealed that rate of nutrient uptake differ for different fungi. Table 4 shows nutrient composition of different mycorrhizal fungi collected from St Lucia trial site.

Table: 4

Nutrient composition of the common mycorrhizal fungi

Mycorrhizal fungi	Macro-elements (%)					Micro-elements (mg/kg)				
	N	P	K	Ca	Mg	Fe	Mn	Zn	B	Cu
C. cibarius	3.22	0.62	3.60	0.23	0.13	555	50	89	12	97
L. deliciosus	4.92	0.72	2.24	0.23	0.11	1238	62	134	12	102
L. edulis	3.18	0.62	3.61	0.20	0.13	668	37	90	12	98
A. zambiana	4.44	0.84	4.65	0.25	0.13	184	20	100	9	24
Cantharellus sp.	3.22	0.44	3.43	0.23	0.10	483	20	64	12	50

To examine the relationship between mycorrhizal fungi population and tree species diversity of the study sites, tree species diversity indices were calculated for all the study sites (Table 5).

Table 5.

Comparison of tree species diversity, dominance and fungal spp. composition

Site name	Mat	Tsik	Nzve	Guyo	Nyam	Chit	Rowa	Nem I	Nem II	St. Lks
Tree biodiversity indices*	0.52	0.59	0.63	0.64	0.33	0.64	0.59	0.56	0.49	0.35
Fungal spp. (No.)	12.00	13.00	7.00	10.00	2.00	23.00	19.00	18.00	10.00	17.00
Dominance index	0.48	0.41	0.37	0.36	0.67	0.36	0.41	0.44	0.51	0.65

*Calculated using Simpson's Diversity Index (D_v) formula: (D_v = 1- D; where D = $\sum(n_i/N)$ where n_i is the number of individuals (i) in the sample and N is total individuals of all the species in the sample

Site name key: Mat-Matiya, Tsik. -Tsikwa, Nzve-Nzvera, Guyo-Guyo, Nyam-Nyamuzihwa, Chit-Chitakatira, Rowa-Rowa, Nem I-Nemaire I, Nem II- Nemaire II, St. Lks-St. Lukes

Over a period of four months (December, January, February, March), which is the rainfall period in Zimbabwe, fungal population compositions collected from each plot were found to be distinct with a few species found commonly across the sample sites (see Table 6). Rainfall data for the study sites are shown in Appendix IV.

By using the Simpson's Similarity Coefficient test, most of the ten experimental plots exhibited high variability in their fungal diversity with only 11 of 45 comparison combinations scoring similarity indices above 50% (Table 6). Mycorrhizal fungi species populations for the study sites identified over the three year study period are shown in Appendices I, II and III.

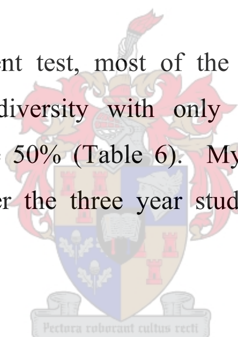

Table 6.

Comparison of fungal community similarity within and between ten sample sites based on fortnightly sampled species in the 1998/99 rainfall season[1]

Number of fungi species	24	19	18	10	17	11	13	7	7	10
Sites	Ch	Row	Nem I	Nem II	St Lks	Mat	Tsi	Nzv	Ny	Guy
Ch		10	12	4	8	10	4	4	0	6
Row	46.5		6	4	6	8	6	4	0	4
Nem I	57.1	32.4		6	8	6	6	4	0	8
Nem II	23.5	27.6	42.9		8	4	4	0	0	4
St Lks	39.0	33.3	45.7	59.3		6	6	0	0	1
Mat	57.0	51.6	41.4	38.1	42.9		8	7	0	8
Tsi	21.6	37.5	38.7	34.8	40.0	66.0		6	0	8
Nzv	25.8	30.8	32.0	00.0	00.0	70.0	60.0		0	6
Ny	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0
Guy	35.3	27.6	57.1	40.0	7.4	76.2	69.6	70.6	0.0	

[1]Number of fungi species per site is shown in the row above the column names. Fungal species found in common between sites are shown above the diagonal. Similarity Indices values (shown below the diagonal) are calculated as follows: Simpson's Similarity Coefficient (c) = 2w x 100/(a+b), Where a = No. of species in stand 1, b = No. of species in stand 2, w = No. of species held in common

These mycorrhizal fungi produced their sporocarps during the humid periods shown in Figures 2 (a), (b), (c) and (d). This is the period when there is adequate residual moisture[1].

1 Residual moisture is the differences between precipitation (Prep) and potential evapotranspiration (PET) which is the moisture not removed from the soil by drainage or evaporation. This is depicted by the area below the PET graph lines in Figures 2 (a), (b), (c) and (d).

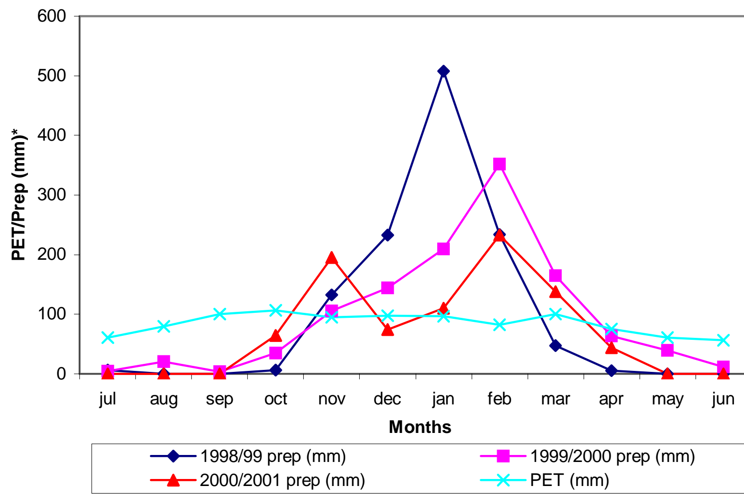

Fig. 2 (a) Graphic presentation of soil residual* moisture from Rusape meteorological station representing three experimental sites Nemaire I, Nemaire II, St Lukes

* See footnote 1 for explanation of PET and Prep

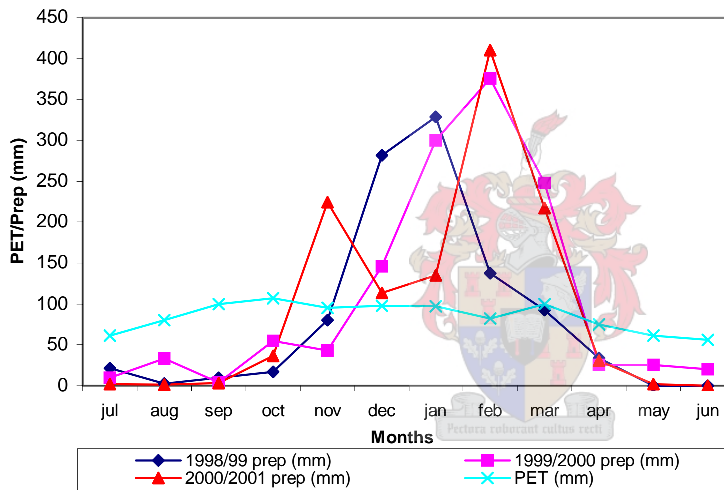

Fig. 2 (b). Graphic presentation of soil residual moisture* from Mutare meteorological station representing two experimental sites, Rowa and Chitakatira

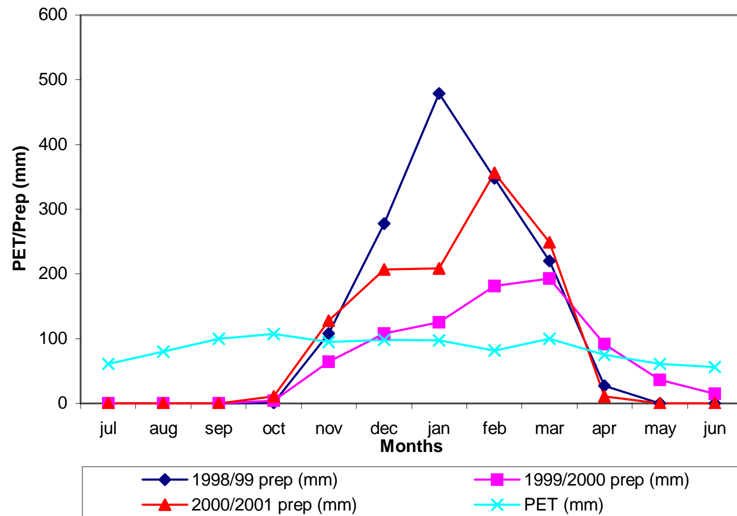

Fig. 2 (c) Graphic presentation of soil residual moisture* from Mvurwi meteorological station representing two experimental sites, Nyamuzihwa and Guyo

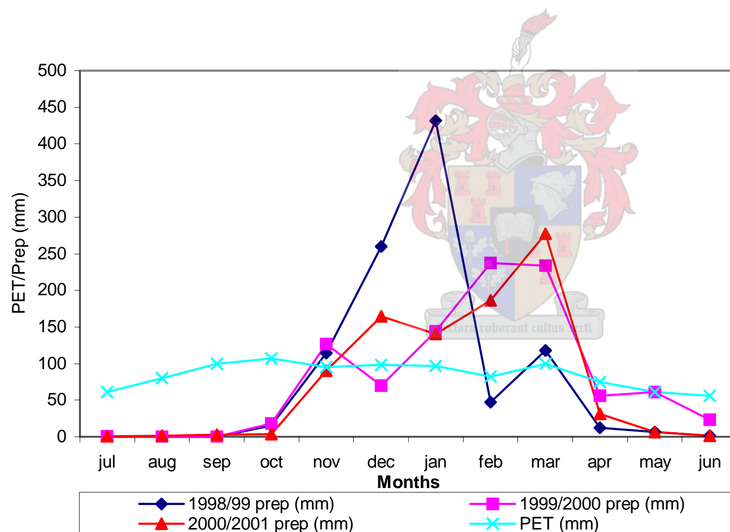

Fig. 2 (d) Graphic presentation of soil residual moisture* from Henderson meteorological station representing three experimental sites Matiya, Tsikwa and Nzvera

2.4 Discussion

Mycorrhizal fungi species in the ten study sites were found to be distinct populations with a few species found commonly across the sample sites (Table 6). Factors influencing fungal population composition at the different sites could probably include rainfall, air temperature, host plant density and various soil factors, such as temperature, nutrient levels, pH, soil structure and carbon content (Laiho, 1970; Marx *et al*., 1970; Singh, 2000; Slankis, 1974). Table 3 (a) shows the regression analysis of soil factors and rainfall on similarity coefficeint indices of mycorrhizal population species between the pairs of the compared sample plots. Furthermore, regression equation for the soil factors is shown in Table 3 (b). According to the equation, seventy six percent of the variation in the Similarity Coefficient Index was explained by pH, levels of nitrogen, phosphorus, potassium, calcium and amount of rainfall. However, only pH and potassium had a significant effect on the Index ($P < 0.05$). Table 3 (c) shows that the two variables are not serially correlated, thus strengthening their independent effects on the Index.

Importance of pH on fungal growth is extensively reported (Dahm *et al*., 1997; Hung and Trappe, 1983; Slankis, 1974). Harley (1969) reports of optimum mycorrhizal colonisation at a pH range of 4 to 6 ($CaCl_2$), which all ten sites in this study fall within. It should, however, be considered that species pH preferences vary even within this range although there are species that occur outside this range. *Boletus* species prefer a pH of 3 ($CaCl_2$) and some *Paxillus involutus* strains grow optimally in pH of 3.1 or even 6.4 depending on other growth factors. *Boletus granutus* and *Boletus variegatus* isolates produced maximum mycelial growth at pH 5-6 in a medium composed of inorganic salts, glucose, asparagine, peptone and thiamine. However, in a PDA agar with the same salts added, maximum growth was attained at pH 3 (Slankis, 1974). Variation in optimum pH among different strains of the same species suggests that mycorrhizal fungi may adapt to soil pH. The biochemical pH reactions have been analysed by Theodorou and Bowen (1969), who postulated that in acid soils with abundant NO_3, fungal colonisation is inhibited by NO_3 at the hyphal plant cell penetration. Further research is, however, needed to investigate the role played by nitrates possibly by examining specific effects of nitrogen. It is known that nitrogen is an important component of the amino group (NH_2) that make up amino acids and these have been proven to be inhibitory to fungi auxin production. Auxins have been known to hydrolyse starch to sugars, which have

long been considered important for successful mycorrhizal fungal colonisation (Lister *et al.*, 1968; Slankis, 1965). Other factors, which have been reported to influence production of sugars in tree root systems, are nitrogen and phosphates (Lister *et al.*, 1968).

Although nitrogen and phosphorus did not show significant influence on the mycorrhizal fungi development in this study, previous investigations by various workers (Agerer *et al.*, 1998; Dahm *et al.,* 1997; Danielson and Visser, 1989; Marx *et al.*, 1977b) did establish their importance. Work on determination of optimum nitrogen and phosphate levels by various workers is discussed by Slankis (1974) where nitrogen and phosphate combination levels resulted in a corresponding increase in soluble sugars, sucrose, glucose, fructose and raffinose. Although varying sugar levels were produced under different treatment combinations, mycorrhizal formation was not variable between treatments, possibly indicating that factors other than sugars are necessary for successful colonisation. Role of sugars in mycorrhizal formation has long been studied by several workers, but their results were variable (Handley and Sanders, 1962; Lister *et al.*, 1968; Slankis, 1965). Slankis (1969) working on pine tree roots proved that under the highest nutrient regimes, mycorrhizal development was inhibited despite high sugar levels in the roots. This finding was in agreement with findings by Lister *et al.* (1968).

Other theories question the hypothesis that nitrogen and phosphorus influence root sugar levels. Slankis (1974) also discusses findings by various workers who concluded that surplus sugars in the roots did not enhance mycorrhizal formation but that accumulation of these sugars was triggered by auxins produced by mycorrhizal fungi. This theory was based on experiments by Alexander (1938) and Bausor (1942), where artificially added auxins enhanced hydrolysis of starch into sugars. This process was, however, only possible under certain acceptable lighting conditions. Work by Slankis (1969) indicated that mycorrhizae colonisation processes are not initiated in pine roots when synthetic auxins are added under low light intensities even when soluble sugars are added to their nutrient solution. It was therefore concluded that in addition to other factors, light intensity is also important for fungal auxin production. Other compounds that are known to influence auxin production by fungi are amino acids (Melin, 1953). Slankis (1974) further discusses the importance of continuous production and transfer of auxins at optimum levels into the tree roots for successful mycorrhizal development. Conclusion can therefore be drawn that factors that

disturb this production and transport processes of auxins at optimal levels would inhibit mycorrhizal formation. With auxins being the primary factor predisposing plant cell wall to fungal hyphal penetration, sugars can therefore be considered important mostly as carbon source required for fungal growth and not for the fungal colonisation processes. This could possibly explain the regression analysis results [Table 3 (b)] where nitrogen and phosphorus were noted to have insignificant influence on fungal species diversity as identified by fruiting bodies. It should, however be considered that mycorrhizal fungi which have optimum development conditions different from those in the study sites could actually be growing actively in the soil but fail to produce sporocarps. Jonsson *et al.* (1999), using ITS-Restriction Fragment Length Polymorphism (RFLP), demonstrated that species composition of the below-ground community of ectomycorrhizal fungi did not reflect the population of sporocarps produced, indicating that there are species, which although present in the soil, fail to fruit under certain conditions.

However, K as the other important major element in plant growth, has been noted to have significant influence on the mycorrhizal species diversity ($P < 0.05$). Potassium is known to be an important element required for plant growth but not much is known on how it affects mycorrhizal fungal species in their colonisation processes. It is, nevertheless, reasonable to assume its importance because it plays an important part in the plant phloem transport system. Canny (1984) discusses the hypothesis that potassium ions are involved in the pumping, loading and translocating of sucrose in the phloem transport system. Of interest is the observation that all major growth substances such as auxins have been found in phloem sap and that when IAA is artificially injected into the system, it moves within the phloem at the same speed as the sucrose (Canny, 1984). The detail on how K loads growth substances is an area still requiring further investigation. It is, however, reported by Hill and Hill (1973) and Jeschke (1970) that there are enzymes (ATPase) that release energy from ATP, and these are activated by monovalent cations such as K^+ and Na^+ .Energy released from ATP by ATPases can therefore be assumed to be important for translocating auxins to the roots. As previously mentioned, auxins increase plant cell permeability and therefore resulting in the root cells being more susceptible to fungal hyphae penetration. Distribution efficiency of auxins to the roots could therefore be considered an important factor in mycorrhizal colonisation. Conclusion can therefore be drawn that K ions are a key factor in auxin translocation, which influences mycorrhizal formation.

Other factors that were not included in this study that could possibly influence fungal development are soil structure and carbon content (Simard *et al.,* 1997; Skinner and Bowen, 1973). Soils that are compact have poor aeration and therefore affect oxidation processes that are important for development of fungal species. Soil carbon content is important because fungi require carbon for their nutrition and this carbon can either be supplied by the soil or by the tree species with which the fungi are mycorrhizally associated with (Simard *et al.*, 1997). In a situation that the fungus interconnects with more than one tree species implies transfer of solutes from one tree to another irrespective of the genera they belong to. Source of soil carbon is most likely predominantly from microbial activities including saprophytic fungi. The possibility of saprophytic fungi supplying carbon to mycorrhizal fungi highlights an area of research which requires further investigation in an effort of understanding miombo myco-ecology.

Another factor of interest is the influence of host plant diversity on the mycorrhizal fungi composition in a given area. Biodiversity indices of study sites exhibit a weak positive correlation coefficient ($r^2 = 0.3$) with the respective total number of fungal species of each site in a season (Table 5).

The weak correlation between biodiversity indices of the study sites and the respective total number of fungal species of each site in a season exhibited in this study (Table 5), reflected low influence of mycorrhizal fungal communities on plant species population composition. This finding is however, not conclusive considering that there were mycorrhizal fungi that were present in the ecosystem but had not produced sporocarps during the time of the survey. Influence of mycorrhizal fungi on the plant species population composition has been demonstrated in arbuscular mycorrhizal fungal species (Marcel *et al.*, 1998). It is possible therefore that miombo ectomycorrhizal fungi populations could also play a similar ecological function. As these fungi have been reported to have a wide range of host trees (Munyanziza, 1994; Redhead, 1968), it could be considered that common association of particular tree species growing in specific sites could strongly be linked by the endemic mycorrhizal fungi in the area. Interconnection of trees of different species has been reported to be through common mycelia. Transfer of carbon, nitrogen and phosphorus through the interconnecting mycelia has been measured frequently in laboratory experiments. These studies, using

isotope tracers, show that the strength of one way transfer of solutes can be influenced by shading of receiver plants and fertilising donor plants with phosphorus. Solutes were noted to flow from the fertilised donor plants to the shaded plants that have lesser photosynthetic products, indicating that movement of solutes may be governed by source-sink relationships (Simard *et al*., 1997). In nature, this relationship could be extrapolated to the association of the newly germinated seedlings that would be growing as under-storey, thereby receiving nutrients from older trees through the interconnecting mycorrhizal fungi mycelia. This possibly explains the successful regeneration of *U. kirkiana* in the wild compared to the poor establishment of seedlings in former crop fields, which often have low mycorrhizal mycelia networks (Mwamba *et al.*, 1992).

The complexities of myco-ecology are difficult to understand, possibly because of the many variables such as different levels of pH, phosphorus, potassium, nitrogen, iron and calcium that occur in nature. Furthermore, climatic factors, changes in host physiological conditions and the variation in mycorrhizal fungal efficiency between species make the association more complex. This could probably explain the difficulties of enhancing sporocarp production under nursery conditions (Fries, 1978). The manipulated soil conditions may fail to meet standards of natural soil conditions because of various reasons, some of which could be that under natural conditions certain types of fungi co-exist under particular conditions performing ecological functions collectively and interact with the tree species occurring in a given locality. The other reason could be the low-level sugars produced by seedlings as compared to mature trees. As production of these sugars by plants is through photosynthesis processes, it can be assumed that environmental factors, such as temperature and light, that influence photosynthesis also have indirect effects on mycorrhizal fungi.

Pectora roborant cultus recti

Influence of temperature on fungal growth has been studied extensively (Cline *et al*., 1987; Hacskaylo *et al.*, 1965; Marx *et al.,* 1970; Theodorou and Bowen, 1971; Theron, 1991). However, in this study it has not been considered independently but as an interactive factor influencing evapotranspiration[1], which when looked at in conjunction with rainfall can give

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[1] Calculated using Penman's formula: $ET_R = c\ \{[\Delta/(\Delta+\gamma)](R_n-G) + [\gamma/(\Delta+\gamma]f(U)(e_s-e_a)\}$; where c = Correction factor taking into account day-night changes ,Δ= slope of the vapour pressure-temperature curve(mbar^{o}C^{-1}), γ = pschrometeorologicalric constant (m bar^{o}C^{-1}), R_n = net radiation in equivalent evaporation units (mm day $^{-1}$), G = soil heat flux density (mm day^{-1}), f(U) = wind function (mm mbar^{-1} day^{-1}), e_s = saturation vapour pressure of air at a reference height (mbar), e_a = actual vapour pressure of air at a reference height (mbar)

an indication of soil residual moisture or humid period, which is important for fungal growth. The humid periods shown in Figures 2 (a), (b), (c) and (d) are indicative of moisture regimes that resulted in the production of fruiting bodies by particular fungi species in the study sites over a period of three years (Appendices I, II and III).

However, residual soil moisture does not independently influence fungal growth but other soil factors are also important (Slankis, 1974). The complexity of interaction of all these factors sets up a high ecological and taxonomic selective pressure of fungal species in a given area. This pressure will obviously vary even within a season taking cognisance of periodic temperature and rainfall variations. For the four months period of sporocarp collection in the three study periods, distinct populations were noted in each collection, with a few species held in common between sites and times of collection (Appendices I, II, III). Variation between fungal populations between collections possibly exhibits an ecological inter-dependence of mycorrhizal fungal species. As fungi growth rates are dependent on environmental factors, any changes that occur would be expected to influence mycorrhizal efficiency of different fungal species.

Species differences in mycorrhizal efficiency in nutrient uptake has been reported by Vetter *et al.* (1997), whereby *Russula* and *Amanita* species were noted to vary in their zinc content, supposedly reflecting differences in nutrient transporting efficiency. Results of this study could either reveal that certain fungi possibly accumulate more zinc in their sporocarps than others do and therefore could be considered poor zinc transporters or that the fungal species with higher zinc content are the more efficient species. Similarly, the rate of uptake of an element such as iron, which is known to be important in plant chlorophyll formation, may also vary between different mycorrhizal fungi species. Terry and Low (1982) reported that whenever the supply of iron is deficient, there is a close positive correlation between the total iron content of the leaves and the chlorophyll content. Mycorrhizal fungi function of transporting nutrients from the soil across to the roots of host plants is a very complex system involving soil, fungi and host factors. As soils in Zimbabwe are generally known to be inherently low in available phosphate (Nyamapfende, 1991), it would be of interest to determine phosphate transport efficiency of different fungal species. Table 4 shows nutrient content of selected commonly found mycorrhizal fungi sporocarps. This does not, nevertheless, establish whether a fungus with high nutrient content is a nutrient sequester or

is efficient in nutrient translocation. Further studies should be undertaken to establish P uptake efficiency of selected mycorrhizal fungi.

Findings of such investigation would be useful in developing a nursery inoculum with high P uptake efficiency. In the preparation of such inoculum it should be considered that these fungi in nature do not exist in isolation and that there are indications of strong collaborative functioning. Fungal species that have been identified to have complimentary and sequential functions should be used to prepare inoculum.

As functions of mycorrhizal fungi are also influenced by physiological conditions of the host tree (Mishustin, 1951; Shemakhanova, 1962), it could be deduced that photosynthetic changes in a given day would have a direct effect on the nutrient demand and therefore influence functions of the fungi. *In vivo* studies on *Gonyaulax polyedra* showed that rate of photosynthesis changed due to intensity of chlorophyll '*a'* fluorescence from 4.1$\mu mol\ O_2\ h^{-1}\ 10^{6}\ cell^{-1}$ at 00.10 hours to 6.7$\mu mol\ O_2\ h^{-1}\ 10^{6}\ cell^{-1}$ at 21.25 hours (Sweeney *et al*., 1979). As the photosynthesis process expends energy that is released when the ATP bond is broken (ADP+Pi), there would be a higher phosphate demand by the plant when photosynthesis increases. Variation in the phosphate demand occurring as a result of changes in the rate of photosynthesis, is supposedly met by functioning of the fungal species that efficiently enhance P uptake in both low and high photosynthesis rates.

Changes in soil temperature in a given day can influence mycorrhizal physiological reactions as different fungi require different optimum temperatures for their growth (Theron, 1991). Understanding of the complexities of the impact of physiological changes in one tree species on the mycorrhizal reactions could be further complicated by physiological demand by other tree species which are mycorrhizally linked with the tree under study. Variation in the efficiency of different fungi in the transportation of different elements would therefore imply collaborative functions of different fungi in a given population. Different fungi possibly complement each other in nutrient transportation, with a particular fungus being a specialist in uptake and transporting a specific element. In the domestication of *U. kirkiana* whereby a combination of selected edible mushrooms is used as an inoculum, it is preferable that efficiency of each fungal species be determined so that only fungi that have complementary functions are used in the preparation of the inoculum.

This study therefore established that climate and biophysical factors that occur in the different study sites created ecological selection pressure that resulted in certain mycorrhizal fungi populations forming fruiting bodies. Mycorrhizal fungi population identified by their fruit bodies only occurred during the months of December, January, February, and March. Mycorrhizal fungi that were commonly found across the study sites would therefore be considered highly adaptable species managing to grow and fruit under varying climatic and soil conditions. These fungal species include species such as *A. zambiana, A. phalloides, C. cibarius, and L. deliciosus.* The interactive analysis between precipitation and potential evapotranspiration in respect to the sporocarps formation, also indicated that humid periods are the most conducive periods for production of fruiting bodies by mycorrhizal fungi. The study did not, nevertheless, reveal the complex biochemical reactions that occur under these conditions but managed to identify the importance of soil pH and K in the production of sporocarps by the mycorrhizal fungi. The role of other nutrients such as N and P, although established to be important in previous studies, was not conclusive in this study. These nutrients could be important in various physiological processes, for instance, N, assessed in the form of NH_2 is known to inhibit mycorrhizal fungi colonisation, and P is an important component of ATP compounds which provide energy for various host plant-fungal physiological association. Conclusion could be drawn that N and P could be important for mycorrhizal fungi colonisation processes but have less significant influence on the production processes of fruit bodies as evidenced by their lack of influence in this study. Table 7 (a) and (b) summarises the processes involved in the mycorrhizal colonisation and their possible effects.

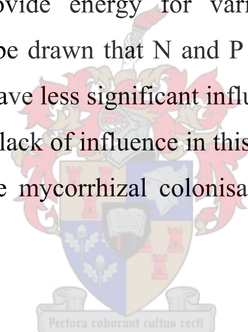


Table 7 (a).

Summary of effects of soil factors on the mycorrhizal fungi colonisation processes

Factor	Processes	Possible effects
pH	(i) Certain mycorrhizal fungi adapt to different soil pH levels (ii) In acidic soils high levels of NO_3 result in inhibition of mycorrhizal fungal colonisation processes (iii) Directly or indirectly pH influences the development of reproductive structures/fruit bodies of mycorrhizal fungi	Certain species of mycorrhizal fungi commonly occur across different ecological sites Use of nitrate fertilisers in acidic soil conditions affect mycorrhizal fungi colonisation processes and therefore affect production of fruit bodies Production of fruit bodies
K	K activates ATPase enzymes that release energy from ATP compound and this energy is assumed to be important for the translocation of auxins to the roots. Auxins increase plant cell permeability and therefore resulting in the root cells being more susceptible to fungal hyphae penetration	Distribution efficiency of auxins is dependent on the K levels and this influences mycorrhizal formation
N	Role of N has not been established in this study	Amino group (NH_2) has been reported to be inhibitory to fungi auxin production. Auxins hydrolyse starch to sugars which enhance mycorrhizal fungi colonisation
P	Role of P has not been established in this study	P is an important component of ATP compounds which is a source of energy for translocation of auxins support other host plant physiological processes

Pectora roborant cultus recti

Table 7 (b).

Summary of possible climatic and biological effects on the mycorrhizal fungi colonisation processes

Factor	Processes involved	Possible effects
Climatic (temperature and rainfall)	Temperature and rainfall were assessed on their role in determining soil residual moisture in a given location	The humid period, i.e. the period with high residual moisture, enhanced production of mycorrhizal fungi fruit bodies
Host plant population diversity	Mycorrhizal fungi interlink different tree species in a given site through root to root inter-mycelial connection	Activities that disturb an ecosystem also disturb production of mycorrhizal fungal fruit bodies

This study revealed that under ideal climatic conditions, occurrence and distribution of mycorrhizal fungi can be influenced by ecosystem management and soil amendments such as manipulating pH levels and varying major soil nutrients. It was also established that fires do alter soil nutrient levels and therefore exerting influence on mycorrhizal fungi population. Furthermore, this work also established that myco-ecological selection pressure that was identified to have occurred as a result of the climate and biophysical factors, was indicative of the importance of identifying the right mycorrhizal fungi inocula for a given location. This is particularly important when establishing *U. kirkiana* plantations.

3 MACRO AND MICRO-NUTRIENT DYNAMICS IN THE MIOMBO WOODLAND AND THEIR INFLUENCE ON SEEDLING GROWTH OF *U. KIRKIANA*

3.1 Introduction

The relationship of mycorrhizal fungi species and the soil nutrient status that was established in Chapter 2 highlighted the importance of understanding nutrient dynamics of woodland ecosystems. Although K and pH were the only investigated soil factors that had significant influence on the fungal species diversity, influence of biophysical and environmental factors that could possibly affect biochemical reactions, were also discussed. The biophysical factors include host plant physiological status and various soil factors, e.g. nutrient levels, pH, organic matter content and soil structure. Palgrave (1995) and Ngulube *et al*. (1995) discuss a wide range of sites suitable for growth of *U. kirkiana.* In general, this tree species grows in mixed communities of the *Brachystegia-Julbernardia* woodland vegetation type either as a dominant or co-dominant species, and is usually found in association with other species such as *Isoberlinia angolensis, Syzygium guineense,* etc., forming dense groves and favouring freely draining sandy soils that occur in middle slopes of escarpments. A soil profile of shallow gravelly soils overlying weathered rock is typical in these situations. It is however, also evident that when such sites are covered by hydromorphic soils that experience seasonal flooding, tree growth is impeded. Other factors that characterize *U. kirkiana* growing areas are low cation exchange capacity, low organic matter content and low levels of macro-nutrients, N, P and K (Mwamba, 1983; Nyamapfende, 1991; Watson, 1964). Soil reaction is acidic with pH ($CaCl_2$) between 4 and 6, but the pH range 5-5.5 being the most ideal (Mwamba, 1983).

Pectora roborant cultus recti

Establishment and growth of *U. kirkiana* has been of interest to several researchers (Högberg, 1986; Mwamba *et al.*, 1992; Mwamba, 1994; Ngulube *et al*., 1995; Piearce, 1988). Although mycorrhizal fungi have been known to be an important biological factor for successful seedling establishment, there is need to understand the physical and biological processes of the woodland ecosystem where successful regeneration through seedlings occurs naturally. One of the important roles of mycorrhizal fungi is that of improving plant nutrient uptake, particularly P and N (Cumming, 1996; Jeutschke *et al*., 2000; Kottke *et al.,* 1995; Wallander, 1995). There are extensive discussions on how ecological factors influence mycorrhizal

activities but these have often not been conclusive. For instance, work by Slankis (1974) indicated that neither relatively high concentrations of N and P, nor severe deficiencies of these elements necessarily arrest mycorrhizal colonisation. He further highlights importance of soil conditions such as pH, organic matter content and various other factors in the development of mycorrhizae. Work by Newbery *et al*. (1997), studying P dynamics in a lowland African rain forest, also established that there was no difference in P foliar content between low mycorrhizal (LEM) and high mycorrhizal (HEM) plots, suggesting that any effect of ectomycorrhizal fungi was not reflected in the concentrations of the element in the plant tissue, despite the fact that the soil P status was different between LEM and HEM forests. This observation implies that physiology of trees determine their nutrient uptake and not the level of nutrients in the soil. With tree physiological processes being influenced by climatic factors such as rainfall and temperature, and other soil growth factors, it would be of interest to analyse the relationship of the foliar and soil nutrient status of *U. kirkiana* woodlands under varying environmental conditions in relation to natural regeneration.

Regeneration in a natural woodland ecosystem is greatly influenced by soil nutrient status and various processes that occur as a result of activities of symbiotic rhizospheric micro-organisms that enhance nutrient uptake (Högberg, 1986). These biochemical processes are dependent on the soil and environmental factors such as moisture content, oxygen content, soil temperature and plant physiological status. To successfully domesticate *U. kirkiana* at sites outside its natural habitat, it is necessary to establish and compare soil nutrient levels with foliar nutrients for the wet and dry seasons. Nutrient uptake is a dynamic process that is highly influenced by soil and climatic factors. When climatic factors are conducive, semi-deciduous trees experience leaf flush and expansion with the resultant increase in nutrient demand. Uptake of nutrients by trees is however also dependent on critical factors such as amount of nutrients at the root surface, root length and distribution. Furthermore, there are other biophysical factors that can modify uptake rate of these nutrients, e.g. root hairs, mycorrhizal fungi, pH, plant exudates and soil bacteria (Tinker, 1989). It is however, generally difficult to determine which of these factors are the most important in a particular soil type. Factors such as soil depth, texture, structure, organic matter content, nutrient composition and biochemical processes have also been found to determine root distribution and therefore indirectly influence nutrient and moisture uptake. (Atkinson *et al*., 1983; Nye and Tinker, 1977).

Low levels of P and N are often limiting plant production in the tropics. P is particularly low in acidic soils because it is bound by Fe and Al into insoluble compounds (Sanchez, 1976 quoted by Högberg, 1982), whereas N is often lost through volatization and leaching. P is an important element for photosynthesis, respiration, energetic metabolism and carbohydrate metabolism (Mitsuru *et al*., 1996), whereas N is strongly linked to plant growth through its influence on the carbon-nitrogen balance, which is important for the development of leaves and maintenance of high photosynthetic rate.

This experiment was therefore carried out to examine the nutrient dynamics of varying ecological conditions of *U. kirkiana* woodlands growing in different geographical areas of the country. In particular, this work sought to investigate the relationship between the soil nutrient status with the foliar nutrient content in relation to the associated mycorrhizal fungi species. If mycorrhizal fungi play an important role in the nutrient dynamics, it would be possible to analyse and compare nutrient cycles between periods of active mycorrhizal growth and periods of low mycorrhizal activity. On the basis of the hypothesis that natural regeneration of indigenous tree species is generally successful because of the ecological processes that occur in the ecosystems, this study was undertaken in order to establish the soil-plant nutrient dynamics in a natural *U. kirkiana* woodland ecosystem. These findings would be useful in developing similar conditions in the domestication initiatives of this important indigenous fruit tree species.

The specific objectives of the study were as follows:

(i) Analyse soil-plant nutrient dynamics in different *U. kirkiana* woodland stands.

(ii) Assess survival and growth of seedlings on different soil types.

(iii) Evaluate effect of N and P mineral fertilisers on seedling biomass.

3.2 Methods and materials

3.2.1 Analysis of soil-plant nutrient dynamics in a natural ecosystem

3.2.1.1 Soil and foliar analyses

The ten sites that were used in the previous study (Chapter 2) on the survey of mycorrhizal fungi sporocarps were chosen for the study. The sites lie between longitude 31^0 00’ and 33^0 00’ E and between latitudes 17^0 00’ and 19^0 00’ S. This is the highland miombo woodland

area, in which pockets of *U. kirkiana* dominate or occur in mosaic association with other miombo tree species in Zimbabwe. Soils and leaves of *U. kirkiana* tree species were sampled during the calendar year of 1999 from the ten 20 m x 20 m plots marked in each respective study site. Soil analysis results from Chapter 2 were used in this study. Foliar sampling was done twice, once during February (the rainy period) and the other during June (the dry winter period) when there was low mycorrhizal activity.

Foliar samples were taken by bulking leaves from mid-point of tree canopies of respective sample trees, for all ten plots. Sampling from mid-point of the tree canopy was chosen because it would represent a balance between the young leaves at the top of the canopy and the older leaves at the bottom of the tree canopy. Högberg (1986) used this sampling technique in nutrient cycle studies in savanna woodlands.

To analyse nutrient content of the *U. kirkiana* leaves, the foliar analytical methods as described by Schönau (1981), were used. To determine levels of K and trace elements, leaves were washed, oven-dried at 65^0C and the samples were ground using an electric grinder and filtered with a 0.15 mm sieve. Several samples, each weighing 1 g, were put into porcelain crucibles and then placed in a muffle furnace for 16 hours at 450^0C. After washing, the samples were cooled at room temperature. Six millilitres of 25% HCL and 5 drops of concentrated nitric acid were added to each sample. Drying of the samples was carried out under ultra-violet light. The reason for adding nitric acid and 25% HCL was to oxidize all elements in the samples. After drying, additional 6 ml of 25% HCL was added to each sample and the solution was then filtered using Whatman No. 2 filter papers. The solution was collected in 50 ml volumetric flasks. The solution was analysed by spectrophotometry whereby the elements in the leaf extracts were identified by the position of emission and absorption lines and bands in the spectrum.

Phosphorus content was determined by placing of 1g ground foliar sample into a silica basin which was then saturated with Calcium acetate solution. The sample was placed in a cold furnace and ignited overnight (16 hours) at a temperature of 600^0C. The sample was allowed to cool and 10 ml of 2.4 Normal perchloric acid was added and covered with a watch glass and placed on a water bath for 30 minutes. The sample was transferred into a 100 ml volumetric flask and distilled H_2O was added to the mark. This solution was filtered and a 10

ml sub-sample was transferred to a 50 ml volumetric flask into which 10 ml of vanadomolydate reagent was added and distilled water was used to fill to the mark. Reading was done by use of a spectrophotometer at a wavelength of 400 nm.

For N analysis a ground foliar sample was weighed (0.2 g) into a Kjeldahl flask and 0.1 g of selenium mixture was added as well as 5 ml of concentrated H_2SO_4. The mixtures were gently heated on a digestion rack until the solution was clear, or colourless for about 30 minutes. The mixture was transferred to the distillation flask through the side arm. The ammonia gas was collected in a 100 ml conical flask containing 5 ml of boric acid (50 ml of the sample was collected). Ammonia was titrated in the conical flask with diluted sulphuric acid.

Pearson's Correlation and multiple regression analyses were carried out to examine relationships between soil factors (pH, nutrients) and foliar nutrient status, using model:

$$y_i = \beta_0 + \sum_{j=1}^{p-1} \beta_j X_{ij} + e_i, i = 1,2,\ldots,n$$

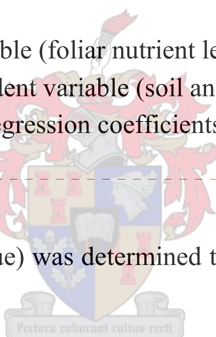
Where:

y_i = i^{th} observation of the dependent variable (foliar nutrient level)

X_{ij} = the ith observation of the jth independent variable (soil and climatic factors)

β = parameters $\beta_1, \beta_2, \ldots, \beta_{p-1}$ (partial regression coefficients)

p = number of parameters



A common regression coefficient (R-squared value) was determined to enable comparison of several pairs of data from different locations.

3.2.1.2 Climatic factors

Annual precipitation for the year 1999, mean maximum and minimum monthly temperatures for both February and June were determined for the ten study sites. Correlation and multiple regression analyses were carried out to understand influence of temperature and precipitation on foliar nutrients. Annual rainfall data from Chapter 2 were used in this analysis with mean maximum and minimum monthly temperatures of the months of February and June being the other variables (see Appendix V).

3.2.2 Seedling survival and growth on soils collected from different sites

The ten sites that were used for carrying out soil-plant nutrient analysis were also chosen for collecting soil for use in the seedling growth studies. Soil was collected by bulking the diagonally sampled soils in each plot in December 1999, when the country was generally considered to be wet. Bulked samples from the top 10 cm soil from each site was bagged separately and transported to the experimental site, Forest Research Centre, Harare. This site falls under the Henderson meteorological region within which three of the study sites (Nzvera, Matiya and Tsikwa) were located. Ten 12 cm diameter polythene pots with a length of 25 cm were filled with bulked soil sample from the respective experimental plots. From each soil sample, a sub-sample was taken for nutrient analysis (see par. 3.2.1.1). *U. kirkiana* seedlings raised in sterilised pine bark media were transplanted into these pots at two-leaf stage of growth. One seedling was planted in each pot. Ten seedlings per each site-soil combination were used as treatment. The soil media surface in each pot was covered with aluminium foil to minimise mycorrhizal fungi cross infection between the different soils. The pots were laid out in a completely randomised design with ten treatments replicated ten times. The first growth assessment was carried out after a four-week period. This was undertaken by measuring shoot height and also counting number of leaves on each shoot. These assessments were continued at four-week intervals attaining a total of four assessments. Seedling survival for different treatments was also assessed.

3.2.3 Effect of nitrogen and phosphorus on seedling biomass

A 4 x 4 factorial experiment was used to test the effect of N and P on seedling biomass. Sixteen N-P fertiliser treatment combinations were prepared, i.e. four levels of nitrogen (N=0 g, N=3.5 g N=7.0 g, N=10.5 g per container) and four levels of phosphorus (P=0 g, P=2.1 g, P=4.2 g, P=6.3 g per container). Each treatment combination was repeated ten times. Ammonium nitrate commercial fertiliser with 34.5% nitrogen was used as the source of nitrogen and for phosphorus, single super-phosphate with 20.5% phosphorus was used. Polythene pots with diameter of 12 cm and length of 25 cm were filled with fertilised soil. Soil used in this experiment was loamy sand with the following nutrient composition: 15 mg/kg N, 1 mg/kg P, 0.05 $cmol_c$/kg K, 1.21 $cmol_c$/kg Ca, 0.43 $cmol_c$/kg Mg and a pH of 4.2 ($CaCl_2$). The fertiliser combinations were mixed with soil before pot filling. At two-leaf stage of growth, seedlings raised in sterilised pine bark medium were washed, clipped and transplanted into these pots with one seedling planted per pot. The potted seedlings were

arranged in a completely randomised design in a glass-house that had day temperature ranging from 25^0C to 36^0C. Watering was carried out when necessary to meet the moisture requirements of the seedlings. Seedlings received full sunlight for 8-hour photoperiod per day. The few weeds that grew in the pots were removed by hand. After 12 months of growth, shoots and roots of the different treatments were oven-dried for 96 hours at 45^0C and their biomass determined.

Data on the seedling total dry mass from the sixteen treatment combinations were subjected to an analysis of variance (ANOVA) with Genstat statistical software using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ik} + \varepsilon_{ijk}$$

Where **:**

Y_{ijk} = observed response when Factor A is at the ith level and Factor B is at the jth level for the kth replicate

μ = overall mean;

α_i = effect of the ith level of Factor A **,** i = 1,2…,a

β_j = effect of the jth level of Factor B, j =1,2…,b

$(\alpha\beta)_{ij}$ = effect of the interaction between i^{th} level of Factor A and j^{th} level of Factor B

ε_{ijk} = random error component

Following the F-test, where significance was detected, the least significant difference was used to separate the means at the 5% level of significance.

Pectora roborant cultus recti

3.3 Results

3.3.1 Analysis of soil-plant nutrient dynamics in a natural ecosystem

Soil nutrient status for each site was the same with those shown in Chapter 2 (Table 2). Two values of N were presented, the initial-N being available N in the soil sample at time of collection and the Incu-N being nitrogen that is released when soil is incubated at 30^0C for a period of two weeks. This temperature was used to simulate conditions during the crop-growing season. The results showed high nutrient variation between respective sites, particularly P, N, Ca and Mg, which were remarkably high at Guyo and Nyamuzihwa. The high P levels at these two sites did not however, seem to influence their foliar concentration,

as P leaf concentrations were not very different between the respective sites for both samples collected during the dry and wet periods [Tables 8 (a) and (b)].

Table 8 (a).

Foliar nutrient concentrations during the rainy period (February)

	Foliar macro-nutrient					Foliar micro-nutrient (mg/kg)				
Site	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe	Mn	Zn	B	Cu
Chitakatira	1.72	0.14	0.98	0.46	0.31	96	94	19	6	6
Rowa	1.64	0.17	1.25	0.50	0.30	57	90	18	12	9
Nemaire I	1.32	0.17	0.90	0.40	0.28	63	122	41	9	9
Nemaire II	1.42	0.16	0.93	0.46	0.29	90	109	42	10	5
St Lukes	1.62	0.16	0.78	0.34	0.36	61	54	14	9	9
Nzvera	1.38	0.16	0.99	0.44	0.25	58	74	26	11	6
Tsikwa	1.56	0.11	0.76	0.35	0.34	60	38	12	6	8
Matiya	1.48	0.22	1.04	0.44	0.38	70	84	21	10	5
Guyo	1.60	0.17	0.95	0.40	0.48	48	99	51	12	6
Nyamuzihwa	1.58	0.14	0.85	0.51	0.25	42	80	27	11	5
Mean	1.53	0.16	0.94	0.43	0.32	50	84.4	27.1	9.6	6.8

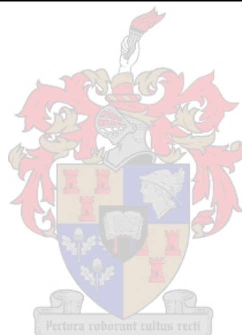


Table 8 (b).

Foliar nutrient concentrations during the dry winter period (June)

	Foliar macro-nutrient					Foliar micro-nutrient (mg/kg)				
Site	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe	Mn	Zn	B	Cu
Chitakatira	1.76	0.11	0.79	0.66	0.34	61	109	25	10	7
Rowa	1.54	0.13	1.16	0.54	0.31	143	88	25	12	23
Nemaire I	1.44	0.17	0.90	0.58	0.28	132	120	33	4	6
Nemaire II	1.30	0.13	0.85	0.71	0.28	143	109	34	10	6
St Lukes	1.48	0.13	0.64	0.60	0.50	147	76	19	6	7
Nzvera	1.48	0.11	0.66	0.69	0.23	118	108	27	16	4
Tsikwa	1.52	0.09	0.75	0.58	0.26	90	58	16	7	6
Matiya	1.30	0.15	0.41	0.91	0.39	133	174	18	18	6
Guyo	1.42	0.12	0.70	0.64	0.28	308	91	19	15	3
Nyamuzihwa	1.24	0.13	0.96	0.69	0.46	365	84	31	12	7
Mean	1.45	0.21	0.78	0.59	0.33	164.0	101.7	24.7	11.0	7.5

Similarly, the high P soil level at the Tsikwa plot also did not seem to influence its foliar content. Although the soil samples were not analysed for Fe, there was an evidently higher Fe foliar concentration in samples collected during the dry period than those collected in the wet period [Tables 8 (a) and (b)], especially for Guyo and Nyamuzihwa.

The relationship between soil factors and foliar nutrients as indicated by their simple correlation coefficients (Pearson's) are shown in Table 9 (a) for the rainy period in February and Table 9 (b) for the dry winter period in June.

The correlations can be broadly grouped into four categories, i.e. low or high correlations and the positive and negative correlations. The foliar nutrient elements that showed the highest positive correlation coefficient with soil factors was Fe during the winter period, followed by B which did not vary much between the wet and dry period.

Pectora roborant cultus recti

Table 9 (a).

Simple correlation coefficients for soil factors and foliar nutrient concentrations for the rainy period (February)

Soil factor	FN	FP	FK	FCa	FMg	FFe	FMn	FCu	FZn	FB
SCa	0.24	-0.23	-0.06	0.26	0.45	-0.24	0.24	0.13	0.61	0.43
SK	0.17	-0.05	-0.48	0.04	0.23	-0.25	-0.13	-0.09	0.33	0.22
SMg	0.20	-0.16	-0.25	0.39	0.06	-0.30	0.10	-0.16	0.39	0.32
SN_1	0.21	-0.18	-0.21	0.29	0.12	-0.63	0.06	0.12	0.37	0.43
SN_2	0.27	-0.05	-0.33	0.09	0.33	-0.36	0.02	0.20	0.50	0.29
SP	0.22	0.01	0.00	0.12	0.59	-0.54	-0.06	0.18	0.31	0.47
STC	0.23	-0.20	-0.14	0.30	0.33	-0.27	0.19	0.03	0.55	0.40
pH	0.00	-0.34	0.60	0.68*	-0.20	-0.17	0.37	-0.01	0.32	0.72*

* Significant at 5 %

Key: F-Foliar, SN_1-Soil initial nitrogen, SN_2-Soil incubated nitrogen, S-soil, TC-Total cations

Table 9 (b).

Simple correlation coefficients for soil factors and foliar nutrient concentrations for the dry winter period (June)

Soil factor	FN	FP	FK	FCa	FMg	FFe	FMn	FCu	FZn	FB
SCa	-0.42	-0.19	0.08	0.22	0.08	0.80**	-0.06	-0.30	0.02	0.42
SK	-0.52	0.10	0.00	0.21	0.32	0.73*	-0.06	-0.47	0.01	0.19
SMg	-0.56	-0.13	0.25	0.19	0.31	0.86**	-0.19	-0.25	0.23	0.23
SN_1	-0.46	-0.09	0.24	0.00	0.32	0.96**	-0.27	-0.18	0.12	0.21
SN_2	-0.35	-0.10	0.20	-0.04	0.12	0.82**	-0.41	-0.34	0.03	0.15
SP	-0.53	-0.14	-0.12	0.37	0.18	0.77**	0.04	-0.19	-0.36	0.58
STC	-0.48	-0.16	0.14	0.22	0.17	0.84**	-0.12	-0.30	0.09	0.36
pH	-0.33	-0.25	0.72*	-0.13	-0.17	0.37	-0.10	0.58	0.45	0.29

* Significant at 5 % ** Significant at 1 %

Key: F-Foliar, SN_1-Soil initial nitrogen, SN_2-Soil incubated nitrogen, S-soil, TC-Total cations

With Zn there was a reverse situation, i.e. during the rainy period, a stronger correlation occurred than during the dry period, indicating that water or other factors that are active during the rainy period are important for Zn uptake. The overall relationship between soil

factors and foliar nutrient concentrations as analysed by multiple regression analysis is given in Tables 10 (a) and (b).

Table 10 (a).

Results of multiple regressions of soil factors on individual foliar nutrient levels for the rainy period (February)

Foliar nutrient	Regression constant, explaining variable and partial regression coefficient	R^2
N	+1.8 -.03(STC) +.002(SP) +.005(SN_2) -.002(SN_1) +.10(SMg) -.56(SK) -.06(pH)	0.13
P	+2.11 -.49(STC)* +.035(SP)* +.039(SN_2)* -.029(SN_1) +1.09(SMg)* -2.69(SK) -.30(pH)	0.95
K	-.201 +.09(STC) -8.92(SP) -.006(SN_2) +.002(SN_1) -.16(SMg) -.29(SK) +.26(pH)	0.92
Ca	+.12 +.02(STC) -.001(SP) -.003(SN_2) +8.50(SN_1) +.031(SMg) -.144(SK) +.07(pH)	0.88
Mg	+.51 +.02(STC) +.00(SP) +.00(SN_2) -.00(SN_1) -.08(SMg) -.04(SK) -.05(pH)	0.87
Fe	+177.0* +10.28(STC) -.86(SP) -.27(SN_2) -1.02(SN_1)* +12.38(SMg) -58.06(SK) -20.27(pH)	0.97
Mn	+105.0 +31.4(STC) -2.06(SP) -1.36(SN_2) +.60(SN_1) -31.58(SMg) – 100.57(SK) +3.37(pH)*	0.81
B	-24.6 +1.39(STC) -.02(SP) -.15(SN_2) +.16(SN_1) –5.98(SMg) +18.52(SK) +6.12(pH)	0.96
Zn	+4.61 +12.60(STC) -.70(SP) -.24(SN_2) +.28(SN_1)* -23.11(SMg)* -7.75(SK) +3.40(pH)	0.60
Cu	+4.15 -.19(STC) +.039(SP) +.15(SN_2) +.04(SN_1) -4.00(SMg) -6.82(SK) +.81(pH)	0.84

* Significant at 5%

Key: SN_1-Soil initial nitrogen, SN_2-Soil incubated nitrogen, S-Soil

Table 10 (b).

Results of multiple regressions of soil factors on individual foliar nutrient levels for the winter period (June)

Foliar nutrient	Regression constant, explaining variable and partial regression coefficient	R^2
N	+2.53 +.12(STC) -.00(SP) +.007(SN_2) -.001(SN_1) -.040(SMg) -.98(SK) -.15(pH)	0.67
P	-.11 +.01(STC) -.00(SP) -.00(SN_2) +.00(SN_1) -.12(SMg) +.76(SK) +.02(pH)	0.48
K	-.79 -.129(STC) -.001(SP) +.013(SN_2) -.003(SN_1) +.34(SMg) -1.44(SK) +.39(pH)	0.91
Ca	+.90 +.08(STC) +.001(SP) -.01(SN_2) -2.38(SN_1) -.04(SMg) +.62(SK) -.08(pH)	0.94
Mg	+.39 +.005(STC) -8.09(SP) -.005(SN_2) +.003(SN_1) -.015(SMg) +.65(SK) -.03(pH)	0.51
Fe	-279.33 +9.8(STC) +.39(SP) -1.46(SN_2) +4.48(SN_1) -53.68(SMg) +248.36(SK) +64.90(pH)	0.97
Mn	+214.9 +38.23(STC) -.71(SP) -3.67(SN_2) +.61(SN_1) -39.61(SMg) +81.01(SK) -22.36(pH)	0.93
B	-17.07 +3.47(STC) +.09(SP) -.35(SN_2) +.119(SN_1) -7.84(SMg) +25.52(SK) +4.14(pH)	0.72
Zn	+2.78 +3.42(STC) +.09(SP) -.35(SN_2) +.119(SN_1) -7.84(SMg) +25.52(SK) +4.14(pH)	0.72
Cu	-42.78 -2.82(STC) +.16(SP) +.13(SN_2) -.05(SN_1) +5.49(SMg) -26.33(SK) +12.31(pH)	0.75

Key: SN_1-Soil initial nitrogen, SN_2-Soil incubated nitrogen, S-Soil

This shows that between 60% and 97% of the variation in foliar nutrient concentrations during the wet period, with the exception of nitrogen, could be explained by the investigated soil factors, while in winter these percentages were between 48% and 97%. Combinations of factors that had significant influence ($P<0.05$) were exhibited during the wet period and these were those influencing foliar P, Fe, Mn and Zn [Table 10 (a)].

3.3.2 Climatic factors

The effects of precipitation on foliar nutrient levels showed a similar trend as those of the soil factors with significant effect being observed on foliar Fe. Simple correlation coefficients (Pearson's) are shown in Tables 11 (a) and (b) for the rainy and dry periods respectively.

Table 11 (a).

Simple correlation coefficients for climatic factors and foliar nutrient concentrations for the rainy period (February)

Climatic factors	FB	FCa	FCu	FFe	FK	FMg	FMn	FN	FP	FZn
Precipitation[1]	0.31	-0.03	0.17	-0.64*	-0.42	0.30	0.00	-0.10	-0.04	0.52
Temperature[2]	-0.61	-0.27	-0.32	-0.00	-0.07	-0.03	-0.51	-0.36	0.54	-0.40

* Significant at 5%

1- Annual precipitation

2-Mean of maximum and minimum monthly temperatures

Key: F Foliar

Table 11 (b).

Simple correlation coefficients for climatic factors and foliar nutrient concentrations for the dry winter period (June)

Climatic factors	FB	FCa	FCu	FFe	FK	FMg	FMn	FN	FP	FZn
Precipitation[1]	0.23	0.16	-0.51	0.89**	-0.12	0.18	-0.15	-0.65*	-0.04	0.00
Temperature[2]	-0.39	-0.47	0.42	0.18	0.60	0.25	-0.22	0.25	0.23	0.42

*Significant at 5%, ** Significant at 1%

1- Annual precipitation

2-Mean of maximum and minimum monthly temperatures

Key: F Foliar

Pectora roborant cultus recti

Correlation between foliar Fe and rainfall was negatively statistically significant during the rainy period ($P<0.05$) and became positively statistically highly significant during the dry period ($P<0.01$). There was also a highly statistically significant negative relationship between the total annual rainfall and foliar nitrogen during the dry period. Climatic data for the months of February and June in 1999 are shown in Appendix VI.

Multiple regression analysis between climatic factors and foliar nutrients showed Fe had a statistically significant negative relationship with temperature and a highly significant positive relationship with precipitation during the dry period [Table 12 (b)]. No strong relationship was established during the rainy period [Table 12 (a)].

Table 12 (a).

Results of multiple regressions of climatic factors on individual foliar nutrients for the rainy period (February)

Foliar nutrient	Explaining variable and partial regression coefficient	R^2
B	-42.081 + 0.279(Avmt) +0.936(Maxtemp) + 0.264(Annprep)	0.49
Ca	+0.681 - 0.031(Mintemp) + 0.008(Maxtemp) + 0.000(Annprep)	0.34
Cu	+32.889 – 0.783(Mintemp) – 0.591(Maxtemp) + 0.010(Annprep)	0.12
Fe	+94.361 – 1.496(Mintemp) + 4.231(Maxtemp) – 1.358(Annprep)	0.46
K	+0.583 – 0.055(Mintemp) + 0.063(Maxtemp) – 0.004(Annprep)	0.44
Mg	+0.352 – 0.001(Mintemp) – 0.008(Maxtemp) + 0.002(Annprep)	0.11
Mn	+540.693 – 13.373(Mintemp) – 7.890(Maxtemp) – 0.721(Annprep)	0.29
N	+2.291 – 0.089(Mintemp) + 0.024(Maxtemp) – 0.000(Annprep)	0.54
P	-5.264 +0.175(Mintemp) + 0.094(Maxtemp) + 0.006(Annprep)	0.30
Zn	+239.057 – 3.167(Mintemp) – 7.951(Maxtemp) + 0.395(Annprep)	0.43

Key: Annprep-Annual precipitation, Avmt-Average monthly temperature, Mintemp-Monthly minimum temperature, Maxtemp-Monthly maximum temperature, Annprep-Annual precipitation

Table 12 (b).

Results of multiple regressions of climatic factors on individual foliar nutrients for the dry winter period (June)

Foliar nutrient	Explaining variable and partial regression coefficient	R^2
B	-49.532 + 0.720(Mintemp) + 1.363(Maxtemp) + 0.317(Annprep)	0.49
Ca	+0.323 + 0.038(Mintemp) – 0.002(Maxtemp) + 0.002(Annprep)	0.24
Cu	-46.964 – 2.224(Mintemp) + 3.241(Maxtemp) – 0.052(Annprep)	0.51
Fe	939.556* – 26.861(Mintemp)* – 54.565(Maxtemp)** + 7.109(Annprep)**	0.92
K	+0.095 – 0.116(Mintemp) + 0.050(Maxtemp) + 0.003(Annprep)	0.48
Mg	+0.926 – 0.018(Mintemp) – 0.022(Maxtemp) + 0.00(Annprep)	0.12
Mn	+25.354 + 6.985(Mintemp) + 3.257(Maxtemp) – 0.438(Annprep)	0.09
N	-0.491 – 0.036(Mintemp) + 0.106(Maxtemp)* – 0.002(Annprep)	0.57
P	+0.922 – 0.003(Mintemp) – 0.022(Maxtemp) – 0.003(Annprep)	0.30
Zn	+0.922 – 0.003(Mintemp) – 0.022(Maxtemp) – 0.003(Annprep)	0.30

* Significant at 5%, ** Significant at 1%

Key: Annprep-Annual precipitation, Avmt-Average monthly temperature, Mintemp-Monthly minimum temperature, Maxtemp-Monthly maximum temperature, Annprep-Annual precipitation

3.3.3 Survival and growth of seedlings on soils collected from different sites

Of the four assessments, only at the third and fourth assessments did shoot height differ statistically significantly ($P< 0.05$) between treatments. There was no significant difference in the number of leaves per plant between treatments. This would possibly imply that at this stage of growth, photosynthetic partitioning is in favour of shoot rather than foliage development. Mean growth assessments for the ten study sites measured by mean leaf number per shoot and shoot height, are graphically shown in Figures 3 (a) and (b). The variation observed at the third and fourth assessments could be as a result of varying nutrient levels in the different soil types, as shown by the multiple regression analysis (Table 13). Nitrogen, potassium, and calcium had statistically significant influences on seedling height growth. The marked low seedling growth rate was observed with seedlings grown in soil from Tsikwa, Guyo, Nemaire II and Nyamuzihwa trial sites, and this is graphically shown in Figures 3 (a) and (b). Seedlings grown in soils from Guyo, Nyamuzihwa, Nzvera, and Tsikwa experienced a die-back which in some instances resulted in seedling mortality. This growth pattern is graphically shown in Fig. 3 (b).

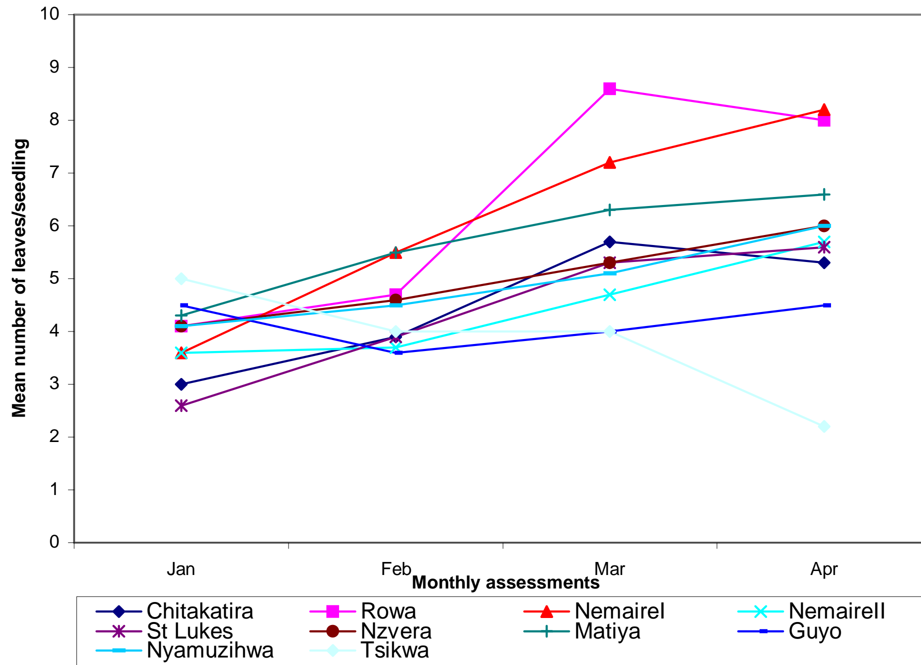


Fig. 3 (a) Mean number of leaves per *U. kirkiana* seedling in different soil types.

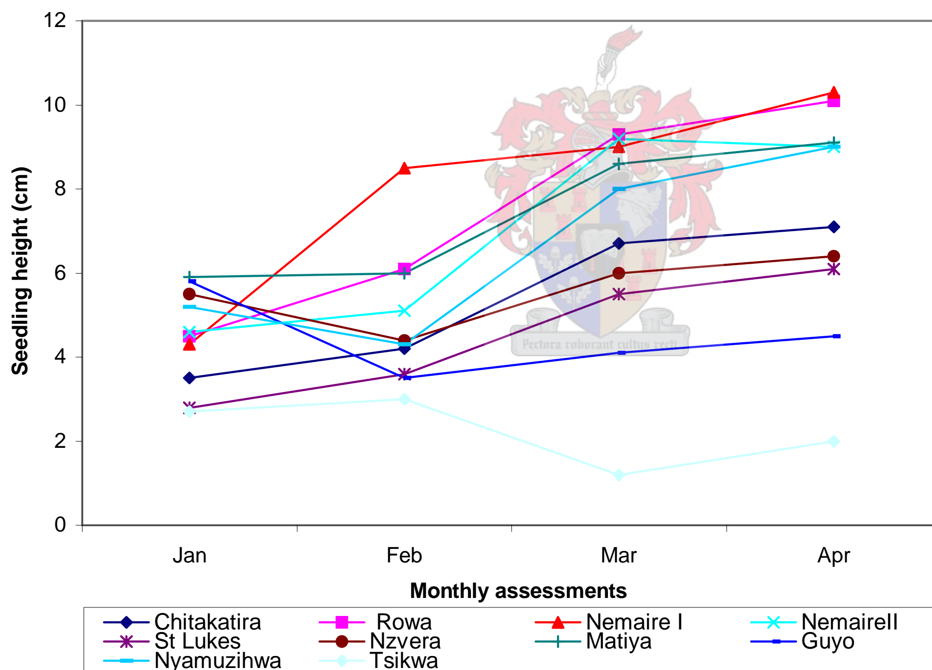


Fig. 3 (b). *U. kirkiana* seedling shoot growth rates in different soil types.

Table 13 (a).

Regression analysis for shoot height model

Source	DF	SS	MS	F	P
Regression	8	48.8383	6.1048	915.10	0.026*
Error	1	0.0067	0.0067		
Total	9	48.8450			

Table 13 (b).

Regression equation for the third shoot growth assessment, relating shoot growth to variable soil factors (nutrients, pH)

Shoot height = 6.62 - 0.034 N_1 + 1.37 pH + 0.212 N_2 - 0.002 P - 24.2 K - 2.56 Ca + 3.03 Mg + 0. 002 TC

Predictor	Coef.	Std Dev.	t-Ratio	Prob.
Constant	6.618	0.768	8.61	0.074
N_1	-0.034	0.006	-5.65	0.112
pH	1.367	0.136	10.06	0.063
N_2	0.212	0.004	48.13	0.013*
P	-0.002	0.004	-0.40	0.756
K	-24.178	0.572	-42.26	0.015*
Ca	-2.556	0.056	-45.44	0.014*
Mg	3.031	0.140	21.63	0.029*
TC	0.002	0.004	0.37	0.777

S=0.08168 R^2 = 100% R^2 (adj.) = 99.9

* Significant at 5%
Key: N_1-Initial nitrogen, N_2-Nitrogen after soil incubation

3.3.4 Effect of nitrogen and phosphate on seedling biomass

There was a significant difference ($P < 0.05$) in total oven-dry mass for N and P fertiliser levels, respectively. There was also significant interaction between the two nutrients [Table 14 (a)]. There was a positive response in biomass production when P application was increased from 4.2 g to 6.3 g without N application. A negative response was observed when 10.5 g of N was applied in combination with low P levels, that is, 0, 2.1 and 6.3 g [see Table 14 (b)]. High seedling mortality (35%) was observed in seedlings fertilised with 10.5 g of nitrogen. High mortality of seedlings made it difficult to interpret growth rates. As a result of the missing values due to the seedling mortality, the sum of squares in the analysis of variance do not add up to the total sum of squares [Table 14 (a)].

Table 14 (a).

Result of analysis of variance for the effect of N and P on the seedling biomass

Source of variation	DF	SS	MS	VR	Pr >F
Nitrogen	3	3.75	1.25	4.39	0.009 **
Phosphorus	3	4.56	1.52	5.35	0.003 **
P x N	9	10.58	1.17	4.13	0.001**
Residual	44	12.52	0.28		
Total	59	17.23			

* Significant at 5%   ** Significant at 1%

Table 14 (b).

Comparison of different levels of N and P (total dry mass) using LSD (5%)

Treatment#	P0	P1	P2	P3
N0	0.44	0.43	0.07	1.28
N1	1.35	1.00	0.59	0.82
N2	0.88	0.63	0.69	0.26
N3	0.75	0.35	0.38	0.90

LSD (5%) combination means = 0.48

LSD (5%) Nitrogen means = 0.24

LSD (5%) Phosphorus means = 0.24

#N0=0 g, N1= 3..5 g, N2= 7.0 g, N3= 10.5 g, P0=0 g, P1=2.1 g, P2=4..2 g, P3=6..3 g per container

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3.4 Discussion

The strong positive significant correlation of all soil factors (except pH) with foliar Fe during the winter period [Table 9 (b)] reflects presence of a factor that enhanced uptake of this element during this period and failure of this factor to exert its influence during the rainy period. During the rainy period there was actually a negative correlation of all soil factors to foliar Fe. These were however, not statistically significant [Table 9 (a)]. A possible explanation is that there might have been competition for Fe during the rainy period. Saprophytic fungi or other soil microbes that actively grow during this period could actually be competing with plants. Furthermore, certain mycorrhizal fungal species have been noted to either accumulate or enhance uptake of Fe by plants. Table 4 in Chapter 2 shows Fe content of sporocarps of mycorrhizal fungal species collected during the rainy period, which is the period when conditions are most ideal for microbial growth. It is evident that sporocarps have higher Fe content than the foliage sampled during the rainy period. There was nevertheless an increase in foliar Fe content during the dry winter period.

It is therefore possible that during the rainy period there is sequestration of Fe and other nutrients by the actively growing mycorrhizal fungi which become released during dry periods when the mycelia degenerate. The data further show variation of uptake of Fe between fungal species. Jonasson *et al*. (1999) reviewed various research findings from cold climates and concluded that microbes sequester nutrients during the growing season, when they presumably build up their populations and create a strong nutrient sink. These nutrients are then released during the non-growing season. It is therefore possible that under hot, wet periods of the sub-tropic regions, similar processes do occur. References to support this view could not be found. More research work is therefore recommended in this area.

Variation in the type and strength of the relationship between soil factors and foliar Fe between dry and wet periods [Tables 9 (a) and (b)] could also possibly be explained by oxidation-reduction processes. Ferric (Fe^{+++}) and ferrous (Fe^{++}) ions have very different solubility properties. Ferric iron precipitates in alkaline environments as ferric hydroxide. Ferric iron may be reduced under anaerobic conditions to the more soluble ferrous form. Under some anaerobic conditions, however, sufficient H_2S may be evolved to precipitate iron as ferrous sulphide. Flooding of soil, which creates anaerobic conditions, favours the

accumulation of ferrous iron. In aerobic habitats, such as well-drained soil, most of the iron exists in the ferric state**.** In the Fe biogeochemical reactions, bacteria species that are considered important in the oxidation-reduction reactions are *Bacillus* spp. and *Pseudomonas* spp. (Atlas and Bartha, 1987). These reactions have not, however, been studied in this work**.**

As soils where *U. kirkiana* tends to grow are generally of fersiallitic origin (Celander, 1983) and therefore high in Fe, it can be concluded that similar oxidation-reduction processes do occur when conditions are suitable. The other possible reason for high Fe foliar levels detected during the dry-period, could be that Fe has low mobility and therefore is not easily translocated from old leaves to younger leaves when new leaves develop. When *Uapaca* tree species defoliate some of the newly formed leaves towards the winter period, most of the older leaves higher in Fe content remain intact. Leaf fall in miombo tree species is known to be caused by dry conditions that set in after the rainy season (Chidumayo, 1997), and this generally coincides with cessation of root growth of most miombo tree species (Rutherford, 1983). As leaf sampling was done at the canopy's mid-point, the leaves that were picked during the winter period were the older leaves that had high Fe levels. It can therefore be deduced that high Fe foliar concentrations during the dry period enables the *U. kirkiana* to improve photosynthetic efficiency when some of the leaves would have been senesced. Iron is known to be necessary for chlorophyll formation and therefore important for photosynthesis (Terry and Low, 1982). Improved photosynthesis rates during the winter period would therefore be of value to the trees carrying a heavy fruit set.

Similarly, the variation in correlation coefficients for soil factors and other foliar nutrient concentrations during the rainy period [Table 9 (a)] and those for the dry period [Table 9 (b)] possibly also indicate general influence of climatic factors on uptake and translocation of nutrients. The relative stronger correlation between soil factors and foliar Zn levels during the rainy period [Table 9 (a)] than the dry period, could be indicative that water or other factors that are activated during the rainy period are important for Zn uptake. The soil factor that could possibly influence Zn uptake during the rainy period could be its efficient uptake and translocation by fungi to plants. Although several mycorrhizal fungi have been proved to accumulate Zn in their sporocarps (Vetter *et al.*, 1997), it does not in this situation appear to have detrimental effects.

Of major interest was the general weak correlation of soil factors to foliar P and K, for both the wet and dry period. Soil pH was the only soil factor that was significantly correlated ($P < 0.05$) to the foliar Ca during both the wet period and K during the dry period. Although there was a relatively strong negative correlation of soil factors to foliar N during the dry period, these were not statistically significant [Table 9 (b)]. There was nevertheless, an indication of climatic influence on the foliar N during the dry winter period. For instance, temperature had a statistically significant influence on foliar N [Table 12 (b)]. This could be explained by that reduced temperature causes reduction in plant metabolic processes rate and result in the decrease in plant demand. Chidumayo (1997) indicated that N tree leaf stock during the dry season, that is characterised by low temperatures, was 17.2 kg ha^{-1} and this dropped to 3.9 kg ha^{-1} at the end of the rain season which is the active growing period. He also indicated that P foliar content dropped by 80%. This foliar nutrient decline was explained as a mechanism of reducing loss of nutrients from the trees through defoliation by withdrawing nutrients from the leaves into the plant's main physiological system before leaf fall. Influence of temperature on nutrient uptake is very complex considering that temperature regimes experienced by roots and shoots can vary widely within the same site. Furthermore, influence of temperature on the roots and shoots is also different. For instance, influence on the root systems would involve complex microbial reactions and hormone production whereas, influence on the shoot would cause changes in the transpiration and photosynthesis that affect plant growth. Contrary to the miombo situation, Jonasson *et al.* (1999) report that warming increased N and P foliar concentrations in the deciduous shrubs but declined in the main ecosystem components of the vascular plants. Shrubs and mosses have a stronger sink than the main vascular trees (Jonasson *et al*., 1999).

Other than soil factors, certain elements also influence uptake or translocation of other elements within the plant physiological system. Appendix V shows statistically significant correlations between foliar elements (eg. Cu and K), probably indicating that beside soil factors, certain foliar elements also influence the uptake or translocation of other elements within the plant system. Wallander and Wickman (1999) established that uptake of Mg in *Pinus sylvestris* seedlings was enhanced when there was severe K deficiency. It is also likely that the correlations could be coincidental because there are various other factors such as rainfall and temperature that could have collective influence on a range of elements. There are several other factors that could influence this relationship. These could include soil

biochemical reactions that are also affected by environmental factors such as rainfall and temperature. Activities of mycorrhizal fungi and other chemical reactions have been reported to influence phosphorus status in the plant tissue. Cumming (1996), working on *Pinus rigida,* reports that mycorrhizal colonisation increases P concentration in roots, and under limiting conditions it is retained within the root system for the production of ATP compounds. This retention is, however, at the expense of phosphorus translocation to foliage. Nealson (1983) mentions that availability of P is restricted by its tendency to precipitate in the presence of divalent metals such as Ca^{++}, Mg^{++} and Fe^{++} at neutral to alkaline pH. He further highlights that within many habitats, P is combined with Ca^{++}, rendering it insoluble and unavailable to plants and microbes. Chidumayo (1994) also reports changes in P foliar concentrations between seasons in the miombo woodland tree species. One of the reasons given is that P gets reabsorbed from the leaves to the main plant physiological system prior to leaf fall during the dry period. This is a mechanism of reducing the loss of valuable nutrients from miombo tree species. The other factor he discusses is that during the rainy period, foliar nutrient concentrations in trees are low because of competition by herbaceous growth and microbial nutrient sequestration. Kottke *et al.* (1995) also noted that Al and P increase in mycelia that are actively growing. It has, however, been indicated that in some situations micro-organisms aid plants by mobilising P while in other situations they compete with plants for available P reserves (Atlas and Bartha, 1987).

Although seasonal fluctuations of P uptake, storage and translocation by the ectomycorrhizal fungi have not been previously demonstrated quantitatively (Chidumayo, 1994; Nealson, 1983), results from investigations by Connell and Lowman (1989) and Högberg (1986) prove that ectomycorrhizal fungi take up P at times when trees are not actively growing and later translocate it to the host tree when needed. This mycorrhizal fungal activity has been used to explain the dominance of ectomycorrhiza-forming tree species in seasonal tropical forests where supply of nutrients is discontinuous (Alexander, 1983; Harley and Smith, 1983). This process was recently verified by Lussenhop and Fogel (1999), who measured the P content of sheaths of the ectomycorrhizal fungus *Cenococcum geophilum* growing in association with *Pinus strobus.* These measurements were carried out in different seasons, and there was clear evidence of phosphorus seasonal variation in root sheaths. In the seasonal tropical woodlands the P pathway is rather different. The P that is sequestered in the root sheath and mycorrhizal

fungi mycelia is released into the soil when lateral roots and mycelia degenerate during the dry conditions (Newbery *et al*., 1997).

Nutrient dynamics in the *U. kirkiana* woodland, assessed by foliar and soil nutrient analyses in different seasons, could be an important indicator of influence of climatic factors in plant nutrient uptake. These climatic factors could either exhibit a direct or indirect influence by affecting the biochemical reactions that affect the uptake of certain nutrients. It is nevertheless advisable to take foliar samples during the rainy period when the tree is not experiencing severe defoliation. The studies on effects of different soil types on seedling growth indicated that Ca, N and K had statistically significant negative effects ($P<0.05$) on the shoot height (Table 13). Similarly, the effects of N and P mineral fertilisers on seedling biomass was significant ($P < 0.01$). Table 14 (a) shows analysis of variance for the experiment. The results indicate that there was positive significant response in P levels. This response trend could be attributed to the low P level of the experimental soil (see par. 3.2.3). Boaler (1966) observed that seedlings of the miombo tree species did not respond easily to mineral fertiliser application when applied to miombo soil that had 10 mg P/kg. He established that the rate of growth of seedlings growing in soil that had 30 mg P/kg was not different from seedlings growing in the normal miombo soil that had 10 mg P/kg. Considering the possibility that threshold value for miombo could be below 10 mg P/kg, further work should be undertaken to establish the optimum P requirement for *U. kirkiana.* However, high levels of foliar Mn and Zn observed in the trial sites Nemaire I and Nemaire II had a corresponding positive relationship with the growth rate of seedlings planted in these respective soils. It can also be deduced that foliar analysis could be used to identify the most suitable soils for raising *U. kirkiana* seedlings. The possibility of using foliar fertiliser to supply micro-elements should also be examined particularly in nurseries where seedlings could experience trace element deficiency due to soil compaction occurring as a result of over-watering.

In this experiment there was no direct evidence of P being a limiting factor in the seedling growth. Phosphate levels in all the test sites (Table 2), with the exception of Nemaire I, were relatively high when compared to 10 mg/kg used by Boaler (1966). Figures 3 (a) and (b) show seedling growth rates on soils from the different sites. The lowest growth rate was with seedlings grown in Tsikwa soil. This could possibly be explained by the relatively low soil N

level. Low levels of micronutrients such as Mn, Zn and B in leaf samples from Tsikwa could be indicative of their deficiency in the soil. Their deficiency could therefore have contributed to the poor growth of seedlings. The second lowest growth rate was with the Guyo soil followed by that of Nemaire II and Nyamuzihwa. Surprisingly all three these soils had generally high levels of N and P, probably suggesting influence of other factors which had not been tested. This influence could probably include effect of divalent metals such as Ca^{++}, Mg^{++} and Fe^{++}, which have been known to precipitate P into unavailable forms. Furthermore, Ellis (1999) indicated that Ca ions reduce the availability of Fe to plant uptake. It can therefore be concluded that the low growth rate of seedlings growing in the soils from Guyo, Nyamuzihwa and Nemaire II was due to relatively high Ca levels (Table 2). It is also known that under alkaline conditions Fe combines readily with P to form insoluble precipitates rendering both P and Fe unavailable (Ellis, 1999). For instance, Nyamuzihwa and Guyo soils had the lowest foliar Fe, 42 mg/kg and 48 mg/kg, respectively during the rainy period [Table 8 (a)]. As Fe plays an important role in plant chlorophyll formation, its deficiency results in growth reduction since it directly affects photosynthesis. Terry and Low (1982) reported that, whenever the supply of Fe is deficient, there is a close positive correlation between the total Fe content of the leaves and the chlorophyll content. Levels of the other elements such as K and Mg in Nyamuzihwa and Guyo did not differ markedly from the rest of the test soils.

Another interesting observation noted in this experiment was the seedling die-back observed for soils from four sites: Guyo, Nyamuzihwa, Nzvera and Tsikwa, that occurred between January and February [Fig. 3 (b)]. This die-back is thought to be associated with poor mycorrhizal activity (Mwamba *et al*., 1992). Munyanziza (1994) also argued that such die-back could occur because of the water-logging that results from soil compaction that occurs as a result of regular watering in the nursery. Soil compaction was not established in this experiment. Another explanation advanced to explain this seedling die-back is that seedlings use nutrient reserves in the cotyledons and later from the potted soil but as the plants grow bigger, the demand becomes larger but the limited potted soil supply would become depleted. Seedling die-back in nature is known to be caused by water stress (Boaler, 1966; Chidumayo, 1991; Trapnell, 1959). Annual shoot die-back that occurs for the first 8 to 10 years in most of the miombo tree species is considered an important mechanism for building up nutrients and energy reserves in the root system (Boaler, 1966; Celander, 1983). It may therefore be necessary to carry out further studies on the benefits of shoot die-back, particularly looking at

the development of root systems under different nursery management techniques. The role of mycorrhizal fungi in the build-up of root nutrient reserves is an interesting area for further research. Importance of the trace elements, particularly Mn, Zn and B, which are relatively low in Tsikwa soil when compared to other sites and therefore presumably resulting in poor plant growth, is also an area for further investigation. In addition, influence of N should also be examined because it is reflected to be the lowest in the Tsikwa soil.

Other than the low N level in Tsikwa soil, there is no marked differences in N levels in the rest of the soils, probably indicating little direct influence of N on the seedling growth variations in the different soils. This is however, contrary to the work by Högberg (1982) who highlighted importance of N and P for the growth of miombo tree species. This is indicative of the limitations of the use of soil analysis in determining plant N requirement, as total nitrogen content in the soil bears little relationship to mineral N availability for plant uptake (Chidumayo, 1994). Similarly, total P in the soil may give an indication of the overall status of a soil but it does not provide an accurate indication of the available P. Availability of P depends on interactive complex processes that are influenced by pH, the Al and Fe concentrations, and the activities of the microflora.

In the soils that are generally low in fertility, use of commercial inorganic fertilisers has been noted to be benefical to *U. kirkiana* seedling gowth. Table 14 (b) shows means of the oven dry total seedling biomass for the different N and P treatment levels when grown in low fertility soil. There was significant difference in the dry mass for the different treatments indicating that commercial fertilisers would be beneficial to *U. kirkiana* when grown in poor soils. Further studies should nevertheless be carried out to establish the optimum fertilisation levels.

Even though N and P are generally known to be important nutrients for growth of miombo tree species (Högberg, 1982), this study highlighted the importance of other factors that influence their availability and uptake by plants. For instance, levels of divalent metals such as Ca^{++}, Mg^{++} and Fe^{++} in the soil have been proven to critically negatively influence availability of P to plants. Furthermore, the study revealed the need to establish the most suitable time to collect foliar samples for analysis. This can be done by continuously carrying out foliar nutrient analyses throughout the year to establish the period of slow

change in concentrations of nutrients in the leaves and this would be considered the stable physiological condition that would be most suitable to collect foliar samples for analysis.

In summary, it was established that influence of soil factors on foliar Fe was only significant during the dry period. Influence of soil factors on foliar N and P was generally weak, with the exception of pH, which had a positive significant influence on foliar K during the dry period and also had a positive effect on foliar Ca and B during the rainy period. Foliar N was, however, negatively influenced by moisture during the winter period. Furthermore, the study showed that certain elements could also have either positive or negative influence on the uptake or translocation of other elements within the plant physiological system. For instance, foliar Ca had a positive effect on the foliar B and Mn during the dry period. K and Cu also exhibited a positive significant relationship. Nutrient translocation and mobility within an ecosystem is also influenced by mycorrhizal fungi that grow in association with trees in a given area. Mycorrhizal fungi have particularly been noted to be important for the uptake of P.

As findings in this study demonstrated an increase in seedling biomass when P was increased from 4.2 g to 6.3 g per seedling, it was concluded that use of commercial P fertilisers would be beneficial in the domestication of *U. kirkiana* tree species when grown in P deficient soils. It was also noted that seedling growth was different for different soil types. It is therefore recommended that soil and leaf analyses should be used as a guide to determine suitable sites for new plantings. Soil and foliar analyses could also be used in choosing soils that could be used in the nurseries. Considering that trace elements, particularly Mn, Zn and B were identified to be important for seedling growth, it is recommended that when necessary, these could be applied to the seedlings through foliar sprays. Considering that there is a positive relationship in the uptake of Ca and Mn, it is advisable that their optimum synergistic levels for growth of *U. kirkiana* be established.

4 GROWTH RESPONSE OF *U. KIRKIANA* SEEDLINGS TO VARIOUS MYCORRHIZAL FUNGI

4.1 Introduction

The study on the 'Ecology and pattern of mycorrhizal fungi distribution in the *Uapaca kirkiana* woodlands' (Chapter 2) indicated the presence of particular fungi populations, but it did not qualitatively or quantitatively exhibit their ecological efficiency and compatibility with *U. kirkiana.* The quantitative assessment of the efficiency of mycorrhizal fungi involves determining the ability and extent individual fungi enhance nutrient and water uptake by plants, lengthen life of host tree roots, protect the host tree root systems against soil-borne pathogens, and also their ability to increase tolerance of plant roots to various adverse soil conditions (Bowen, 1994; Perrin *et al*., 1996). The ecological complexities of the mycorrhizal fungi occurring in nature, nevertheless, make controlled laboratory experiments evaluating the above individual fungal functions of limited practical benefit. It is the cumulative effect and interactions of these factors rather than their individual effect that influence plant biomass production or seedling growth. As mycorrhizal fungi are known to be important for seedling establishment because of their wide range of functions (Harley and Smith, 1983), the most practical way of assessing their efficiency is by determining plant growth response to their influence. Several mycorrhizal fungi plant growth response studies have been carried out elsewhere for different tree species (Amaranthus and Perry, 1987; Marx and Artman, 1979; Marx *et al.,* 1977a; Ruehle, 1982; Stenström *et al*., 1990).

Variations in symbiotic efficiency of mycorrhizal fungi have been proved in various experiments. Ohga and Wood (2000), working on *Larix kaemferi* established that five fungal species had varying effects on seedling growth assessed by root and shoot length. Similarly, work by Singh and Lakhanpal (2000) studying mycorrhizal fungi *Boletus edulis* and *Russula brevipes* revealed variation in their effect on seedling biomass and root collar diameter. Related studies on different tree species and mycorrhizal fungi have been carried out elsewhere (Ba *et al.,* 1999; Gorissen and Kuyper, 2000; Pera *et al.,* 1999).

Differences in the influence of mycorrhizal fungi species on the growth of host plants could be due to variation in their percentage root infection. It has also been established in pure culture experiments that mycorrhizal fungi also differ in their ability to use various carbon

and nitrogen compounds, growth substances requirements, and their relation to pH, temperature, and oxygen supply (Hacskaylo *et al*., 1965; Lundeberg, 1970). Results from the plant growth response experiments by various workers have however, tended to be inconsistent. This could be due to several factors, such as the influence of different artificial laboratory or green house nursery conditions. Spores of certain fungal species fail to germinate under certain nursery conditions. In addition, fungal mycelial growth and infection processes are generally highly influenced by aeration, soil structure and many other factors such as pH, soil nutrient levels, and temperature (Laiho, 1970; Marx *et al*., 1970; Slankis, 1974). Given that *U. kirkiana* seedlings have proved difficult to raise under nursery conditions, this research was undertaken to establish from the identified mycorrhizal fungi the most efficient species in enhancing seedling survival and growth under such conditions.

The specific objectives of the study were the following:

(i) Assess influence of mycorrhizal fungi on seedling growth.

(ii) Evaluate colonisation efficiency of the selected mycorrhizal fungi species.

4.2 Methods and materials

4.2.1 Preparation of mycorrhizal fungi inoculum

Five mycorrhizal fungi that were commonly found in the ten study sites were chosen for this experiment, i.e. *Amanita zambiana, Cantharellus cibarius, Cantharellus* sp., *Lactarius deliciosus,* and *Lactarius edulis.* Sporocarps of these fungi were collected from *U. kirkiana* woodland and air-dried in khaki pockets. The respective dried sporocarps were ground with a laboratory grinder and sieved through a 2 mm mesh to produce a fine inoculum powder. Twenty grams of each of the fungi spore collections was used for nutrient analysis using spectrophotometry (Koroleff, 1976). The remaining powder was kept in vials at 4^0C in an incubator (Theron, 1991) for one week and used as inocula for the experiment.

4.2.2 Inoculation treatments

Eight inoculation treatments were formulated as follows: (i) *Amanita zambiana,* (ii) *Cantharellus cibarius,* (iii) *Cantharellus* sp., (iv) *Lactarius edulis,* (v) *Lactarius deliciosus,* (vi) Cocktail (mixture) of the five mycorrhizal fungi species, (vii) Control-1 (autoclaved cocktail of mycorrhizal fungi), (viii) Control–2 (uninoculated).

Five grams of the respective inoculum were placed at 4 cm depth of the sterilised sand media in polythene pots that had a diameter of 5 cm and depth of 12 cm. These eight treatments were replicated ten times and arranged in a completely randomised block. *U. kirkiana* seedlings raised in sterilised pine bark were transplanted into the inoculated and control polythene pots when the first pair of leaves had developed. One seedling was planted per pot making a total of 10 plants per replication per treatment. These seedlings were raised in a glass-house that had day temperature that varied between 25^0C and 36^0C. Seedlings were fertilised by weekly application of liquid fertiliser (75 ml per pot) which had the following nutrient composition; 5N, $6P_2O_5$, $7K_2O_5$, Mg (0.1%), Zn (0.03%), Cu (0.02%), B (0.03%) and S (0.15%). Watering was carried out when necessary to meet their moisture requirements. All seedlings received full sunlight for an 8-hour photoperiod per day. As the growth media had been sterilised, few weeds occurred and were removed by hand. Seedling growth assessments were carried out on a monthly basis for a period of four months. Growth parameters that were assessed were: shoot height, leaf number, total fresh and dry mass.

Macroscopic features were used to examine fine roots to determine presence or absence of mycorrhizal colonisation when the seedlings were harvested after four months. The presence or absence of hyphae and rhizomorphs and the branching habit of fine roots were noted. Sub-samples of fine roots of each treatment were examined, using the trypan blue stain method and percentage infection estimated by use of stereo-microscope to determine the ratio of 'number of mycorrhizal short roots: total number of short roots examined'. Final shoot heights were measured at harvest. In addition, root and shoot mass were recorded after drying to a constant mass at 70^0C. Analysis of variance was used to identify significant responses. Data on leaf number, fungi colonisation percentage and seedling total dry mass from the eight treatments were analysed (one-way analysis) with SAS statistical software using the following model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where:

Y_{ij} = j^{th} observation in the ith treatment

μ = overall mean

α_i = effect of ith treatment, i = 1,2,3,…,8

ε_{ij} = random error component

As defoliation, die-back and seedling mortality were also considered as effects of the various treatments, missing values were not extrapolated, but only complete data sets were analysed. Forty observations for the leaf number assessment, 57 observations for seedling biomass assessment and 55 observations for mycorrhizal root colonisation were analysed. Following the F-test, where significance was detected, Tukey's test was used to separate the means at the 5% level of significance.

4.3 Results

4.3.1 Mycorrhizal fungi nutrient composition

Results of the mycorrhizal fungi nutrient analysis in Chapter 2 were used in this experiment.

4.3.2 Seedling growth assessment

One month after transplanting, most seedlings had three leaves above the premordial leaves. At the second assessment, seedlings inoculated with a cocktail of mycorrhizal fungi were showing symptoms of drying and all those seedlings had died by the time of the third assessment. The analysis of variance for the effect of various treatments on survival and the growth factors indicate that there were statistically significant differences in the percentage seedling survival ($P<0.01$) and in the number of leaves ($P<0.05$) between treatments (Table 15) but no differences in shoot length were noted. Table 16 shows seedling shoot and leaf development assessments for the different treatments.

Table 15.

Analysis of variance for number of leaves at four months stage of growth for different mycorrhizal fungi treatments

Source	DF	SS	MS	F-value	Pr>F
Treatment	6[1]	29.74	4.95	2.59	0.0355*
Error	34	65.04	1.91		
Total	40	94.78			

R-square	Coeff Var	Root MSE	Leaves Mean	* Significant at 5%
0.31	23.34	1.38	5.92	

1 Only seven entries were analysed as treatment vi resulted in 100% seedling mortality

Table 16.

Summary of statistics on the growth assessment of four months old *U. kirkiana* seedlings inoculated with different mycorrhizal fungi

Treatment (Inoculum)	Treatment code	Seedling survival (%)	Mean leaf number*	Mean shoot height (cm)*	Max. height (cm)	Min. height (cm)
Cantharellus cibarius	1	70	6.2ab	6.33ab	9.0	4.0
Lactarius edulis	2	80	5.0bc	5.33b	7.0	3.5
Lactarius deliciosus	3	70	7.2a	7.40a	9.0	5.0
Amanita zambiana	4	40	6.3ab	7.33ab	9.0	6.0
Cantharellus sp.	5	60	6.2ab	6.50ab	8.0	4.0
Mycorrhizal fungi # (cocktail)		0	-	-	-	-
Control-2 (uninoculated)	6	80	4.5c	5.08b	7.8	4.0
Control-1 (autoclaved fungi cocktail)	7	100	6.4ab	6.71ab	8.1	5.2

Data are not included because all seedlings had died at the final assessment.

*Values within the column with the same letter are not statistically significantly different at 5%. Tukey's Studentised Range Test was used to compare the means.

Seedlings inoculated with an inoculum of autoclaved mycorrhizal fungi (mixture), had 100% survival rate whereas the seedlings inoculated with the live inoculum mixture of mycorrhizal fungi had 100% mortality. *L. deliciosus* inoculated seedlings had the highest number of leaves compared to the other treatments followed by the control-1 (autoclaved mycorrhizal fungi cocktail). The superiority of influence of *L. deliciosus* on the number of leaves was also noted to have a corresponding positive inflluence on the seedling height. Seedlings inoculated with *L.deliciosus* attained mean height of 7.40 cm followed by seedlings incoculated with *A.zambiana* which attained a seedling mean height of 7.33 cm.

4.3.3 Percent root colonisation and biomass production

There were statistically significant differences between treatments in percentage root colonisation by mycorrhizal fungi ($P<0.05$). Analysis of variance for this experiment is shown in Table 17. *A. zambiana* had the highest percentage colonisation of over 60%,

followed by *Cantharellus* sp. which had 52%, and the rest had values of 40% and lower (Table 18).

Table 17.

Analysis of variance for different mycorrhizal fungi percentage root colonisation

Source	DF	SS	MS	F-Value	Pr>F
Treatment	4	6943.02	1735.75	3.24	0.0193*
Error	51	27360.86	536.48		
Corrected Total	55	34303.88			

R-Square	Coeff Var	Root MSE	Inf. (%)
0.20	48.55	23.16	47.69

* Significant at 5%

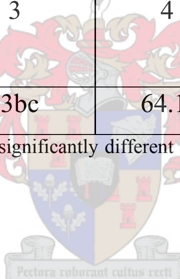
Table 18.

Root colonisation percentages of different mycorrhizal fungi*

Treatment	Mycorrhizal fungi species				
	C. cibarius	*L. edulis*	*L. deliciosus*	*A. zambiana*	*Cantharellus* sp.
Treatment code#	1	2	3	4	5
% Colonisation	40.17bc	40.11bc	34.43bc	64.19a	52.10ab

* Values within the row with the same letter are not statistically significantly different at 5%. Tukey's Studentised Range Test was used to compare the means.

\# See Table 16



Effects of different treatments on the seedling biomass production were also analysed using ANOVA. The results indicated that the total dry mass of the different treatments were statistically significantly different at $P<0.01$. Table 19 shows the results of the analysis of variance for total dry mass.

With respect to biomass production, seedlings inoculated with the autoclaved mycorrhizal fungi cocktail had the highest total dry mass followed by those inoculated with *A. zambiana,* while the control-2 (uninoculated) had the least total dry mass (Table 20).

Table 19.

Analysis of variance for the total dry mass of the four months old seedlings inoculated with different mycorrhizal fungi treatments

Source	DF	SS	MS	F value	Pr>F
Treatment	6	1.50	0.25	12.08	0.001**
Error	51	1.05	0.02		
Corrected Total	57	2.55			

R-square	Coeff Var	Root MSE	Total dry mass.
0.69	49.74	0.05	0.10

** Significant at 1%

Table 20.

Seedling biomass production for different mycorrhizal fungi treatments at 4-months stage of growth

Treatment (Inoculum)	Code	Mean total dry mass* (g)	Mean dry shoot mass (g)	Mean dry root mass (g)	Shoot-Root ratio
Cantharellus cibarius	1	0.36bc	0.29bc	0.07bc	4.10
Lactarius edulis	2	0.30c	0.24c	0.06c	4.00
Lactarius deliciosus	3	0.30c	0.25c	0.05c	5.00
Amanita zambiana	4	0.47b	0.36b	0.12b	3.00
Cantharellus sp.	5	0.38bc	0.30bc	0.08bc	3.73
Mycorrhizal fungi cocktail #	-	-	-	-	-
Control-2 (uninoculated)	6	0.28c	0.23c	0.05c	4.60
Control-1 (autoclaved fungi cocktail)	7	0.76a	0.51a	0.25a	2.04

Data are not included because all seedlings had died at the final assessment.

* Values within the column with the same letter are not statistically significantly different at 5%. Tukey's Studentised Range Test was used to compare the means.

Pectora roborant cultus recti

The good growth of seedlings inoculated with autoclaved inoculum was probably because of beneficial effects of additional micronutrients found in the fungi combination (see Table 4 in Chapter 2 for nutrient composition of different fungal species).

In assessing the influence of individual mycorrhizal fungi on biomass production, it is evident that mycorrhizal root percentage colonisation is an important factor. For example, *A. zambiana* had both the highest fungi colonisation (Table 18) and the highest mean biomass (Table 20). Such results tend to suggest that under certain conditions, seedlings inoculated with selected mycorrhizal fungi species could perform better than the uninoculated ones.

4.4 Discussion

In the comparative analysis of influence of the various mycorrhizal fungi on the *U. kirkiana* seedling growth, there were statistically significant differences ($P<0.05$) between different treatments. The improved growth of seedlings inoculated with autoclaved inoculum which accumulated the highest mean total dry weight of 0.76 g was probably due to the beneficial effect of additional combined effects of micro-nutrients found in the inoculum (see Table 4 in Chapter 2 for nutrient composition of different fungi). This implies that the mycorrhizal fungi used in this treatment are 'accumulator species', sequestering more nutrients than they supply to the host plant. Iron, the element not supplied by liquid fertiliser but found in all test fungi, has been noted to be an important plant element in Chapter 3, where macro and micro-nutrient dynamics were studied in a natural *U. kirkiana* woodland ecosystem. Contribution of macronutrients found in the autoclaved inoculum would mostly be insignificant when compared to the micronutrients, which are required in small quantities. Variation in the influence of individual mycorrhizal fungi could be due to inherent differences in the colonisation efficiency, mycelial growth rates and response to environmental factors.

Among the five mycorrhizal fungi inoculants, *L. deliciosus* exhibited the greatest influence on the seedling height, followed by *A. zambiana*. *L. edulis* had the least influence (Table 16), probably reflecting its poor symbiotic efficiency. Symbiotic efficiency of mycorrhizal fungi has been suggested by Bowen (1987) to be dependent on the combination of influence of environment on pre-infection phases such as spore germination and mycelial growth through the soil and the root susceptibility. Factors that can influence variation in basidiospore

germination percentages between different fungi could be their respective differences in their responses to root exudates. Several workers have established that certain types of fungi spores germinate when they come in contact with root exudates, and the other group involve species whose spores are not stimulated by root exudates (Fox, 1986; Fries, 1987; Theodorou and Bowen, 1987). The first group has been termed 'early stage' fungi and the second group the 'late stage' fungi. It can therefore be assumed that when spores are used as inoculum, late stage fungi would not prolifically form mycorrhizae at an early stage. Fleming (1983) suggested that infection by late stage fungi occur through mycelia. It is therefore important that investigations be carried out to establish whether organic material or microorganisms associated with organic material stimulate spore germination of late stage fungi, or whether exudates from older parts of roots stimulate them. The 'early stage' fungi have been reported to include species of the genera *Hebeloma*, *Laccaria* and *Pisolithus*, and the 'late stage' fungi include species of *Amanit*a, *Russula*, *Lactarius* and *Leccinum*. In this experiment, *Lactarius deliciosus* and *A. zambiana,* although belonging to the late stage fungi group, produced the best seedling growth responses when compared to other species, probably indicating that their spores managed to germinate and infect the seedling root systems. This highlights that the theory of 'early' and 'late stage' fungi still needs to be further researched. Furthermore, reduction of other beneficial micro-organisms through sterilisation could also have affected performance of certain fungi species. Mycorrhizal fungi function in collaboration with various other microbes. For instance, Duponnois *et al.* (1993) identified mycorrhizal helper bacteria that promoted ectomycorrhizal establishment of *Laccaria lacuna* but inhibited mycorrhizal formation by other fungi.

Influence of individual mycorrhizal fungi on the physiology of seedlings being measured by biomass accumulation, showed *A. zambiana* to be significantly superior ($P<0.05$) to L*actarius edulis*, *L. deliciosus* and control-2 (Table 20). When compared to other mycorrhizal fungi treatments, seedlings inoculated with *A. zambiana* had the lowest shoot-root ratio of 3, indicating an increase in root volume. This increase in root volume could be as a result of its relatively high root percentage colonisation of 64.19 %.

Although it has been shown that differences exist between different fungal species in their influence on the growth of *U. kirkiana* seedlings*,* it is recommended that the identified fungi be further evaluated under different environmental conditions. Work on various mycorrhizal

fungi has been reported to have variable results under different conditions. For example, inoculation with *Paxillus involutus* greatly improved growth and survival of pine seedlings in the field (Laiho, 1970), whereas in the experiment by Lundeberg (1967), the same fungus suppressed seedling growth. Likewise the fungus *Cenococcum graniforme*, which is very drought tolerant and therefore has been recommended for dry areas (Trappe, 1964), has inhibited growth of pine seedlings in some nursery experiments. The observed variation could most likely be explained by individual fungi species differences in their tolerance to various nursery conditions. Different fungi have different optimum environmental conditions and therefore cannot perform the same under artificial nursery conditions. It should therefore be noted that when screening fungi species in controlled artificial environments, it should not be assumed that they would perform the same in nature. On the other hand, it is also difficult to accurately study functions of the respective fungi in nature because of the dynamics of microbial populations in nature and their complex biochemical reactions that occur in the soil. The complexity of myco-ecology in natural systems makes it difficult to isolate and accurately study various factors and assess their effects on the performance of respective fungi species. It can therefore be concluded that laboratory or nursery comparative studies of individual mycorrhizal fungi efficiency would be unlikely to provide reliable information on their field functional capabilities, but it is still a useful technique for identifying potential species.

Despite the evident beneficial effect of individual mycorrhizal fungi, the high mortality (100%) experienced by seedlings inoculated with a mixture of mycorrhizal fungi was indicative that under certain conditions mycorrhizal fungi can exert negative effects on the seedlings. Similar occurrence has been reported by Dominik (1961, cited by Slankis, 1974), who indicated that under adverse nursery conditions the dominance of *Boletus luteus* caused mortality of pine seedlings. There are other studies that have reported situations when profuse growth of mycorrhizal fungi resulted in suppressed growth (Dosskey *et al*., 1990; Stenström *et al*., 1990). Bowen (1994) also highlighted that under certain conditions, mycorrhizal fungi could cause photosynthetic drain on the host plant. It was suggested that this photosynthetic drain on the host plants often happen when there is an imbalance in the symbiotic relationship. Munyanziza and Oldeman (2000) also established in their studies that cotyledons are the sole source of carbon needed for early mycorrhizal colonisation in miombo tree seedlings such that when cotyledons of seedlings are shed too early, the

mycorrhizal fungi would wholly depend on the host seedlings for their carbon requirements. This condition would therefore cause photosynthetic drain on the seedlings. Wright *et al.* (2000) also carried out comparative analysis of carbon physiological processes between mycorrhizal and non-mycorrhizal seedlings of *Betula pachela* and demonstrated that seedlings colonised by *Paxillus involutus* had significantly reduced biomass after six months when compared with the uninoculated. Similar results in nursery studies on the inoculation of pine seedlings with *Pisolithus tinctorius* have attributed this suppressed seedling growth to the drain of photosynthate (sucrose) due to the mycorrhizal demand. This mycorrhizal demand on the host has been estimated to be 10% of the carbohydrates produced by the tree hosts (Harley, 1973).

Although these conditions may exert negative effects on the seedlings, it is, nevertheless, most unlikely that they would have resulted in the total mortality of the *U. kirkiana* seedlings. It has been established that mycorrhizal fungi demand on the host plant can be considerable but the benefits from the association under natural conditions usually outweigh the energy cost by the fungus. The host plant may be able to compensate for an energy drain to the fungus by increasing its photosynthesis (Bowen, 1978).

Various other arguments have been advanced to explain mortality of miombo tree species seedlings under nursery conditions. Munyanziza (1994), working on miombo tree species, investigated the effects of fertilisation on *Pterocarpus angolensis* and also evaluated the effects of different watering regimes on *Afzelia quanzensis* seedlings.

Fertilisation studies established that the normal shoot: root ratio of miombo tree species is disrupted when commercial N and P fertilisers are used in the nursery. It was noted that whereas the shoot: root ratio of *Pterocarpus angolensis* seedlings growing in a natural ecosystem had ratios below 2, this ratio shifted to above 2 when fertilised with N and P. Disturbance of the shoot: root ratio in the miombo tree species through various nursery management techniques such as manipulating root and root environment is considered to act against the natural seedling surviving strategy (Munyanziza, 1994). In nature, the miombo tree species develop a strong taproot, which is allocated a larger share of photosynthetic biomass. The annual shoots die back for 8 to 10 years until sufficient food and energy reserves have accumulated in the root system (Boaler, 1966; Celander, 1983; Chidumayo,

1993). It has been suggested that it is a mechanism by which seedlings develop food reserves in the root system for them to survive during stress periods. Shoot die-back of miombo tree seedlings in natural ecosystems has been known to be triggered by droughts, and other environmental stresses (Chidumayo, 1991, 1992; Ernst, 1988). This die-back does not generally lead to the physiological death of seedlings. The taproot survives the adverse dry periods and new roots and shoots emerge when rain resumes. It serves as a reservoir of water and nutrients (Boaler, 1966). Simard *et al.* (1997) and Molina *et al.* (1992) further highlighted that taproots have access to deeper water and nutrients via inter-hyphal connections with the root systems of larger trees. It can therefore be concluded that in this study, the root systems were confined to limited growing medium in the polythene pots and as such they would not receive a supplementary supply of photosynthetic products through the inter-mycelial root connection that exists in nature. Table 20 shows the shoot: root ratios for the various treatments ranged from 2.04 to 5, indicating that there was more shoot growth than the root development for all the treatments. It can therefore be concluded that use of liquid fertiliser with the following nutrient composition: 5%N, 6%P_2O_5, 7%K_2O_5, Mg (0.1%), Zn (0.03%), Cu (0.02%), B (0.03%) and S (0.15%), disrupted the shoot: root ratio in all the treatments. Nevertheless, it cannot be concluded that disruption of the shoot: root ratio caused mortality of the various treatments because there is no correlation between shoot: root ratio and the percent survival ($r^2 = 0.02$).

Watering regime studies on *Afzelia quanzensis* revealed that moisture stress affected the photosynthetic capacity of seedlings (Munyanziza, 1994). This indirectly affected the amount of carbon available for the fungal symbiont. Lack of this carbon would therefore affect mycorrhizal fungi colonisation processes. This has not been examined in this study.

Pectora roborant cultus recti

As the cause of the seedling mortality in this experiment cannot be conclusively ascertained, further studies need to be carried out to examine the effect of various possible adverse nursery conditions. An area that requires further investigation is the effect of factors such as soil compaction, fertilisation, pH, and moisture stress on the association of mycorrhizal fungi with the host seedlings and their survival. Studies to establish the optimum inoculum level for various mycorrhizal fungi should also be undertaken.

The study has, nevertheless, established that different mycorrhizal fungi vary in their influence on seedling growth. From the mycorrhizal fungi species under study, *L. deliciosus* was noted to have the greatest influence on seedling height but less effective on the seedling biomass, where *A. zambiana* proved superior to the rest of the species. Furthermore, these mycorrhizal fungi have been found to vary in their root colonisation efficiency. *A. zambiana* had the highest colonisation percentage followed by *Cantharellus* sp. Mycorrhizal fungi *L. deliciosus* had the lowest colonisation percentage.

It is therefore recommended that when promising mycorrhizal fungi have been identified under laboratory and nursery conditions, they should be further evaluated under different site conditions, particularly in areas where the seedlings are to be established. It may also be important to examine the behaviour of introduced mycobionts after out-planting because different soils have been shown to vary in their receptivity to different mycorrhizal fungi (Duvert *et al*., 1990; Plenchette *et al*., 1989). Although results in this experiment indicated that all the tested mycorrhizal fungi, i.e. *A. zambiana*, *Cantharellus cibarius*, *Cantharellus* sp., *L. edulis* and *L. deliciosus* did stimulate seedling growth, there is still need to understand ecological processes involved. Areas that require further attention include, understanding effectiveness of different types of inocula (mycelia, spores), and the quantitative study of mycelial growth into the soil of these mycorrhizal fungi and other selected species found in the *U. kirkiana* ecosystem.

5 EFFECT OF ECTOMYCORRHIZAL FUNGI IN THE PROTECTION OF *U. KIRKIANA* SEEDLINGS AGAINST ROOT PATHOGENS

5.1 Introduction

The study on the growth response of seedlings to selected mycorrhizal fungi demonstrated their general beneficial effects but their specific role in the management of soil-borne diseases in *U. kirkiana* has not been established. The high seedling mortality experienced in the various *U. kirkiana* experiments has generally been attributed to poor mycorrhizal colonisation (Högberg, 1982, 1986; Mwamba *et al*., 1992). Mycorrhizal fungi among many other beneficial ecological reactions, have mostly been known to improve seedling survival and growth by enhancing uptake of nutrients and water and protecting the root system against soil-borne pathogens (Harley and Smith, 1983).

Although mortality of *U. kirkiana* seedlings has been considered to occur as a result of low available phosphate (P) in the miombo soils (Nye and Greenland, 1960; Trapnell *et al*., 1976), symptoms exhibited, however, indicate presence of pathogenic agents. Deficiency of P in *U. kirkiana* seedlings is often noted by the yellowing of leaves that eventually turn necrotic leading to death of the seedling. In nurseries, these symptoms are often accompanied by hypocotyl rot which spreads to the entire root system. Symptoms of hypocotyl rot develop as dark brown water-soaked lesions at the base of hypocotyls that possibly cause the die-back (Khangura *et al*., 1997).

Soil pathogens that are commonly found in most Zimbabwean soils and attack a wide range of plants, include fungi such as *Phytophthora* spp., *Pythium* spp., *Rhizoctonia* spp. and the bacteria belonging to the common *Pseudomonas* genus (Cole *et al*., 1998). Etiology and epidemiology of these soil-borne diseases have generally been known to be influenced by soil and physiological host plant factors. Seedling resistance to these infections is most often attributed to improved nutrition that boost host defences or to direct inhibition of pathogen growth and activity by both soil minerals and biological agents (Duff and Defago, 1997).

With the extensive recognition of a relationship between plant nutrient status and the development of root diseases (Duff and Defago, 1997; Von Broembsen and Deacon, 1997), it can be postulated that mortality of *U. kirkiana* seedlings possibly occurs as a result of both

the direct effects of nutrient deficiency and indirectly by making seedlings more susceptible to infections.

The benefits of mycorrhizal fungi in protecting plant roots against soil-borne pathogens are well documented (Marx, 1970, 1973; Quales, 1999). Reported studies on the activities of specific mycorrhizal fungi are, however, more on exotic commercial tree species such as pine (Gorissen and Kuyper, 2000; Lussenhop and Fogel, 1999). Limited work on the role of mycorrhizal fungi in root disease management in the miombo ecosystem has been undertaken. This work was therefore undertaken to identify mycorrhizal fungi that are effective in protecting *U. kirkiana* seedlings from commonly found soil-borne pathogens.

Specific objectives of the study were the following:

(i) Assess effect of mycorrhizal fungi in the management of *U. kirkiana* root diseases.

(ii) Evaluate effect of macro and microelements on the growth of pathogenic fungi.

5.2 Methods and materials

5.2.1 Effect of mycorrhizal fungi on the development of *U. kirkiana* root diseases

5.2.1.1 Mycorrhizal fungi inoculum preparation

Freshly collected fruiting bodies of *Amanita zambiana, Cantharellus ciberius, Lactarius deliciosus and Lactarius edulis,* were air-dried in paper bags. Inocula were prepared by grounding the whole fruit bodies of the respective fungi into a fine powder using a laboratory grinder. Mycorrhizal fungi colonisation efficiency data from Chapter 4 (par. 4.3.3) was used in this experiment. The inocula were kept in vials at 4^0C (Theron, 1991) for a week before use. To establish nutrient composition of each of the four mycorrhizal fungi, the following methods of analysis were used: Available P was analysed using the method described by Koroleff (1976), N was analysed using the Kjeldahl method and the exchangeable bases were analysed by the method described by Hesse (1971).

5.2.1.2 Soil pathogen inoculum preparation

Rhizoctonia solani

An isolate of *Rhizoctonia solani* was collected from *U. kirkiana* growing in the nursery. Four week-old seedlings showing typical symptoms of damping-off were collected and their roots

were gently washed under running tap water to remove adhering soil. The roots were then cut into 0.5 cm long pieces and placed in the incubator at 25^0C for 48 hours. After 48 hours, colonies resembling *R. solani* were sub-cultured onto fresh Potato dextrose agar (PDA) containing 10 mg/kg aureomycin hydrochloride (PDA+A). After two days, hyphal tips from all *Rhizoctonia*-like colonies were transferred onto fresh PDA+A and incubated at 25^0C, which is the recommended temperature for the growth of *Rhizoctonia* species (Khangura *et al*., 1999). The inoculum was maintained on PDA in 9 cm diameter petri dishes. The inoculum was subcultured weekly and maintained at 23^0C during the period in which experiments were conducted. Pathogenicity of this isolate on *U. kirkiana* was tested on germinating seeds on 2% water agar in petri dishes. Five petri dishes were each seeded in the centre with a PDA disc (7 mm in diameter) of the inoculum. Two days after seeding the petri dish with *R. solani,* six *U. kirkiana* seeds were evenly spaced on the agar surface in a circle halfway between the centre and the edge of the dish. The petri dish was placed in the incubator at 23^0C for seven days. The growing *R. solani* reached the seeds just as they began to germinate. Notes on the disease development were taken daily, starting after seven days and these included symptoms such as discolouration and necrotic lesions.

Phytophthora parasitica

The fungal pathogen *Phytophthora parasitica* was isolated from a diseased tobacco plant by the Zimbabwe Tobacco Research Board Plant Pathology unit. The inoculum was maintained on PDA in 9 cm diameter petri dishes. The inoculum was sub-cultured on PDA weekly and maintained at moderate growth rate at 22^0C, which is a temperature below the optimum range 25-27^0C (Griffin, 1979), during the period in which experiments were conducted. Pathogenicity of this isolate on *U. kirkiana* was tested in seedlings raised in sand media by injecting a suspension of mycelia of the pathogen at the seedling root collar level.

Pseudomonas solani

A *Pseudomonas solani* isolate was obtained from the Tobacco Research Board and was sub-cultured in 9 cm diameter petri dishes on Kelman's Tetrazolium chloride (TZC) medium. This was prepared as follows: 10 g peptone, 5 g glucose, 1 g casein hydrolysate, 0.05 g tetrazolium chloride and 15 g agar mixed in one litre of sterile deionised distilled water. The pH ($CaCl_2$) of the medium was established at 7.2 by slowly adding 2M NaOH. The medium was autoclaved at 121^0C for 15 minutes. The culture was incubated at 24^0C for ten days.

Pathogenicity of this pathogen on *U. kirkiana* was established by placing seeds on the bacterial inoculum. Development of the disease was determined by observing symptoms such as radicle and foliage discolouration.

5.2.1.3 Experimental layout and inoculation procedure

Two hundred 5 cm diameter polythene pots with height of 12 cm were filled with sterilised sand growth media. These pots were made up of the following treatment combinations of mycorrhizal fungi and pathogenic agents replicated ten times: RT1, RT2, RT3, RT4, PT1, PT2, PT3, PT4, BT1, BT2, BT3, BT4, CT1, CT2, CT3, CT4, CP, CR, CB and CC. The three pathogenic agents were R- *Rhizoctonia solani,* P- *Phytophthora parasitica,* B- *Pseudomonas solani,* C- Control (seedlings not inoculated with any of the mycorrhizal fungi) and CC- Control (Non-mycorrhizal seedlings not challenged with any of the pathogens). Mycorrhizal fungi were as follows: T1- *Cantharellus ciberius*, T2- *Lactarius edulis,* T3- *Lactarius deliciosus,* T4- *Amanita zambiana.* These treatment combinations were laid out in a completely randomised design.

Five grams of the respective mycorrhizal fungi inocula material were placed at 4 cm depth of the sand media in each polythene pot as per experimental layout. Seedlings raised in sterilised pine bark were transplanted into the polythene pots inoculated with mycorrhizal fungi when the first pair of leaves had developed. These seedlings were watered when necessary to meet their moisture requirements. Three petri dishes of each of the three pathogens were flooded with sterile distilled water, the inoculum was scraped using a spatula and the suspension was mixed with sterile water making up volumes of 250 ml per pathogen. Each suspension was then homogenised using a rotary shaker (150 rpm) for a period of 20 minutes. At the second month of the growth period after transplanting, seedlings were inoculated with the three pathogens, *Rhizoctonia solani*, *Phytophthora parasitica* and *Pseudomonas solani.* A syringe was used to inject 5 ml of the inoculum at the seedling root collar level. Influence of disease infection levels on the physiology of the seedlings was assessed by biomass accumulation after four months of growth. Plants were oven dried for 96 hours at 45^0C and their biomass determined.

5.2.1.4 Pathogenicity assessment

After four months from the time of transplanting, seedlings were harvested and the roots were thoroughly washed and rated for taproot rot on a 0 to 5 scale; (0 = no lesions observed, 1 = small lesion on taproot, 2 = necrosis up to 30% of taproot, 3 = necrosis covering 31 to 60% of the taproot, 4 = necrosis covering 61 to 99% of the taproot, 5 = lesion completely severing taproot). Plants were also rated on a 0 to 5 scale for rot of lateral roots (0 = no lesions observed, 1 = 1 lateral root girdled, 2 = 2 to 5 lateral roots girdled, 3 = 6 to 10 lateral roots girdled, 4 = 11 to 15 lateral roots girdled, 5 = >15 lateral roots girdled). Percent disease index was calculated using the following formula used by Khangura *et al*. (1999):

Percent disease index formula = [Σ(No. of plants in disease category) x (numerical value of disease category) x 100]/[(No. of plants in all categories) x (maximum value on rating scale)]

5.2.2 Effect of macro and microelements on soil-borne pathogens

Micro and macro-elements amendments have been used commercially on a limited scale to manage certain soil-borne diseases (Duff and Defago, 1997). Disease reduction is most often attributed to improved nutrition that boosts host defenses or to direct inhibition of fungal growth and activity. Pathogen suppression may also result indirectly from amendment-mediated modification of chemical and physical properties such as soil and rhizosphere pH (Simon and Sivasithamparam, 1989) or from modification of host root exudates to disfavour pathogenic activity (Huber, 1989). As mycorrhizal fungi are considered important for enhancing nutrient uptake by plants, it is possible that this improved nutrient uptake does also help develop the host plant defence mechanisms against root diseases. Plant tolerance to root and other diseases has been reported to be enhanced by manipulation of soil levels of Ca, Zn and Cu (Babich and Stotzky, 1978; Donaldson and Deacon, 1993; Halsall and Forrester, 1977). This experiment was therefore undertaken to assess direct effects of these three nutrients to the two pathogenic fungi, *Phytophthora parasitica* and *Rhizoctonia solani* and also to establish the nutrient composition of different mycorrhizal fungi in order to examine their possible role in the management of soil-borne pathogens. The bacterial pathogen *Pseudomonas solani* was not included in this assessment because it would be difficult to comparatively analyse bacterial growth patterns with those of the fungi.

Pure salts used in the study were calcium chloride, copper sulphate and zinc sulphate. These salts were selected on the basis of their previous use *in vitro* efficacy studies on various fungi (Babich and Stotzky, 1978; Kao and Ko, 1986). To assess effect of these salts on the growth of *Phytophthora parasitica* and *Rhizoctonia solani*, the respective salts were prepared in sterile deionised distilled water, and were added to autoclaved warm (45 to 55^{0}C) Malt Extract Agar (MEA) to provide a final concentration of 1000 mg of each respective element per litre (Biggs, 1999). The media was poured into 9 cm diameter petri dishes. MEA not supplemented with salts was included as a control. All the treatments were replicated ten times. To assess the effect of the salts on the mycelial growth of the two pathogenic fungi, 5 mm diameter discs of MEA fully covered with mycelia of the respective pathogen were placed in the centre of each experimental petri dish. Growth of mycelia was assessed daily for four days by measuring the mycelia diameters. Where no growth was observed diameter measurement was scored as zero.

Data on the shoot length and total dry mass from the sixteen treatments and the data on the mycelial growth were analysed using one-way analysis of variance by means of SAS statistical software. The applicable model is:

$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$

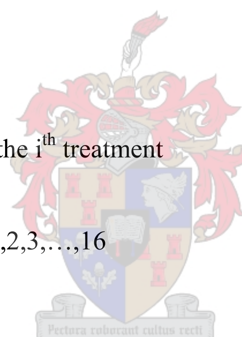
Where:

Y_{ij} = j^{th} observation taken under the ith treatment

μ = overall mean

α_i = effect of ith treatment, i = 1,2,3,…,16

ε_{ij} = random error component



Significant responses were tested at 5 % level.

5.3 Results

5.3.1 Mycorrhizal fungi colonisation efficiency assessment

Mycorrhizal fungi colonisation efficiency was assessed after four months of seedling growth by destructive sampling and examining fine roots of the control seedlings (CT1, CT2, CT3, and CT4) that were not challenged with any pathogens. To estimate percentage infection, sub-samples of the fine roots of each treatment were stained with trypan blue and examined

under a stereo-microscope to determine the ratio of 'number of mycorrhizal short roots: total number of short roots examined'. Mycorrhizal fungi colonisation efficiency for the different fungal species were anlysed using ANOVA and the results indicated that these were statistically significantly different at $P < 0.01$ (Table 21). Table 22 shows percentage root colonisation of the different mycorrhizal fungi.

Table 21

Analysis of variance for different mycorrhizal fungi percentage root colonisation

Source	DF	SS	MS	F-Value	Pr>F
Treatment	4	7714.99	1928.74	7.43	0.0001*
Error	50	12982.61	259.65		
Corrected Total	54	20697.61			

Table 22.

Different mycorrhizal fungi root colonisation percentages

	Mycorrhizal fungi species				
	C. cibarius	*L. edulis*	*L. deliciosus*	*A. zambiana*	*Cantharellus* sp.
Treatment code	1	2	3	4	5
% Colonisation	39.69b	42.33b	33.8b	66.21a	53.35ab

* Values within the row that have the same letter are not significantly different at 5%.

5.3.2 Pathogenicity assessment

Analysis of the pathogenicity of the three pathogens; *Rhizoctonia solani*, *Phytophthora parasitica*, and *Pseudomonas solani* on seedlings inoculated with different mycorrhizal fungi further shows their varying responses to different species of mycorrhizal fungi [Fig.4 (a) and (b)]. Although *A. zambiana* had the highest colonisation efficiency (Table 22), its protective effect was highest only against bacterial lateral root infections and less so against the two pathogenic fungi *Rhizoctonia solani* and *Phytophthora parasitica.*

Following disease assessments, three to five root pieces with lesions were placed onto 2% water agar in petri dishes to re-isolate the *Rhizoctonia solani* and *Phytophthora parasitica* isolates to fulfil Koch's postulate. This is a technique of establishing the causative agent of a disease by re-isolating the pathogen from an inoculated diseased plant.

5.3.3 Effect of mycorrhizal fungi on the development of *U. kirkiana* root diseases

A comparative disease development trend in lateral roots and taproots for various treatments is clearly demonstrated graphically in Figures 4 (a) and (b). From the histograms it is evident that seedlings infected with *Rhizoctonia solani* had low taproot infection levels when compared to those challenged with *Phytophthora parasitica* and *Pseudomonas solani.* On the other hand, there were low lateral root disease infections in the seedlings that were challenged with pathogenic agents *Phytophthora parasitica* and *Pseudomonas solani* as compared to seedlings inoculated with the pathogenic fungi *Rhizoctonia solani,* which had high disease infection levels ranging from 31.2% to 66.9%.

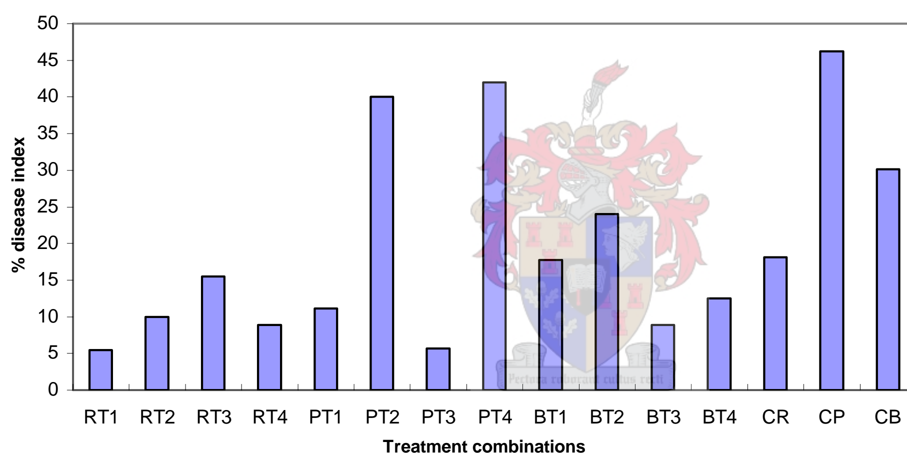


Fig. 4 (a). Infectivity of *Phytophthora parasitica* , *Pseudomonas solani* and *Rhizoctonia solani* on seedlings inoculated with different mycorrhizal fungi (taproot rot)- see par. 5.2.1.4 for explanation of treatment codes

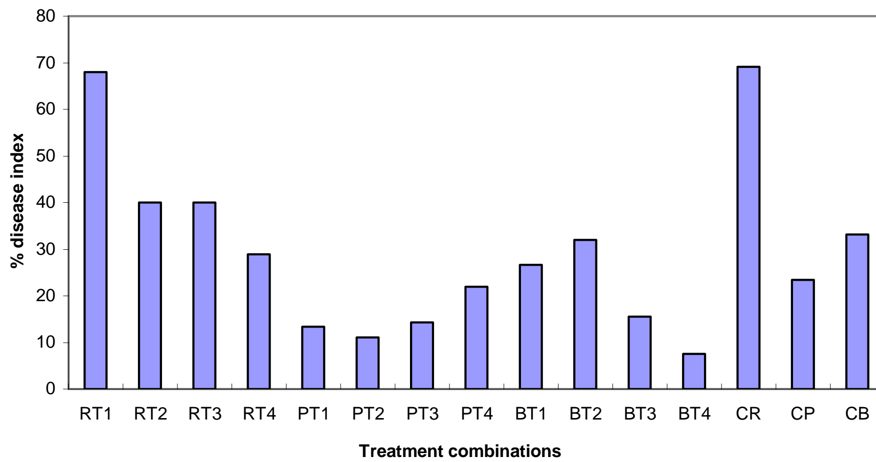


Fig. 4 (b). Infectivity of *Phytophthora parasitica, Pseudomonas solani* and *Rhizoctonia solani* on seedlings inoculated with different mycorrhizal fungi (lateral root rot)- see par. 5.2.1.4 for explanation of the treatment codes

Figures 4 (a) and (b) exhibit a marked difference in percentage disease infection between taproot and lateral roots. Lateral root rot percentage infections were generally higher than the taproot rot infection, probably showing that lateral roots are more prone to infections to the three pathogens than taproots. On more specific comparison, the seedlings inoculated with mycorrhizal fungus *C. ciberius* had the second highest lateral root-rot percentage infection (66.9%) after the control which had 69.1% infection when infected with *Rhizoctonia solani* isolate, most likely indicating poor lateral root protection by this particular mycorrhizal fungus. Lowest lateral root-rot percentage infection (9.5%) occurred with seedlings inoculated with mycorrhizal fungus *A. zambiana* when infected with *Pseudomonas solani.* The lowest taproot percentage infection was with *C. ciberius* inoculated seedlings (5.4%) that were infected with *Rhizoctonia solani.* For the control experiments, *Rhizoctonia solani* had 18.1% infection level whereas *Pseudomonas solani* and *Phytophthora parasitica* had 46.2% and 30.1%, respectively for the taproot infection, reflecting a general higher infection trend for seedlings not inoculated with mycorrhizal fungi. *Rhizoctonia solani* had, nevertheless, the highest infection levels in lateral roots, probably indicating parts of the root system where it has the most competitive advantage over the other two pathogens.

The mycorrhizal fungus that had the lowest colonisation efficiency was *L. deliciosus* and it generally had moderate protection against all three pathogens. Its root protection efficiency against *Phytophthora parasitica* was however, remarkably better than that of *A. zambiana*

which had a disease infection level of 24%. On the other hand, this mycorrhizal fungus offered less protection against *Rhizoctonia solani,* reflecting lateral root infection level of 42% as compared to *A. zambiana* inoculated seedlings that had *Rhizoctonia solani* infection level of 31.2%. Effect of the different treatments on the seedling biomss was analysed and the results indicated that they were statistically significantly different at $P<0.01$ (Table 23). Effects of different treatment combinations on the seedling total dry mass, root biomass and shoot biomass are presented in Table 24.

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Table 23.
Analysis of variance of effect of the sixteen mycorrhizal fungi-pathogen treatment combination on the total dry mass of *U. kirkiana* seedlings after four months of growth

Source	DF	SS	MS	F Value	Pr>F
Treatment	15	5.63	0.37	2.31	0.0066*
Error	114	18.55	0.16		
Corrected Total	129	24.18			

R-Square	Coeff Var	Root MSE	Total dry mass
0.23	47.37	0.40	0.85

* Significant at 5%

Table 24.

Effect of different treatment combinations on *U. kirkiana* seedling biomass production after four months of growth

Leaf number		Total dry mass (g)		Root dry mass (g)		Shoot dry mass (g)	
Treat-Ment[1]	Mean	Treat-ment	Mean	Treat-ment	Mean	Treat-Ment	Mean
PT1	12.12a	RT3	1.22a	RT3	0.37a	RT3	0.85a
BT2	11.87ab	PT3	1.11ab	PT3	0.35ab	PT1	0.84a
RT3	11.55ab	PT1	1.07ab	RT4	0.33ab	PT3	0.76ab
CT3	10.60abc	PT2	1.03abc	PT2	0.32ab	PT4	0.74abc
BT1	10.00abc	RT4	1.00abc	CR	0.29ab	PT2	0.71abcd
PT3	9.71abc	PT4	0.96abc	BT2	0.28ab	RT4	0.67abcde
CP	9.60abc	BT2	0.95abc	RT2	0.28ab	BT2	0.67abcde
PT2	9.57abc	CC	0.87bc	RT1	0.26ab	CC	0.64abcde
RT2	9.00abc	RT2	0.84bcd	CB	0.26ab	BT4	0.61abcde
RT1	9.00abc	CR	0.82bcd	PT1	0.23ab	CT1	0.57abcde
BT3	9.00abc	BT4	0.81bcd	CC	0.23ab	RT2	0.56bcde
CT2	8.75abc	CT1	0.78bcd	CT4	0.22ab	CR	0.53bcde
CT1	8.55abc	CT4	0.74bcd	PT4	0.22ab	CT4	0.52cde
PT4	8.44abc	BT3	0.68cd	CT1	0.21ab	BT3	0.47cde
CT4	8.12abc	RT1	0.68cd	BT3	0.21ab	CT3	0.42de
BT4	7.50abc	CB	0.68cd	CT3	0.21ab	RT1	0.42de
CB	7.33abc	CT3	0.63d	BT4	0.20ab	CB	0.42de
RT4	7.00bc	BT1	0.55d	BT1	0.20ab	CP	0.37de
CR	6.30c	CP	0.52d	CP	0.15b	CT2	0.37de
CC	6.10c	CT2	0.52d	CT2	0.15b	BT1	0.35e

* Values within a column with the same letter are not statistically significantly different at 5%. Tukey's Studentised Range Test was used to compare the means.

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1 See par. 5.2.1.4 for the description of treatment combinations

Treatment combination RT1 had statistically significantly lower biomass ($P < 0.05$) than treatment combinations RT3, PT1, and PT3 (Table 24) but was not significantly different from the rest of the treatments including the control treatments (CT1, CT2, CT3, CT4, CP, CR, CB and CC). Evidently there is more significant variation in total dry mass and shoot dry mass than with the root dry mass.

The treatment combinations RT3, RT4, PT1, PT2, and PT3 had significantly high biomass when compared to the following treatments: CT3, BT1, CP, and CT2 (Table 24). Mycorrhizal fungi T1 (*Cantharellus ciberius*), T2 (*Lactarius edulis*), T3 (*Lactarius deliciosus*) and T4 (*A. zambiana*) had significant positive effects on the seedlings that were inoculated with *Phytophthora parasitica.*

5.3.4 Effect of macro and microelements of soil-borne pathogens

Analysis of variance for the effect of micro and macro-elements on mycelial growth of *Rhizoctonia solani* and *Phytophthora parasitica* is shown in Tables 25 (a) and (b). From the analysis of variance it was shown that Ca, Cu and Zn were statistically significantly different in their effect on the growth of *Rhizoctonia solani* and *Phytophthora parasitica.*

Table 25 (a).

Analysis of Variance for effect of micro and macro-elements on mycelial growth of *Rhizoctonia solani* after four days of growth

Source	DF	SS	MS	F Value	Pr>F
Treatment	3	1348.11	449.37	15.97	0.0001**
Error	36	1013.24	28.14		
Corrected Total	39	2361.36			

R-Square	Coeff Var	Root MSE	Mycelia mean area
0.57	77.47	5.30	6.84

** Significant at 0.1%

Table 25 (b).

Analysis of Variance for effect of micro and macro-elements on mycelial growth of *Phytophthora parasitica* after four days of growth

Source	DF	SS	MS	F Value	Pr>F
Treatment	3	40.35	13.45	26.74	0.0001**
Error	36	18.10	0.50		
Corrected Total	39	58.45			

R-Square	Coeff. Var	Root MSE	Mycelia mean area
0.69	38.98	0.70	1.82

** Significant at 0.1 %

Table 26.

Effects of Ca, Cu and Zn on growth of *Rhizoctonia solani* and *Phytophthora parasitica*

Pathogenic fungi	Mycelial mean area of growing in different elements (cm^2)			
	Ca	Cu	Zn	Control
Rhizoctonia solani	43.69a	9.69b	0.00b	56.17a
Phytophthora parasitica	10.82a	0.45b	8.98a	8.87a

*Values within each row followed by the same letter are not significantly different (P<0.01) tested using Tukey's studentised Range Test.

Zn and Cu inhibited mycelial growth of *Rhizoctonia solani* while Ca did not markedly differ from the control (Table 26). However, Zn was not as effective against *Phytophthora parasitica* but Cu restricted its mycelial growth. Details on the biocidal effect of Ca, Cu and Zn on *Rhizoctonia solani* and *Phytophthora parasitica* are shown in Table 26.

Effectiveness of micro and macronutrients that accumulate in mycorrhizal fungi in the management of the soil-borne pathogens has not, however, been verified. Nutrient composition of different mycorrhizal fungi is shown in Table 4 (Chapter 2). *L. deliciosus* had the highest N, Fe, Mn, Zn and Cu levels. *A. zambiana* had the highest P, K, Ca and Mg. Variation in Ca and Mg levels was minimal in the different mycorrhizal fungi.

5.4 Discussion

The variation in the susceptibility of lateral roots and taproots to different pathogens could possibly imply that the taproots were protected against *Rhizoctonia solani* but were more prone to infections by *Pseudomonas solani* and *Phytophthora parasitica* which caused higher infections of 13.7% and 19.8% respectively. Such a situation could possibly be explained by that *Pseudomonas solani* as a plant vascular system infecting bacteria would show systemic symptoms of its infection process along the vascular vessels in the taproot and stem. Similarly, the relatively high taproot infection in the seedlings infected with *Phytophthora parasitica* could be explained by its disease development process. Infection cycles of *Phytophthora parasitica* occur through motile zoospores (Von Broembsen and Deacon, 1997). It has been established that zoospores locate the most susceptible sites on roots through chemotaxis (Deacon and Donaldson, 1993; Jones *et al*., 1991) and other mechanisms (Morris *et al*., 1992). Zoospores are also transported more readily through water capillary movement through the soil than non-motile cells (Wilkinson *et al*., 1981). This probably explains the competitive advantage of *Phytophthora parasitica* over *Rhizoctonia solani* in the rate of spread of the disease from the primary sources of infection (lateral roots) to the taproots. *Rhizoctonia solani* infection process is dependent on either germ tubes growing from basiodiospores or old mycelia that would need to grow through the growth media to come into contact with the host's roots (Atlas and Bartha, 1987).

Effectiveness of *A. zambiana* in protecting the seedlings against bacterial lateral root rot was not clearly demonstrated in this experiemnt but production of antibiotic compounds by *Amanita* species has widely been reported (Marx 1973). Most of these species were reported to produce both anti-fungal and anti-bacterial compounds. Variation in the mycorrhizal fungi colonisation efficiency and their respective differences in the protection they offer against different pathogens is indicative of importance of choosing the right mycorrhizal fungi for management of a particular plant root pathogen. This is also particularly important when considering that these three pathogens differ in their pathogenicity. Furthermore, inherent differences of the respective mycorrhizal fungi in their effectiveness in protecting roots against the three soil-borne pathogens would also influence disease development. Variation in the ecological competency of different mycorrhizal fungi, that is, their ability to develop mycorrhizal associations and to express beneficial influence to the host plant has been

discussed in Chapter 4. Factors contributing to this variation have been noted to include, *inter alia,* spore germination and mycelial growth rate.

Although some degree of protective effect of different mycorrhizal fungi against soil-borne pathogens in *U. kirkiana* seedlings has been established, the study did not reveal the mechanism on how such protection was achieved. Mycorrhizal fungi are known to protect plants against root pathogens in several ways. These include production of antibiotics by the fungi or by the host and creation of a mechanical barrier by a fungal mantle that protects roots from pathogens (Marx, 1973). Mechanical barrier mechanism has been demonstrated with *Phytophthora cinnamomi* on pine seedlings when pathogenic infection occurred in meristem tissues that were not covered by a fungal mantle, whereas infection failed in meristem tissues that were covered by a mantle. In chemical production mechanism, Marx (1973) discusses the production of inhibitory substances during the host plant metabolic response to pathogenic attack. Antifungal chemicals such as phenols, quinones, various phytoalexins, and numerous other compounds have been found in root tissues during infection processes of both the mycorrhizal and pathogenic fungi (Cruickshank, 1963; Tomiyama, 1963). It has also been established that different mycorrhizal fungi produce different kinds of antibiotics that vary in their effectiveness in inhibiting different pathogens. For instance, antibiotics produced in association of pine roots with mycorrhizal fungi, were active against bacteria and not fungi (Marx, 1973). On the other hand, association of *Eucalyptus marginata* with mycorrhizal fungi *Boletus luteus* and *Lactarius deliciosus* has been reported by Pratt (1971), to produce antibiotics that are active against the pathogenic soil fungus *Phytophthora cinnamomi.*

Other than the mycorrhizal fungi root protection processes and the pathogenicity of the respective pathogens, there are more complex ecological reactions that occur in soil under various conditions that result in the production of diverse chemical compounds that can influence disease development. Duijff *et al*. (1999) and Kloepper *et al*. (1980), established that production of certain biocides are highly influenced by factors such as the host species, climate and soil factors, such as soil microbial population and nutrient status. Influence of soil nutrient levels has been considered important in the management of several soil-borne pathogens. In this experiment, results from the *in vitro* studies indicate that both Zn and Cu were significantly effective against *Rhizoctonia solani.* These elements suppressed growth of

Rhizoctonia solani probably through their anti-fungal properties**.** Copper was also significantly effective against ***Phytophthora parasitica*** (Tables 26). Effect of Calcium, was on the other hand, not signicantly different from the control on both *Rhizoctonia solani* and *Phytophthora parasitica.* Duff and Defago (1997) established that Zn stimulated *in vitro* fungal production of anti-microbial compounds such as 2,4-diacetylphloroglucinol (PHL), pyoluteorin (PLT), and hydrogen cyanide. It is therefore possible that inhibition of *Rhizoctonia solani* mycelial growth by Zn was through these chemical compounds. The ability of Cu to achieve suppression of *Phytophthora parasitica* growth could also be through various biochemical reactions that interfere with its growth processes. Mechanisms on how Cu interferes with the growth of this fungal species have not been examined in this experiment. Further work on whether Cu does also interfere with zoospore production or whether it also stimulates production of anti-fungal compounds should therefore be undertaken. Calcium did not, however, markedly slow down mycelial growth of both *Phytophthora parasitica* and *Rhizoctonia solani*. Von Broembsen and Deacon (1997) established that Ca was effective against *Phytophthora parasitica* and many other zoospore forming pathogens through suppressing production of zoospores, which are important for pathogen infection processes. This would therefore imply that Ca does not have the direct intrinsic fungicidal effect but is able to slow down disease progression by affecting production of zoospores. The effect of Ca in soil-borne disease suppression has been reported by several workers (Deacon and Donaldson, 1993; Jones *et al*., 1991; Zentmyer, 1961). It is therefore possible that mycorrhizal fungi that accumulate Ca contribute in the inhibition of zoospore production by providing the extracellular Ca necessary for immobilising *Phytophthora* spp. zoospores. The other possibility is that mycorrhizal fungi block the most susceptible sites on the roots resulting in zoospores failing to locate them.

Pectora roborant cultus recti

Various other elements have been reported to be important for the production of biocides that also help control disease development. For instance, Fe was reported to be important for hydrogen cyanide production, while molybdenum and magnesium stimulated *in vitro* production of salicylic acid which is also an anti-microbial compound (Voisard *et al*., 1994). It is therefore possible that mobilisation of various micro and macro-elements by different mycorrhizal fungi populations occurring in different soil types could probably explain the variations in susceptibility of different soils to soil-borne pathogens. Further work is,

however, necessary to establish the optimum conditions for production of these compounds particularly in the management of *U. kirkiana* seedling root diseases.

Impact of the interaction of the three respective soil-borne pathogens, *Rhizoctonia solani, Phytophthora parasitica* and *Pseudomonas solani,* and the mycorrhizal fungi on the physiology of the host plant, was assessed by the measure of biomass accumulated by seedlings subjected to various treatment combinations. The statistically significant increase in total dry mass in the seedlings that were infected with *Rhizoctonia solani* and *Phytophthora parasitica* (RT3, RT4, PT1, PT2, PT3, PT4) when compared to the following treatments: BT1, BT3, RT1, CT2, CT3, CB, CR, CP, CT1, CT2, CT3, could be indicative of compensatory growth. Compensatory growth has been reported in an experiment on barley (*Hordeun vulgare*), whereby Crossett *et al*. (1975) demonstrated compensatory response to unfavourable root temperature. Seedlings with roots subjected to an unfavourable soil temperature of 10^0C had shoot dry weight that was statistically significantly greater than the seedlings grown under favourable root temperature (20^0C). Compensatory growth has been explained by Russell (1982), as a factor of 'source-sink' relationships. When a part of a root system is exposed to unfavourable conditions it causes a reduction in the root 'sinks' and therefore changing the balance of source-sink relationship of the plant. The supply of metabolites to those roots which remain is thereby increased, resulting in the stimulation of shoot growth. In this experiment compensatory growth was, however, not noted in the control seedlings that were not inoculated with mycorrhizal fungi, thereby possibly indicating that in *U. kirkiana*, mycorrhizal fungi are also important for compensatory growth under certain soil conditions. The other seedlings that did not exhibit compensatory growth were those infected with pathogenic bacteria *Pseudomonas solani.* Mycorrhizal fungi such as *Cantharellus cibarius*, *Lactarius edulis*, *Lactarius deliciosus,* and *Amanita zambiana* should be investigated for possible use as inocula in indigenous tree nurseries. Particular attention should be given to the mycorrhizal fungus *Cantharellus cibarius,* which showed inconsistent reaction when infected with the pathogenic fungus *Rhizoctonia solani.*

Lack of compensatory growth in seedlings inoculated with *Pseudomonas solani* could possibly be explained by that in bacterial diseases the vascular system is disrupted and therefore, water and nutrient uptake by the plants is impeded. The difference with root rot diseases caused by *Phytophthora* species, would be the rootlet regeneration, which has been

reported to occur when roots are infected with this pathogen (Palzer, 1976). Rootlet regeneration for seedlings infected with *Phytophthora parasitica* would therefore be able to maintain water and nutrient uptake by the plant. With the *Rhizoctonia* species, the disease escape could be explained by that its infection process occurs through germ tubes or old mycelia and therefore would be less aggressive and therefore allowing the plant to accumulate biomass particularly when other conditions such as nutrients, moisture, temperature and pH are optimum.

Findings of this study indicated that use of mycorrhizal fungi in the management of root diseases is dependent on the type of root pathogen. Different pathogens have different pathogenicity and therefore have different disease severity. The study further highlighted that efficiency of mycorrhizal fungi's ability to protect roots against infection is influenced by factors such as colonisation efficiency and the biological differences of the respective mycorrhizal fungi in protecting plant roots. The experiment has also demonstrated that certain macro and micro-elements inhibit growth of pathogenic fungi such as *Rhizoctonia solani* and *Phytophthora parasitica*. Results of this work could probably be used to explain differences in the ability of mycorrhizal fungi to protect roots against root pathogens, particulrly if the mycorrhizal fungi species differ in their nutrient sequestration.

Although these findings highlight the potential use of mycorrhizal fungi in the management and control of soil-borne pathogens, further studies are necessary to establish the behaviour of these mycorrhizal fungal species in the diverse complex ecological processes that occur in different soil types. Etiology and epidemeology of soil-borne pathogens have been reported to vary in different soil types (Duijff *et al*., 1999; Lemanceau, 1989). Findings of this study should therefore contribute to the development of a cost effective and environmentally safe method of managing these and various other problematic pathogens and therefore improve the establishment and survival of *U. kirkiana* seedlings.

Pectora roborant cultus recti

6 GENERAL DISCUSSION

The study on the 'Ecology and patterns of distribution of mycorrhizal fungi in the *U. kirkiana* dominated miombo woodland ecosystem' revealed that among the soil factors, pH and K had significant influence on the mycorrhizal population and their distribution. Nutrients such as N and P did not directly exhibit significant influence but were considered to have an indirect effect on the physiological processes that affect host plant-fungal relationships. P was noted to be essential for the production of ATP compounds, which are an important source of energy for supporting mycorrhizal plant association, whereas N as a component of NH_2, has been noted to be inhibitory to mycorrhizal colonisation. Influence of climatic factors was also established. The humid period, which was assessed by determining the residual moisture, i.e. the difference between evapotranspiration and precipitation, was found to be most ideal condition for the production of fruit bodies by the mycorrhizal fungi. The weak influence exhibited by the host plant population diversity on the mycorrhizal fungi distribution was possibly because these fungal species have a wide host range (Janos, 1980; Simarda *et al*., 1997), and are therefore not greatly influenced by host plant diversity in the different locations.

Other ecological areas that were investigated were the influence of soil nutrient dynamics on the *U. kirkiana* seedling growth. In Chapter 3, the study on the nutrient dynamics in the woodland ecosystem highlighted the importance of soil type in raising seedlings. It was shown that choice of potting soil for raising seedlings could be determined through soil analysis. Soils that have high divalent nutrients such as Ca^{++} and Mg^{++} are not suitable for use as nursery soil**.** This study proved that soils with Ca++ levels exceeding 2 $cmol_c$/kg adversely affected seedling growth. With Mg^{++}, it could be considered that soils with levels above 1$cmol_c$/kg would not be suitable. Foliar analysis could also be used to identify soils for use in raising *U. kirkiana* seedlings. Growth of seedlings that were raised in soils that were collected from woodlands with rainy season foliar levels of Mn, Zn and B falling below 38 mg/kg, 12 mg/kg and 6 mg/kg respectively, was affected detrimentally. Soils from such sites are therefore not recommended for use as nursery soil. Comparative analysis of both soil and foliar nutrient levels is therefore a useful technique for identifying suitable soils for raising *U. kirkiana* seedlings.

The identification of mycorrhizal fungi species that enhance establishment and growth of *U. kirkiana* offers an opportunity for improving nursery technology of raising seedlings. When nursery soil is not sourced from a miombo woodland ecosystem and therefore probably lacking in appropriate mycorrhizal fungi inoculant, the fungi that have been identified to grow in association with *U. kirkiana* in Chapter 2 can be used to inoculate the soil (Appendices I, II, III). To realise optimum benefit of mycorrhizal fungi activity, inoculated seedlings should be kept under ideal nursery conditions. Adverse nursery conditions could negatively affect growth of seedlings possibly leading to their death. To enhance multiproductivity of the orchard, edible mycorrhizal fungi species such as *Amanita zambiana*, *Cantharellus cibarius*, *Lactarius deliciosus* and *Lactarius edulis* can be given priority in the selection of the inoculants. It is also recommended that the mycorrhizal species that have been identified to commonly occur in the different sites and months should be given preference when preparing inocula. These fungi species could be considered to be adaptable under varying soil and climatic conditions and therefore would perform well as inoculant for seedlings that would be distributed to different sites.

Furthermore, application of nutrients through foliar sprays would be advisable in the nursery if the soil has been proven to be deficient in certain nutrients particularly Mn, Zn and B which have been noted to be critical. Foliar application of nutrients in the nursery would be more recommended than soil application because fertilisers applied through the soil could result in the change of pH that would then affect the activities of mycorrhizal fungi and the whole nutrient uptake system. Nursery soil pH can also be altered by use of poor quality irrigation water, particularly if it has high levels of divalent nutrients such as Ca^{++} and Mg^{++} which can inhibit availability of P and Fe. From the experiment on N and P fertilisers, it was concluded that use of commercial P fertilisers would be beneficial in the domestication of *U. kirkiana* when grown in P deficient soils. Soil analyses for the planting sites should therefore be undertaken prior to planting. In carrying out these analyses it should be considered that concentrations of extractable P in miombo soils varies seasonally and with soil depth (Chidumayo, 1992). The concentration is 5-15 mg kg^{-1} soil in the cool dry season (April-August) and is higher (15-45 mg kg^{-1}) during the other seasons. Chidumayo (1992) also reported that N levels vary throughout the year with the ammonium N ranging from 2-30 mg kg^{-1} and that of nitrate N ranging from 2-20 mg kg^{-1}, with no variation with depth. Further

studies should be carried out to establish optimum levels of various nutrients for the sites that have been identified for planting.

The other benefits of the mycorrhizal fungi survey discussed in Chapter 2, are that it highlighted the diversity of fungal species in different areas. This variation in mycorrhizal fungi populations in different sites indicates that establishment and growth of *U. kirkiana* seedlings in these areas would also vary. This finding is therefore useful for provenance research trials whereby genetic material collected from different sites are evaluated across a range of ecological sites. Performance of genetic material should be assessed in consideration of the mycorrhizal fungi population found growing in association with the tree species in a given area. It is therefore recommended that mycorrhizal fungi populations in provenance testing sites should be identified and considered as part of a management package for the identified superior tree genetic material for the respective site. More work should be done to establish fungal-genetic material partner optimalization for particular sites.

In situations where seedling mortality is experienced, particularly where discoloration of hypocotyl is observed, the study has indicated that infection by the common soil-borne pathogens such as *Rhizoctonia solani*, *Phytophthora* spp. and *Pseudomonas solani* could be the cause. Considering that effectiveness of Cu and Zn against these pathogens was only established under laboratory conditions using 1000 mg per litre concentration, it is recommended that further work should be done to determine concentrations that are safe to use as foliar sprays. Another foliar spray that may be beneficial to the *U. kirkiana* seedlings is Fe. Iron has been proven in this study to be an important trace element in the physiology of *U. kirkiana.* It has been considered important because of its role in chlorophyll formation. Iron deficiency therefore results in growth reduction since it directly affects photosynthesis. Iron deficiency is not commonly reported in tropical forestry because most tropical soils are acidic and therefore have high Fe-solubility. Miombo soils are generally classified as ferragious or ferralitic (Celander, 1983). With the emerging interest in propagation of African indigenous tree species, nursery conditions that affect Fe absorption by seedlings could become a major challenge to foresters. Poor soil aeration and high pH has been reported to affect absorption of Fe by plants (Munyanziza, 1994). Aeration depends on soil structure and soil moisture. Structure of potting soil in most nurseries is often lost through sieving of soil before pot filling. This practice should be avoided when dealing with *U.*

kirkiana seedlings. Over-watering should also be avoided as this could result in poor soil aeration and therefore affecting Fe availability. Optimum watering rates for specific sites should be determined by carrying out local level experiments that take into consideration the soil's physical properties and prevailing weather conditions. Use of Fe foliar sprays in the nursery is evidently valuable in nursery conditions that inhibit its uptake by plants. Foliar application of Fe in the nursery could also be beneficial to *U. kirkiana* seedlings as it could provide the seedlings with chlorophyll content that would help sustain the photosynthetic requirements of the seedlings during the winter period when some defoliation occurs. Further research should be carried out to establish appropriate rates for Fe foliar applications.

Use of mycorrhizal fungi in the management of soil borne pathogens such as *Rhizoctonia solani*, *Phytophthora parasitica* and *Pseudomonas solani* have been proven to be beneficial in this study. Effectivenesss of these mycorrhizal fungi varies for different pathogens but it has been established that in addition to the biological direct protection, the seedlings that are colonised by mycorrhizal fungi develop compensatory growth under certain soil conditions including root infections.

The findings of the experiments undertaken in this study contribute to the understanding of the ecological processes that lead to the successful establishment of *U. kirkiana* seedlings. As a general recommendation, it is advisable to carry out both soil and leaf analysis in determining appropriate nursery soils for raising *U. kirkiana* seedlings. Soils with high levels of divalent nutrients such as Ca and Mg should be avoided because of their tendency to cause precipitation of P at neutral to alkaline pH. Mycorrhizal fungi species ocurring in a particular area could also be used to identify the suitability of the soil. Mycorrhizal fungi listed in Appendices I, II and III could be used as a guide in choosing soils with approapriate inocula. To enhance seedling survival and growth in the nursery, foliar sprays with trace elements such as Fe, Mn, Zn, and B could be used. Furthermore, it has been shown that good nursery practices, such as use of the right mycorrhizal fungi inoculum, maintenance of soil structure and appropriate watering schedules, are beneficial for seedling growth and survival in the nursery and later in the field. In addition, particular attention should also be given to the promotion of the natural survival strategy of miombo woodland tree species. This would entail managing seedlings in such a way that promotes more root growth than shoot growth under nursery condtions.

REFERENCES

Agerer, R., Taylor, A. F. S. and Treu, R. (1998). Effects of acid irrigation and liming on the production of fruit bodies by ectomycorrhizal fungi. *Plant and Soil* 199: 83-89.

Akinnifesi, F. K., Kwesiga, F., Mhango, J., Mkonda, A., Chilanga, T. and Swai, R. (2003). Domesticating priority miombo indigenous fruit trees as a promising livelihood option for smallholder farmers in Southern Africa. *Actae Horticulture* 632: 15-30.

Alexander, T. R. (1938). Carbohydrates of bean plants after treatment with indoleacetic acid. *Plant Physiology* 13: 845-858.

Alexander, I. J. (1983). The significance of ectomycorrhizas in the nitrogen cycle. In: Lee, J. A., McNell, S. and Rorison, I. H. (Eds.). Nitrogen as an ecological factor. Blackwell, Oxford, pp. 69-93.

Amaranthus, M. P. and Perry, D. A. (1987). Effect of soil transfer on ectomycorrhiza formation and the survival and growth of conifer seedlings on old, non-reforested clearcuts. *Canadian Journal Forest Research* 20: 914-918.

Anderson, J. M. and Ingram, J. S. I. (1989). Tropical soil biology and fertility programme Methods Handbook. CABS International, Wallingford. 171 pp.

Atkinson, D., Blat, K. K. S., Coutts, M.P., Marsh, P. A. and Rea, D. J. (1983). Tree root systems and their mycorrhizas. Martinus Nijhoff/Dr W. Junk, The Hague, 525 pp.

Atlas, M. R. and Bartha, R. (1987). Microbial ecology: Fundamentals and applications. Benjamin/Cummings Publishing Company, Menlo Park, 533 pp.

Ba, A. M., Sanon, K. B., Duponnois, R. and Dexheimer, J. (1999). Growth response of *Afzelia africana* seedlings to ectomycorrhizal inoculation in a nutrient-deficient soil. *Mycorrhiza* 9: 91-95.

Babich, H. and Stotzky, G. (1978). Toxicity of zinc to fungi, bacteria and coliphages-Influence of chloride ions. *Applied Environmental Microbiology*: 36: 906-914.

Bausor, S. S. (1942). Effect of growth substances on reserve starch. Botany Gazette 104:115-121.

Biggs, A. R. (1999). Effects of calcium salts on apple bitter rot caused by two *Colletotrichum* spp. *Plant Disease* 83: 1001-1005.

Boaler, S. B. (1966). Plant communities and seasonal variation in the vegetation. *Journal of Ecology* 54: 465-479.

Bowen, G. D. (1972). Mineral nutrition of ectomycorrhizae. In : Marks, G. C. and Kozlowski, T. T. (Ed.). Ectomycorrhizae. Their ecology and physiology, Academic, New York, pp.151-197.

Bowen, G. D. (1978). Dysfunction and shortfalls in symbiotic response. In: Horsfall, J. G. and Cowling, E. B. (Eds.). Plant disease - An advanced treatise. Academic Press, New York, pp. 231-256.

Bowen, G. D. (1987). The biology and physiology of infection and its development. In: Safir, G. R. (Ed.). Ecophysiology of VA mycorrhizal plants. CRC Press, Boca Raton, Florida, pp. 28-57.

Bowen, G. D. (1994). Ecology of ectomycorrhiza formation and functioning. *Plant and Soil 159*: 61-67.

Bowen, G. D. and Theodorou, C. (1967). Studies on phosphate uptake by mycorrhizas. Proceedings of 14 th IUFRO Congress, Munich 5: 116-138.

Canny, M. J. (1984). Translocation of nutrients and hormones. In: Wilkins, M. B. (Ed.). Advanced plant physiology. Pitman Publishing Inc., London, pp. 277-296.

Celander, N. (1983). Miombo woodlands in Africa - distribution, ecology and patterns of land use: Working paper 16, Swedish University of Agricultural Sciences International Rural Develoment C entre, 54 pp.

Chidumayo, E. N. (1991). Seedling development of the miombo woodland tree, *Julbernardia globiflora*. *Journal of Vegetation Science* 2: 21-26.

Chidumayo, E. N. (1992). Seedling ecology of two miombo woodland trees. *Vegetation* 103: 51-58.

Chidumayo, E. N. (1993). Responses of miombo to harvesting: ecology and management. Stockholm Environmental Institute, Stockholm, 107 pp**.**

Chidumayo, E. N. (1994). Phenology and nutrition of miombo woodland trees in Zambia. *Trees* 9: 67-72.

Chidumayo, E. N. (1997). Miombo ecology and management. An introduction. Intermediate Technology Publications, London. pp. 30-31.

Chipompha, N. W. S. (1985). Some mushrooms of Malawi. Forestry Research Record No.63, Malawi, 108 pp.

Clarke, J. M., Cavendish, W. and Coote, C. (1996). Rural households and miombo woodlands: use, value and management. In: M.Campbell, B.M. (Ed.). The miombo in transition: Woodlands and welfare in Africa. Centre for International Forestry Research (CIFOR). Bogor, Indonesia, pp. 101-135.

Cline, M. L., France, R. C. and Reid, C. P. P. (1987). Intraspecific and interspecific growth variation of ectomycorrhizal fungi at different temperatures. *Canadian Journal of Botany* 65: 869-875.

Cole, D. L., Masuka, A. J. and Mguni, C. (1998). List of diseases in Zimbabwe. Plant Protection Research Institute, 179 pp.

Connell, J. and Lowman, M.D. (1989). Low diversity tropical rain-forests: Some possible mechanisms for their existence. *American Nature* 134: 88-119.

Crossett, R. N., Campbell, D. J. and Stewart, H. E. (1975). Compensatory growth in cereal root systems. *Plant and Soil* 42: 673-683.

Cruickshank, I. A. M. (1963). Phytoalexins. *Annual Review Phytopathology* I: 351.

Cumming, J. R. (1996). Phosphate-limitation physiology in ectomycorrhizal pitch pine (*Pinus rigida*) seedlings. *Tree Physiology* 16: 977-983.

Dahm, H., Strzelczyk, E., Pachelewski, R. and Redlak, K. (1997). Impact of media and growth conditions on biomass production by the ectomycorrhizal fungus, *Cantharellus cibarius* Fr. Folia Forestalla Polonica Sera A, Lesnictwo 139: 17-26.

Danielson, R. M. and Visser, S. (1989). Host response to inoculation and behaviour of introduced and indigenous ectomycorrhizal fungi of jack pine grown on oil-sands tailings. *Canadian Journal of Forest Research* 19: 1412-1421.

Deacon, J. W. and Donaldson, S. P. (1993). Molecular recognition in the homing responses of zoosporic fungi, with special reference to *Pythium* and *Phytophthora*. *Mycology Research* 97: 1153-1171.

Domink, T. (1961). Studies on mycorrhizae. Scientific Publication for Foreign Cooperation Center, Warsaw, 5: 1-160.

Donaldson, S. P. and Deacon, J. W. (1993). Changes in motility of *Pythium* zoospores induced by calcium and calcium-modulating drugs. *Mycology Research* 97: 877-883.

Dosskey, M.G., Linderman, R. G. and Boersma, L. (1990). Carbon-sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas. *New Phytologist* 122: 269-274.

Drummond, R. B. (1981). The common trees of the central watershed woodlands of Zimbabwe. The Department of Natural Resources, Causeway, Zimbabwe, 256 pp.

Duff, B. K. and Defago, G. (1997). Zinc improves biocontrol of *Fusarium* crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. *Phytopathology* 87: 1250-1257.

Duijff, B. J., Recorbet, G., Bakker, P. A. H. M., Loper, J. E. and Lemanceau, P. (1999). Microbial antagonism at the root level is involved in the suppression of Fusarium wilt by the combination of non-pathogenic *Fusarium oxysporum* and *Pseudomonas putida. Phytopathology* 89: 1073-1079.

Duponnois, R., Garbaye, J., Bouchard, D. and Churin, J.L. (1993). The fungus specificity of mycorrhization helper bacteria (MHB's) used as an alternative to soil fumigation for ectomycorrhizal inoculation of bare-root Douglas-fir planting stock with *Laccaria laccata. Plant and Soil* 157: 257-262.

Duvert, P., Perrin, R. and Plenchette, C. (1990). Soil receptiveness to mycorrhizal association: Concept and method. *Plant and Soil* 124: 1-6.

Ellis, F. (1999). Iron, the first of six micro-nutrients. Wood SA and Timber Times, pp. 10-11.

Ernst, W. (1988). Seed and seedling ecology of *Brachystegia spiciformis*, a predominant tree component in miombo woodlands in South Central Africa. *Forest Ecology Management* 25: 195-210.

FAO (1983). Food and fruit bearing forest species 1: Examples from Eastern Africa. FAO Forest Paper 44/1, 172 pp.

FAO (1990). The major significance of 'minor' forest products. The local use and value of forests in the West African humid forest zone. Community Forest Note No. 6:14-15.

Fleming, L. V. (1983). Succession of mycorrhizal fungi on birch: infection of seedlings planted around mature trees. *Plant and Soil* 71: 263-267.

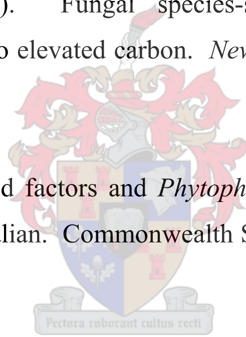
Foster, R. C. and Marks, G. C. (1967). Observations on the mycorrhizas of forest trees. The rhizosphere of *Pinus radiata*. *Australian Journal of Biological Sciences* 20: 915-26.

Fox, F. M. (1986). Groupings of ectomycorrhizal fungi of birch and pine based on establishment of mycorrhizas on seedlings from spores in unsterile soils. *Transactions of British Mycological Society* 87: 371-380.

Fries, N. (1978). Basidiospore germination in some mycorrhiza-forming hymenomycetes. *Transaction of British Mycological Society* 70: 319-324.

Fries, N. (1987). Ecological and evolutionary aspects of spore germination in the higher basidiospores. *Transactions of British Mycological society* 88: 1-7.

Gorissen, A. and Kuyper, T. W. (2000). Fungal species-specific responses of ectomycorrhizal Scots pine (*Pinus sylvestris*) to elevated carbon. *New Phytologist* 146: 163-168.



Griffin , D. M. (1979). Soil water, associated factors and *Phytophthora.* In: Old, K. M. Phytophthora and forest management in Australian. Commonwealth Scientific and Industrial Research Organization, pp. 48-51.

Griffiths, R. P. and Caldwell, B. A. (1992). Mycorrhizal mat communities in forest soils. Mycorrhizas in ecosystems. CAB International, Wallingford, pp. 98-105.

Grundy, I. M., Campbell, B. M., Balebereho, S., Cunliffe, R., Tafangenyasha, C., Ferguson, R. and Parry, D. (1993). Availability and use of trees in Mutanda resettlement area, Zimbabwe. *Forest Economy Management* 56: 243-266.

Gumpo, D. J., Mukamuri, B.B., Muzondo, M. J. and Scoones, I. (1990). Indigenous and exotic fruit trees: Why people want to grow them. Agroforestry for sustainable production: Economic implications. Commonwealth Science Council, London, pp. 185-214.

Hacskaylo, E., Palmer, J. G., and Vozzo, J. A. (1965). Effect of temperature on growth and respiration of ectotrophic mycorrhizal fungi. *Mycologia* 57: 748-756.

Halsall, D. M. and Forrester, R. I. (1977). Effects of certain cations on the formation and infectivity of *Phytophthora* zoospores. Effect of calcium, magnesium, potassium and iron ions. *Canadian Journal of Microbiology* 23: 994-1001.

Handley, W. C. R. and Sanders, C. J. (1962). The concentration of easily soluble reducing substances in roots and the formation of ectotrophic mycorrhizal association: A re-examination of Bjorkman's hypothesis. *Plant and Soil* 16: 42-61.

Harley, J. L. (1969). The biology of mycorrhiza, 2nd edition , Hill, London, 334 pp.

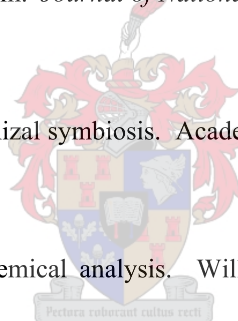
Harley, J. L. (1973). Symbiosis in the ecosystem. *Journal of National Science Council of Sri Lanka* 1: 31-48.

Harley, J. L. and Smith, S. E. (1983). Mycorrhizal symbiosis. Academic Press, London, 483 pp.

Hesse, P. R. (1971). A textbook of soil chemical analysis. William Clowes and Sons, London, 520 pp.

Hill, B. S. and Hill, A. E. (1973). ATP- driven chloride pumping and ATPase activity in the Limonium salt gland. *Journal Membrane Biology* 12: 145-148.

Högberg, P. (1982). Mycorrhizal association in some woodland and forest trees and shrubs in Tanzania. *New Phytologist* 92: 407-415.



Högberg, P. (1986). Nitrogen fixation and nutrient relations in savanna woodland trees (Tanzania). *Journal of Applied Ecology* 23: 675-688.

Högberg, P. (1986). Soil nutrient availability, root symbiosis and tree species composition in tropical Africa (A review). *Journal of Tropical Ecology* 2 : 359-372.

Högberg, P. and Piearce, G. D. (1986). Mycorrhizas in Zambian trees in relation to host taxonomy, vegetation type and succession patterns. *Journal of Ecology* 74: 775-785.

Högberg, P. and Procter, J. (1989). Root symbiosis of trees in savannas. Mineral nutrients in tropical forest and savanna ecosystems. Special Publication No. 9 of the *British Ecological Society* 69: 121-136.

Horan, D. P. and Chilvers, G. A. (1990). Chemotropism-key to ectomycorrhizal formation. *New Phytologist* 116: 297-301.

Huber, D. M. (1989). The role of nutrition in the take-all disease of wheat and other small grains. Soil-borne plant pathogens: Management of diseases with macro and micro-elements. In: Engelhard, A. W. (ed.). *The American Phytopathological Society*, St Paul, pp. 46-74.

Hung, L.L. and Trappe, J. M. (1983). Growth variation between and within species of ectomycorrhizal fungi in response to pH *in vitro*. *Mycologia* 75: 234-241.

Janos, D. (1980). Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest growth. *Ecology* 61: 151-162.

Jeschke, W. D. (1970). Evidence for a K^+ stimulated Na^+ efflux at the plasmalemma of barley root cells. *Planta* 94: 240-245.

Jeutschke, G., Brandes, B., Kuhn, A., Schroder, W. H., Becker, J. S. and Goldbold, D. L. (2000). The mycorrhizal fungus *Paxillus involutus* transports magnesium to Norway spruce seedlings. Evidence from stable isotope labelling. *Plant and Soil* 220: 243-246.

Pectora roborant cultus recti

Jonasson, S., Michelsen, A., Schmidt, I. K. and Nielsen, E. V. (1999). Responses in microbes and plants to changed temperature, nutrient, and light regimes in the Arctic. *Ecology* 80: 1828-1843.

Jones, S. W. Donaldson, S. P. and Deacon, J. W. (1991). Behaviour of zoospores and zoospore cysts in relation to root infection by *Pythium aphanidermatum. New Phytopathology* 117: 289-301.

Jonsson, L., Dahlberg, A., Nilsson, M.C., Zackrisson, O. and Karen, O. (1999). Ectomycorrhizal fungi communities in the late-successional Swedish boreal forests, and their composition following fire. *Molecular Ecology* 8: 205-215.

Kao, C. W. and Ko, W.H. (1986). The role of calcium and micro-organisms in suppression of cucumber damping-off caused by *Pythium splendens* in a Hawaiian soil. *Phytopathology* 76: 221-225.

Khalil, S., Labuschagne, N. and Wingfield, M.J. (1999). *Pinus patula* establishment problem associated with poor ectomycorrhizal development in previously cultivated soils. *Southern African Forestry Journal* 186: 59-65.

Khangura, R., Barbetti, M. J. and Sweetingham, M. W. (1997). Association of *Rhizoctonia* species with hypocotyl rot and damping-off in canola. Proceedings of Biennial Conference, Plant Pathology Society (11th), Perth, Western Australia, 40 pp.

Khangura, R. K., Barbetti, M. J. and Sweetingham, M. W. (1999). Characterization and pathogenicity of *Rhizoctonia* species on Canola. *Plant Disease* 83: 714-721.

Khare, D. K., Khare, P. K. and Mishra, G. P. (1985). Effects of forest fire on soil nutrient levels in a *Tectonia grandis* forest stand. In: Misra, K. C. (ed.). Ecology and resources management in tropics. Vol. 1Bhargava Book Depot, Varanasi, pp. 71-75.

Kibby, G. (1979). Mushrooms and toadstools. A field guide. Oxford University Press. Oxford, 256 pp.

Kloepper, J. W, Leong, J., Teintze, M. and Schroth, M. N. (1980). *Pseudomonas* siderophore: A mechanism explaining disease suppressive soils. *Microbiology* 4: 317-320.

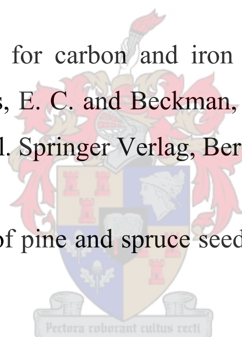
Kormanik, P. P., Bryan, W. C. and Schultz, R.C. (1977). Influence of endomycorrhizae on growth of sweetgum seedlings from eight mother trees. *Forest Science* 23: 500-506.

Koroleff, F. (1976). Determination of phosphorus. In: Tjamos, E.C. and Beckman, C. H. (ed.). Methods of sea water analysis. Verlag Chemie, Veinheim, pp 117-126.

Kottke, I., Pargney, J.C., Qian, X. M. and Disquet, I. (1995). Passage and deposition of solutes in the hyphal sheath of ectomycorrhizas- the soil-root interface. Contribution to forest tree physiology. Institut National de la Recherche Agronomique (INRA) . Paris, pp. 255-271.

Laiho, O. (1970). *Paxillus involutus* as a mycorrhizal symbiont of forest trees. *Acta Forest Fenn*. 106: 1-72.

Lemanceau, P. (1989). Role of competition for carbon and iron in mechanisms of soil suppressiveness to *Fusarium* wilts. In: Jjamos, E. C. and Beckman, C. H. (Eds.). Vascular wilt diseases of plants, basic studies and control. Springer Verlag, Berlin, pp. 386-396.



Levisohn, I. (1954). Aberrant root infections of pine and spruce seedlings. *New Phytologist* 53: 284 pp.

Lister, G.R.; Slankis, G.; Krotkov, G. and Nelson, C. D. (1968). The growth and physiology of *Pinus strobus* L. seedlings as affected by various nutritional levels of nitrogen and phosphorus. *Annals of Botany* 32: 33-43.

Lundeberg, G. (1967). Raw humus as the nitrogen source for pine seedlings. Proceedings of International Union Forest Research Organization (14th Congress), 1967 Vol. 5: p 112.

Lundeberg, G. (1970). Utilisation of various nitrogen sources, in particular bound soil nitrogen, by mycorrhizal fungi. *Studia Forestalia Suecica*. 79: 1-93.

Lussenhop, J. and Fogel, R. (1999). Seasonal change in phosphorus content of *Pinus strobus* - *Cenococcum geophilum* ectomycorrhizae. *Mycologia* 91: 742-746.

Maghembe, J. A. and Seyani, J. H. (1991). Multipurpose trees used by smallholder farmers in Malawi: Results of an Ethnobotanical Survey. Afrena Report No. 42. ICRAF, Nairobi, 39 pp.

Maghembe, J. A., Kwesiga, F., Ngulube, M. and Prins, H. (1994). Domestication potential of indigenous fruit trees of the miombo woodland of Southern Africa. The international conference on: Tropical trees - Potential for domestication, held at Heroit-Watt University, Edinburgh, pp. 22-28.

Marcel, G.A., Heijden, V. D., Boller, T., Wiemken, A. and Sanders, J. R. (1998). Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology* 79: 2082-2091.

Marschner, H. and Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* 159: 89-102.

Marx, D. H. (1970). The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. Resistance of mycorrhizae to infection by vegetative mycelium of *Phytophthora cinnamomi. Phytopathology* 60: 1472.

Marx, D. H. (1973). Mycorrhizae and feeder root diseases. In: Marks, G. C. and Kozlowski, T. T. (Eds.). Ectomycorrhizae, their ecology and physiology. Academic Press, New York, pp. 351-382.

Marx, D. H. and Artman, J.D. (1979). *Pisolithus tinctorius* ectomycorrhizae improve survival and growth of pine seedlings on acid coal spoil in Kentucky and Virginia. *Reclamation Review* 2: 23-31.

Marx, D. H., Bryan, W.C. and Davey, C.B. (1970). Influence of temperature on aseptic synthesis of ectomycorrhizae by *Telephora terrestris* and *Pisolithus tinctorius* on loblolly pine. *Forest Science* 16: 424-431.

Marx, D. H., Bryan, W.C., Cordell, C.E. (1977a). Survival and growth of pine seedlings with *Pisolithus tinctorius* ectomycorrizae after two years on reforestation sites in North Carolina and Florida. *Forest Science* 3: 363-373.

Marx, D. H., Hatch, A. B. and Mendicino, J. F. (1977b). High soil fertility decreases sucrose content and susceptibility of loblolly pine roots to ectomycorrhizal infection by *Pisolithus tinctorius. Canadian Journal of Botany* 55: 1569-1574.

Melin, E. (1953). Physiology of mycorrhizal relations in plants. *Annual Review Plant Physiology* 4: 325-346.

Mishustin, E. N. (1951). Soil microflora and mycorrhiza formation with oak. *Agrobiologiya* 2: 27-39.

Mitsuru, O., Taigen, Z., Kazuo, K., Masatoshi, O. and Toshiaki, T. (1996). Carbon-Nitrogen interaction related to P, K, Ca and Mg nutrients in field crops. *Soil Science Nutrition* 42: 539-552.

Mkonda, A., Akinnifesi, F. K. and Mafongoya, P. (2002). Response of indigenous and exotic fruit trees in Zambia to grafting and airlayering. Proceedings of the 14th Southern African Regional Review and Planning Workshop, 3-7 September 2001, Harare, pp. 136-139.

Moawad, M. (1980). Ecophysiology of vesicular-arbuscular mycorrhiza. Tropical Mycorrhiza Research. Clarendon Press, Oxford, pp. 203-205.

Molina, R., Massicotte, H.B. and Trappe, J. M. (1992). Ecological role of specificity phenomena in ectomycorrhizal plant communities: potentials for interplant linkages and guild development. Mycorrhizas in ecosystems. C. A. B. International, UK. pp. 106-112.

Morris, B. (1987). Common mushrooms of Malawi, Fungiflora, Oslo, 108 pp**.**

Morris, B. M., Reid, B., and Gow, N. A. R. (1992). Electrotaxis of zoospores of *Phytophthora palmivora* at physiologically relevant field strengths. *Plant Cell Environment* 15: 345-353.

Moyo, S., Robinson, P., Katerere, Y., Stevenson, S. and Gumpo, D. (1991). Zimbabwe's environmental dilemma. Balancing resource inequalities. Zimbabwe Environmental Research Organisation (ZERO), 165 pp.

Munyanziza, E. (1994). Miombo trees and mycorrhizae. Ecological strategies, a basis for afforestation. Ph.D. Thesis. Wageningen University, Netherlands, 193 pp.

Munyanziza, E. and Oldeman, R.A.A. (2000). Early spontaneous ectomycorrhizal infection of miombo seedlings in the field in Morogoro, Tanzania: Implication for artificial tree regeneration. *Tanzania Journal of Forestry and Nature Conservation* 73: 73-79.

Mwabumba, I. and Sitaubi, L. A. (1994). Seed pre-treatment, growth and phenology of some indigenous fruit trees of the miombo ecozone at Naungu Forestry Reserve - Malawi. Paper presented at the Regional Conference on potential for improvement of indigenous fruit trees of the miombo woodlands of Southern Africa. Malawi, pp. 23-27.

Mwamba, C. K. (1983). Ecology and distribution of Zambian wild fruit trees in relation to soil fertility. M. Sc. Thesis. University of Ghent, Belgium, 102 pp.

Mwamba, C. K. (1988). Studies on *Uapaca kirkiana* Muell. Arg. (Euphorbiceae) Tree Improvement Research Centre (TIRC), National Council for Scientific Research (NCRSR) Technical Report, Kitwe, Zambia, pp.35-40.

Mwamba, C. K. (1989). An outlook on the role of indigenous fruit trees in agroforestry. Paper presented at the Agroforestry Workshop, 16-19 April 1989, Lusaka, Zambia, 14 pp.

Mwamba, C.K. (1994). Variations in fruits of *Uapaca kirkiana* and effects of in-situ silvicultural treatments on fruit parameters. Paper presented at the Regional Conference on potential for improvement of indigenous fruit trees of the miombo woodlands of Southern Africa, Mangochi, Malawi. 23-27 January 1994, 25 pp.

Mwamba, C. K.; Zgambo, Y and Chongo, G. (1992). Effect of seedling source on post-planting growth of *Uapaca kirkiana.* Muell. Arg. *South African Forestry Journal* 161: 35-40.

Nealson, K.H. (1983). The microbial iron cycle. In: Krumbein W. E (ed). Microbial Geochemistry. Blackwell Scientific Publications, Oxford, pp. 159-190.

Newbery, D. M., Alexander, I. and Rother, J.A. (1997). Phosphorus dynamics in a lowland African rain forest: influence of ectomycorrhizal trees. *Ecology Monographs* 67: 367-409.

Ngulube, M.R. and Mkandawire, M.M. (1997). Short-term storage of *Uapaca kirkiana* seed. *Seed Science and Technology* 25: 565-569.

Ngulube, M. R., Hall, J. B. and Maghembe, J. A. (1995). Ecology of miombo fruit trees: *Uapaca kirkiana* (Euphorbiaceae). *Forest Ecology and Management* 77: 107-117.

Ngulube, M. R., Hall, J .B. and Maghembe, J. A. (1996). A brief review of the silviculture and resource potential of miombo fruit trees: *Uapaca kirkiana* (Euphorbiaceae). *Journal of Tropical Forest Science* 8: 395-411.

Ngulube, M. R. , Hall, J. B. and Maghembe, J. A. (1998). Reproductive ecology *of Uapaca kirkiana* (Euphorbiaceae) in Malawi, Southern Africa. *Journal of Tropical Ecology* 14: 743-760.

Nyamapfende, K. (1991). Soils of Zimbabwe. Nehanda Publishers, Harare, 179 pp.

Nye, P. H. and Greenland, D. J. (1960). The soil under shifting cultivation. Technical Communication 51. Commonwealth Agricultural Bureaux, Harpenden, 156 pp.

Nye, P.H. and Tinker, P.B. (1977). Solute movement in the soil-root system. Blackwell Scientific Publishers, Oxford, 342 pp.

Ohga, S. and Wood, D. A. (2000). Efficacy of ectomycorrhizal basidiomycetes on Japanese larch seedlings assessed by ergosterol assay. *Mycologia* 95: 394-398.

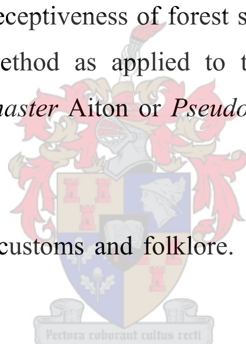
Palgrave, K.C. (1995). Trees of Southern Africa. Struik Publishers, Cape Town, 959 pp.

Palzer, C. R. (1976). Zoospore inoculum potential of *Phytophthora cinnamomi.* Ph. D. Thesis, University of Western Australia, 222 pp.

Pera, J. Alvarez, I.F., Rincon, A. and Parlade, J. (1999). Field performance in northern Spain of Douglas-fir seedlings inoculated with ectomycorrhizal fungi. *Mycorrhiza* 9: 77-84.

Pergler, D.N. (1977). A preliminary agaric flora of East Africa, Kew, Bullet Add. Series 6, HMSO London, 615 pp.

Perrin, E., Parlade, X. and Pern, J. (1996). Receptiveness of forest soils to ectomycorrhizal association: Concept and ectomycorrhizal method as applied to the symbiosis between *Laccaria bicolor* (Maire) Orton and *Pinus pinaster* Aiton or *Pseudotsuga menziesii* (Mirb) Franco. *Mycorrhiza* 6: 469-476.



Piearce, G. D. (1981). Zambian mushrooms-customs and folklore. Bulletin of the *British Mycological Society* 15 : 139-142.

Piearce, G. D. (1988). Mushrooms for pleasure and profit. Research Note No. 41, Division of Forest Research. Kitwe, Zambia, pp. 30-32.

Plenchette, C., Perrin, R. and Duvert, P. (1989). The concept of soil infectivity and a method for its determination as applied to endomycorrhizae. *Canadian Journal of Botany* 67: 112-115.

Powell, W. M.; Ghendrix, F.F. and Marx, D. H. (1968). Chemical control of feeder root necrosis of pecans caused by *Pythium* species and nematodes. *Plant Disease Report* 52: 577.

Pratt, B. H. (1971). Isolation of basidiomycetes from Australian Eucalypt forest and assessment of their anatagonism to *Phytophthora cinnamomi*. *Transactions of British Mycological Society* 56: 243.

Quales, W. (1999). Plant disease biocontrol and ectomycorrhizae. *Integrated Pest Management* 21: 1-10.

Ramachela, K. (1996). Studies on the propagation and establishment of *Uapaca kirkiana* In: Sustainable management of indigenous forests in the dry tropics. Proceedings of an International Conference, Kadoma, Zimbabwe, pp. 168-173.

Rammeloo, J. and Walleyn, R. (1993). The edible fungi of Africa South of Sahara, a literature survey. Meise. National Botanical Garden of Belgium, 5: 62.

Redhead, J. F. (1968). Mycorrhizal associations in some Nigerian forest trees. *Transactions of British Mycology Society* 51: 377-387.

Ross, E. W. and Marx, D. H. (1972). Susceptibility of sand pine to *Phytophthora cinnamomi. Phytopathology* 62: 1197.

Rovira, A. D. (1969). Plant root exudates. *Botany Review* 35: 35-57.

Ruehle, J. L. (1982). Field performance of container grown Loblolly pine seedlings with specific ectomycorrhizae in a reforestation site in South Carolina. *Journal of Applied Forestry* 6: 30-33.

Russell, R. S. (1982). Plant root systems: Their function and interaction with the soil. Mc Graw-Hill Book Company, Berkshire, pp. 54-55.

Rutherford, M.C. (1983). Growth rates, biomass and distribution of selected woody plant roots in *Burkea africana-Ochna pulchra* savanna. *Vegetation* 55: 45-63.

Safir, G. R. and Duniway, J. M. (1991). Evaluation of plant response to colonisation by vesicular-arbuscular fungi. In: Schenck, N.C. (ed.). Methods and principles of mycorrhizal research APS Press. St. Paul Minnesota, USA, pp. 77-83.

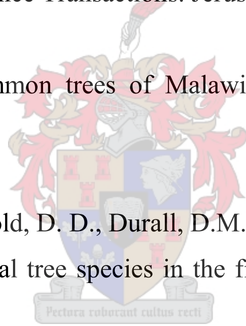
Sambo, E. Y. (1992). Propagation methods for a selection of fruit trees indigenous to miombo ecological zone. Report of visiting scientist. ICRAF, Zomba, Malawi, 135 pp.

Sanchez, P. A. (1976). Properties and management of soils in the tropics. John Wiley, New York, 618 pp.

Schönau, A. P. G. (1981). The effects of fertilising on the foliar nutrients concentrations in *Eucalyptus grandis*. *Fertiliser Research* (2): 73-87.

Shemakhanova, N.M. (1962). Mycotrophy of woody plants. Moscow Academy Nauk 329. (Translation from Russian, Israel Program Science Transactions. Jerusalem, 1967).

Shorter, C. (1989). An introduction to common trees of Malawi. Wildlife Society of Malawi, pp. 76-78.



Simard, S. W., Perry, D. A., Jones, M.D., Myrold, D. D., Durall, D.M. and Molina, R. (1997). Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* 388: 579-582.

Simon, A. and Sivasithamparam, K. (1989). Pathogen-suppression: A case study in biological suppression of *Gaeumannomyces graminis* var. *tritici* in soil. *Soil Biology and Biochemistry* 21: 331-337.

Singh, S. (2000). Effect of edaphic and climatic factors on the development of mycorrhiza in tree nurseries: effect of soil pH, light, and carbon dioxide. *Mycorrhiza News* 11: 3-13.

Singh, L. and Lakhanpal, T.N. (2000). Growth and ectomycorrhizae development in *Cedrus deodara* seedlings inoculated with different vegetative inoculum formations. *Journal of Mycology and Plant Pathology*. 30: 64-67.

Skinner, M. F. and Bowen, G. D. (1973). The penetration of soil by mycelial strands of ectomycorrhizal fungi. *Soil Biology Biochemical* 6: 57-61.

Slankis, V. (1965). The composition of sugars and amino acids in the roots of white pine (*Pinus strobus*) seedlings grown under different nitrogen levels. Proceedings of Canadian Society of Plant Physiology 6: 29 pp. University of New Brunswick, New Brunswick.

Slankis, V. (1969). Formation of ectomycorrhizae of forest trees in relation to light, carbohydrates, and auxins. Mycorrhizae, Proceedings of the First North America Conference on Mycorrhizae. Washington D.C. pp. 151-167.

Slankis, V. (1974). Soil factors influencing formation of mycorrhizae. *Annual Review Phytopathology* 12: 437-457.

States, J.S. (1981). Some criteria in the identification of fungal communities. In: Wicklow, D. T. and Carroll, G. C. (Eds). The fungal community, its organisation and role in the ecosystem. Mercel Dekker, New York, pp. 185-199.

Stenström, E. Ek, M. and Unestam, T. (1990). Variation in field response of *Pinus sylvestris* to nursery inoculation with four different ectomycorrhizal fungi. *Canadian Journal Forest Research* 20: 1796-1803.

Sufi, N. A. and Kaputo, M. T. (1977). Identification and determination of free sugars in masuku fruit (*Uapaca kirkiana*). *Zambia Journal of Science and Technology* 2: 23-25.

Sweeney, B. N.; Prezelin, B. A.; Wong, D. and Govindjee (1979). *In vivo* chlorophyll a fluorescence transients and the circadian rhythm of photosynthesis in *Gonyaulax polyedra*. *Photochemistry Photobiology* 30: 309-311.

Pectora roborant cultus recti

Terry, N. and Low, G. (1982). Leaf chlorophyll content and its relation to the intracellular localization of iron. *Journal Plant Nutrition* 5: 301-310.

Theodorou, C. and Bowen, G. D. (1969). The influence of pH and nitrate on mycorrhizal associations of *Pinus radiata*. *Australian Journal of Botany* 17: 59-67.

Theodorou, C. and Bowen, G.D. (1971). Influence of temperature on the mycorrhizal associations of *Pinus radiata* D Don. *Australian Journal of Botany* 19: 13-20.

Theodorou, C. and Bowen, G. D. (1987). Germination of basidiospores of mycorrhizal fungi in the rhizosphere of *Pinus radiata*. *New Phytopathology* 106: 217-223.

Theron, J. M. (1991). Comparative studies of ectomycorrhizae in South African forestry. PhD (Forestry) thesis, University of Stellenbosch, Stellenbosch, 111 pp.

Thoen, D. and Ba, A. M. (1998). Ectomycorrhizas and putative ectomycorrhizal fungi of *Afzelia africana* and *Uapaca gurneensis*. Mull. Arg. In Southern Senegal. *New Phytologist* 113: 549-559.

Thoen, D. and Ducousso, M. (1989). Fungi and ectomycorrhizas in the Fouta Djallon plateau area. *Bois et Forests-des-tropiques* 221: 45-63.

Tinker, P.B. (1989). Nutrient uptake by plant roots in natural systems. In: Harrison, A.F., Ineson, P. and Heal, O. W. (Eds.). Nutrient cycling in terrestrial ecosystems. Field methods, application and interpretation. Elsevier, Applied Science, London, pp. 322-334.

Tomiyama, K. (1963). Physiology and biochemistry of disease resistance of plants. *Annual Review Phytopathology* I: 295.

Trapnell, C.G. (1959). Ecological results of woodland burning experiments in Northern Rhodesia. *Journal of Ecology* 47: 129-168.

Trapnell, C. G., Friend, M. T., Chamberlain, G. T. and Birch, H. F. (1976). The effects of fire and termites on a Zambian woodland soil. *Journal of Ecology* 64: 577-588.

Trappe, J. M. (1964). Mycorrhizal hosts of *Cenococcum graniforme*. *Lloydia* 27: 100-106.

Tredgold, M. H. (1990). Food plants of Zimbabwe (with old and new ways of preparation). Mambo Press, Gweru, 153 pp.

Tribuskaya, A. Ya. (1955). Investigation of the rhizosphere microflora of pine seedlings. *Mikrobiologiya* 24: 188-92 (In Russian).

Van der Westhuizen, G. C. A. and Eicker, A. (1996). Mushrooms of Southern Africa. Struik Publishers, Cape Town, 208 pp.

Vetter, J.; Siller, I. and Horvath, Z. (1997). Zinc content of sporocarps of basidiomycetous fungi. *Mycologia* 89: 481-483.

Voisard, C. Bull, C. T., Keel, C., Laville, J., Maurhofer, M., Schnider, U., Defago, G. and Haas, D. (1994). Biocontrol of root diseases by *Pseudomonas fluorescens*: Current concepts and experimental approaches: Biotechnology and the release of genetic modified organisms (GMO's). In: O'Gara, F., Dowling, D. N. and Boesten, B. (eds.). Molecular ecology of rhizosphere microorganisms, VCH, Weinheim, pp. 67-89.

Von Broembsen, S. L. and Deacon, J. W. (1997). Calcium interference with zoospore biology and infectivity of *Phytophthora parasitica* in nutrient irrigation solutions. *Phytopathology* 87: 522-528.

Vosnyakovskaya, Yu. M. (1954). Mycorrhiza and its practical value. *Mikrobiologiya* 23: 204-20 (In Russian).

Wallander, H. (1995). A new hypothesis to explain allocation of dry matter between mycorrhizal fungi and pine seedlings in relation to nutrient supply. Nutrient uptake and cycling in forest ecosystems. *Plant and Soil* 168/169: 243-248.

Wallander, H. and Wickman, T. (1999). Biotite and microcline as potassium sources in ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. *Mycorrhiza* 9: 25-32.

Wallenda, T. and Read, D. J. (1999). Kinetics of amino acid uptake by ectomycorrhizal roots. *Plant, Cell and Environment* 22: 179-187.

Watson, J.P. (1964). The characteristics and pedogenesis of a catena on granite in Southern Rhodesia, D.Phil. Thesis, London University, 111 pp.

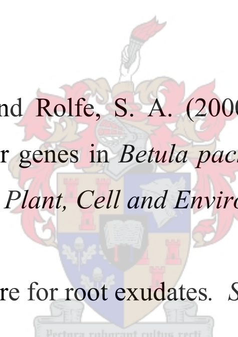
White, F. (1983). The vegetation of Africa. UNESCO National Resources Research 20: 1-356.

Wilkinson, H. T., Miller, R. D. and Millar, R. L. (1981). Infiltration of fungal and bacterial propagules into soil. *Soil Science Society* 45: 1034-1039.

Williamson, J. (1975). Useful plants of Malawi. Government Printers, Zomba, Malawi. pp. 239-240.

Wright, D. P., Scholes, J. D., Read, D. J. and Rolfe, S. A. (2000). Changes in carbon allocation and expression of carbon transporter genes in *Betula pachela* Roth. colonised by the ectomycorrhizal fungus *Paxillus involutus*. *Plant, Cell and Environment* 23: 39-49.

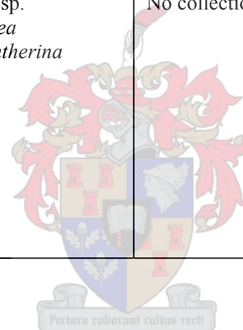
Zentmyer, G.A. (1961). Chemotaxis of zoospore for root exudates. *Science* 133: 1595-1596.



Appendix I

Fungal spp. collected at two-week intervals in the 1998/1999 rainfall season

Area/site	18/12/98	03/01/99	15/01/99	27/01/99	13/02/99	28/02/99	15/03/99
Nemaire I	No collection	No collection	*Russula fellea* *Clavulina cristata* *Lactarius deliciosus* *Lignosus sacer* *Hebeloma* sp. *Boletus* sp.	*Clitopilus pruni* *Hebeloma* sp. *Russula* sp. *Cantharellus* sp.	No collection	*Hypholoma* sp. *Russula* sp. *Lignosus sacer* *Boletus* sp. *Cantharellus* sp.	No collection
Nemaire II	No collection	No collection	*Lactarius* sp. *Agaricus* sp. *Laccaria laccata* *Lignosus sacer*	*Cantharellus* sp. *Laccaria laccata*	No collection	*Lactarius edulis*	No collection
St. Lukes	No collection	No collection	*Leucoagaricus* sp. *Lactarius deliciosus* *Amanita rubescens* *Amanita* sp.	*Clitopillus* sp. *Amanita pantherina* *Cantharellus* sp. *Lactarius deliciosus* *Cantharellus cibarius*	No collection	*Phlebopus* sp. *Lactarius* sp. *Lactarius deliciosus* *Cantharellus cibarius*	No collection
Rowa	No collection	No collection	*Boletus* sp. *Boletus* sp. *Russula* sp. *Russula* sp. *Cantharellus* sp. *Laccaria laccata* *Russula sororia*	*Gymnopilus* sp. *Russula fellea* *Amanita pantherina* *Boletus* sp.	No collection	*Lactarius deliciosus* *Amanita pantherina* *Boletus* sp. *Russula* sp.	*Lactarius edulis*



Appendix I cont'

Chitakatira	*Lactarius edulis* *Russula ciliata* *Boletus* sp. *Cantharellus* sp.	No collection	*Lactarius deliciosus* *Boletus* sp. *Cantharellus* sp. *Boletus* sp. *Russula* sp.	*Amanita pantherina* *Amanita zambiana* *Russula lutea* *Amanita excelsa* *Cantharellus cibarius*	*Amanita excelsa* *Russula* sp. *Cantharellus* sp. *Phlebopus* sp. *Clavulina crtistata*	*Russula* sp. *Lactarius deliciosus* *Boletus* sp. *Amanita* sp.	*Tricholoma* sp.
Nzvera	No collection	*Gyroporus* sp. *Russula sororia* *Russula capensis* *Clavulina cristata*	No collection	No collection	*Amanita rubescens* *Inocybe* sp. *Amanita phalloides* *Russula* sp.	No collection	No collection
Tsikwa	No collection	*Scleroderma* sp. *Lactarius* sp. *Lignosus sacer* *Amanita phalloides* *Inocybe eutheles* *Russula capensis* *Amanita zambiana* *Lactarius deliciosus*	No collection	No collection	*Russula sororia* *Gymnopilus* sp. *Amanita* sp. *Lactarius* sp.	No collection	*Russula* sp. *Amanita phalloides* *Lactarius* sp.
Matiya	No collection	*Amanita phalloides* *Amanita pantherina* *Russula sororia* *Chalciporus* **sp.** *Amanita zambiana*	No collection	No collection	*Amanita phalloides* *Cantharellus* sp. *Boletus* sp. *Paxillus* sp. *Russula* sp.	No collection	*Amanita* sp. *Lactarius* sp. *Clitopillus* sp.
Nyamuzihwa	No collection	No collection	No collection	No collection	*Amanita citrina* *Laccaria laccata*	No collection	No collection
Guyo	No collection	*Amanita zambiana* *Cantharellus* sp. *Boletus* sp. *Stropharia* sp. *Russula* sp. *Lepista cafforum*	No collection	No collection	No collection	No collection	*Cantharellus* sp**.** *Russula sororia* *Amanita phalloides* *Clavulina cristata* *Amanita folidissimus*

Appendix II
Fungal spp. collected at two-week intervals in the 1999/2000 rainy season

Area/site	12/01/2000	30/01/2000	14/02/2000	28/02/2000	15/03/2000	30/03/2000
Nemaire I	No collection	*Russula sororia* *Boletus* sp. *Russula capensis*	*Lactarius deliciosus* *Amanita pantherina* *Cantharellus cibarius* *Amanita zambiana* *Clavulina cristata*	*Hebeloma* sp. *Russula sororia* *Lactarius deliciosus*	R***ussula sororia*** *Amanita zambiana*	No collection
Nemaire II	No collection	*Cantharellus cibarius* *Lactarius* sp.	*Russula* sp. *Russula sororia* *Lactarius deliciosus* *Russula* sp. *Amanita zambiana* *Russula fellea* *Cantharellus cibarius*	*Heboloma* sp. *Boletus* sp. *Russula* sp.	*Boletus* sp.	No collection
St. Lukes	No collection	*Amanita zambiana* *Russula sp.* *Russula delica*	*Amanita zambiana* *Lactarius* sp. *Cantharellus cibarius* *Lactarius deliciosus* *Boletus edulis*	*Boletus edulis* *Russula* sp. *Cantharellus cibarius* *Amanita zambiana*	No collection	Lactarius deliciosus *Boletus* sp**.**
Rowa	*Russula* sp.	*Russula* sp. *Phlebopus* sp. *Russula capensis* *Amanita zambiana*	*Amanita pantherina* *Amanita phalloides* *Russula sororia* *Cantharellus* sp. *Boletus edulis* *Cantharellus cibarius* *Lactarius* sp.	*Amanita zambiana* *Lactarius deliciosus* *Cantharellus* sp. *Russula edulis* *Boletus* sp.	*Boletus* sp. *Russula* sp.	No collection

Pectora roborant cultus recti

Appendix II cont'

Chitakatira	No collection	*Lactarius deliciosus* *Lactarius* sp. *Amanita phalloides* *Russula* sp.	*Lactarius* sp. *Russula* sp. *Amanita zambiana* *Amanita pantherina* *Lactarius deliciosus* *Cantharellus* sp.	*Russula delica* *Lactarius helvus* *Cantharellus* sp. *Lactarius* sp. *Boletus* sp. *Amanita zambiana* *Cantharellus cibarius* *Lactarius* sp. *Russula capensis* *Boletus edulis*	*Lactarius* sp. *Boletus* sp. *Lactarius deliciosus* *Cantharellus* sp.	*Lactarius deliciosus* *Cantharellus* sp.
Nzvera	No collection	*Lactarius* sp. *Russula capensis*	*Clavulina cristata* *Cantharellus cibarius* *Russula capensis*	*Russula sororia* *Lactarius* sp. *Russula capensis* *Clavulina cristata*	*Russula sororia* *Clavulina cristata*	No collection
Tsikwa	No collection	*Amanita zambiana*	*Lactarius deliciosus* *Russula delica* *Boletus edulis* *Boletus* sp. *Russula* sp.	*Russula sororia* *Lactarius* sp.	*Russula* sp.	No collection
Matiya	No collection	*Russula* sp. *Amanita phalloides*	Lactarius deliciosus *Cantharellus cibarius* *Amanita zambiana* *Russula delica*	*Russula fellea* *Lactarius* sp.	*Russula* sp.	No collection
Nyamuzihwa	No collection	No collection	*Boletus* sp. *Lactarius* sp. *Russula* sp. *Cantharellus* sp.	*Amanita pantherina* *Russula* ***capensis*** *Lactarius* sp.	No collection	*Russula* sp.
Guyo	No collection	No collection	*Russula* sp. *Lactarius deliciosus* *Russula capensis* *Clavulina cristata*	*Russula capensis* *Amanita phalloides* *Clavulina cristata*	No collection	No collection

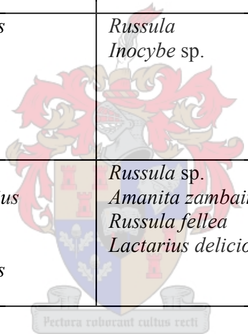
Appendix III
Fungal spp. collected at two-week intervals in the 2000/2001 rainy season

Area/site	16/12/2000	31/12/2000	30/01/2001	14/02/2001	28/02/2001	15/03/2001	30/03/2001
Nemaire I	*Russula sororia* *Boletus* sp. *Russula capensis*	*Heboloma* sp. *Russula sororia* *Tricholoma* sp. *Lactarius deliciosus*	No collection	*Clavulina cristata* *Inocybe euthelis* *Lactarius deliciosus* *Russula capensis* *Cantharellus cibarius*	No collection	No collection	*Russula sororia* Sp. not identified
Nemaire II	*Russula* sp. *Russula sororia*	*Russula* sp. *Russula* sp. *Lactarius deliciosus* *Russula fragilis* *Heboloma* sp. *Russula fellea*	No collection	*Russula sororia* *Boletus* sp. *Inocybe euthelis* *Lactarius deliciosus*	No collection	No collection	No collection
St. Lukes	No collection	*Boletus edulis* *Amanita zambiana* *Cantharellus cibarius* *Russula foeteus* *Russula delica*	No collection	*Amanita zambiana* *Lactarius* sp. *Cantharellus cibarius* *Boletus edulis* *Lactarius deliciosus*	No collection	No collection	*Lactarius deliciosus* *Clitocybe* sp.
Rowa	No collection	*Phlebopus* sp. *Boletus edulis* *Heboloma* sp. *Amanita zambiana*	*Russula* sp.	*Agrocybe* sp. *Stropharia* sp. *Amanita phalloides* *Russula sororia* *Phlebopus* sp. *Cantharellus* sp. *Amanita pantherina* *Russula* sp. *Russula* sp. *Boletus edulis*	*Russula delica* *Lactarius deliciosus* *Clitopillus* sp. *Amanita zambiana* *Boletus* sp. *Russula capensis*	*Cantharellus cibarius* *Russula fellea* *Cantharellus* sp.	No collection

Pectora roborant cultus recti

Appendix III cont'

Chitakatira	*Lactarius deliciosus* *Amanita zambiana* *Lactarius piperatus* *Phlebopus* sp. *Lignosus sacer*	No collection	*Lactarius deliciosus* *Lactarius* sp. *Amanita* sp. *Russula* sp.	*Lactarius deliciosus* *Russula* sp. *Amanita zambiana* *Volvariella* sp. *Lactarius* sp.	*Boletus edulis* *Russula delica* *Cantharellus* sp. *Lactarius* sp. *Boletus* sp. *Amanita zambiana* *Lactarius helvus* *Clitopillus prunulus* *Russula capensis*	*Lactarius turpis* *Boletus* sp. *Lactarius deterrimus* *Lactarius deliciosus* *Cantharellus* sp.	*Lactarius deliciosus* *Cantharellus* sp. *Clitocybe* sp.
Nzvera	No collection	No collection	*Cantharellus* sp. *Russula capensis* *Hebeloma* sp.	*Russula capensis* *Russula delica* *Clavulina cristata* *Russula sororia* *Lactarius deliciosus* *Lactarius* sp. *Boletus* sp.	*Clavulina cristata* *Russula fellea* *Boletus* sp. *Russula sororia* Russula capensis	No collection	*Russula sororia* *Cantharellus* sp. *Leccinum holopus*
Tsikwa	*Russula capensis* *Russula sororia* *Amanita zambaina*	No collection	*Amanita zambaina*	*Lactarius deliciosus* *Russula delica* *Russula claroflava* *Boletus edulis* *Boletus* sp. *Inocybe* sp.	*Russula* *Inocybe* sp.	*Leccinum duriusculum*	*Inocybe eutheles* *Clavulina cristata* *Amanita zambaina* *Lactarius* sp. *Lipista saeva*
Matiya	*Russula sororia*	No collection	No collection	*Russula delica* *Cantharellus cibarius* *Clitocybe* sp. *Boletus* sp. *Lactarius deliciosus* *Amanita zambaina*	*Russula* sp. *Amanita zambaina* *Russula fellea* *Lactarius deliciosus*	No collection	*Clavulina cristata* *Lactarius turpis* *Russula aerginea* *Amanita pantherina* *Boletus* sp. *Cantharellus* sp.


Pectora roborant cultus recti

Appendix III cont'

Nyamuzihwa	No collection	No collection	No collection	*Boletus* sp. *Lactarius deliciosus* *Amanita zambiana* *Lactarius* sp. *Russula capensis* *Russu;la* sp. *Heboloma* sp. *Lactarius* sp.	No collection	No collection	*Chalciporus piperatus*
Guyo	No collection	No collection	No collection	*Russula delica* *Russula nigricaus* *Clavulina cristata* *Lactarius deliciosus* *Boletus* ***sp.***	*Russula fellea* *Clavulina cristata* *Amanita zambiana* *Boletus* sp.	*Boletus* sp. *Russula* sp.	*Boletus* sp. *Russula capensis*

Appendix IV

Rainfall data (mm) for 1998/99, 1999/2000 and 2000/2001 seasons for the four meteorological stations covering the ten respective experimental sites.

Rusape Meteorological Station (For experimental sites: Nemaire I, Nemaire II and St Lukes).

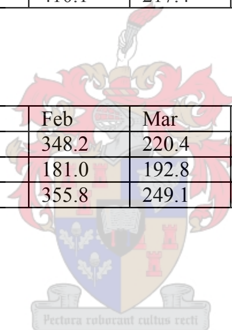
Month/Season	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
1998/99	6	0.0	0.0	5.9	132.6	233.0	508.0	233.8	47.5	5.4	0.0	0.0	1166.2
1999/00	4.7	20.6	3.9	35.3	105.6	143.8	210.0	252.0	165.1	63.2	39.3	11.5	1090.1
2000/01	0.0	0.0	0.0	64.8	195.5	74.2	110.4	232.5	137.5	44.0	0.0	0.0	858.9

Mutare Meteorological Station (For experimental sites: Rowa and Chitakatira).

Month/Season	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
1998/99	21.2	2.7	10.1	16.9	80.4	281.8	329.0	137.4	92.4	33.8	0.0	0.0	1005.8
1999/00	9.9	33.2	3.8	55.0	42.8	146.0	299.8	375.6	247.7	25.6	25.4	20.4	1285.2
2000/01	2.0	1.0	3.0	36.6	224.6	113.6	135.3	410.1	217.4	30.9	2.0	0.0	1176.5

Mvurwi Meteorological Station (For experimental sites: Nyamuzihwa and Guyo).

Month/Season	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
1998/99	0.0	0.0	0.0	0.7	108.2	278.1	478.5	348.2	220.4	27.3	0.0	0.0	1461.4
1999/00	0.0	0.0	0.0	4.1	64.2	108.2	125.2	181.0	192.8	91.2	36.6	15.0	818.3
2000/01	0.0	0.0	0.0	10.5	128.0	206.6	208.7	355.8	249.1	11.0	0.0	0.0	1169.7



Henderson Meteorological Station (For experimental sites: Matiya, Tsikwa, Nzvera).

Month/Season	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
1998/99	0.0	0.0	0.0	15.0	114.5	259.5	431.4	47.0	117.8	12.7	6.3	1.5	1005.7
1999/00	1.0	0.0	0.0	18.0	126.3	70.0	143.9	237.3	233.9	55.9	61.2	23.5	971.0
2000/01	0.7	1.5	2.9	3.5	90.0	164.4	140.6	186.0	277.1	31.4	6.3	1.5	945.5

Appendix V

Simple correlation coefficients between different foliar factors for the rainy period (February) and the dry winter period

Correlation Coefficients for the rainy period (February)

	FB	FCa	FCu	FFe	FK	FMn	FMg	FN	FP	FZn
FB	1.00	0.22	0.31	-0.22	0.20	0.35	0.40	0.26	-0.24	0.32
Fca		1.00	-0.49	0.06	0.64	0.45	-0.47	0.08	-0.50	0.10
Fcu			1.00	-0.42	-0.00	0.02	0.62	0.19	0.08	0.28
Ffe				1.00	0.10	0.28	-0.12	0.03	-0.09	-0.06
FK					1.00	0.43	-0.05	0.09	-0.43	0.03
FMn						1.00	-0.07	-0.34	-0.65	0.75
FMg							1.00	0.34	0.11	0.23
FN								1.00	0.06	-0.43
FP									1.00	-0.40
FZn										1.00

Correlation Coefficients for the winter dry period (June)

	FB	FCa	FCu	FFe	FK	FMn	FMg	FN	FP	FZn
FB	1.00	0.67*	-0.04	0.26	-0.21	0.48	-0.09	-0.27	-0.30	-0.21
Fca		1.00	-0.39	0.05	-0.61	0.79**	0.19	-0.47	-0.05	-0.08
Fcu			1.00	-0.13	0.72*	-0.15	0.06	0.22	0.02	0.05
Ffe				1.00	0.22	-0.19	0.33	-0.61	-0.00	0.14
FK					1.00	-0.54	-0.07	0.13	-0.23	0.42
FMn						1.00	-0.01	-0.25	-0.10	0.12
FMg							1.00	-0.23	0.75	-0.14
FN								1.00	-0.05	-0.25
FP									1.00	-0.22
FZn										1.00

Pectora roborant cultus recti

Appendix VI
Temperature data for February and June 1999

February data

Description	Rusape Meteorological Station For sites: Nemaire I, St Lukes & Nemaire II	Mutare Meteorological Station For sites: Rowa & Chitakatira	Henderson Meteorological Station For sites: Matiya, Tsikwa, & Nzvera	Mvurwi Meteorological Station For sites: Nyamuzihwa, & Guyo
Min temperature (^{0}C)	11.5	10.6	13.1	11.3
Max. temperature (^{0}C)	29.7	30.1	33.2	31.6

June data

Description	Rusape Meteorological Station For sites: Nemaire 1 & Nemaire II	Mutare Meteorological Station For sites: Rowa & Chitakatira	Henderson Meteorological Station For sites: Matiya, Tsikwa, & Nzvera	Mvurwi Meteorological Station For sites: Nyamuzihwa, & Guyo
Min temperature (^{0}C)	5.5	4.5	5.1	7.4
Max. temperature (^{0}C)	21.4	23.5	21.1	22.6