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CONTENTS

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Vesicular arbuscular mycorrhizal fungi prevalence and diversity in Zimbabwean soils

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The prevalence and population levels of VAM fungi in a range of field soil environments in Zimbabwe were determined. The main VAM genera *Acaulospora, Scutellospora, Gigaspora, Glomus, Sclerocystis* and *Entrophospora* were represented in the study sites. The relative abundance was *Glomus* > *Acaulospora* > *Scutellospora* > *Sclerocystis* > *Gigaspora* > *Entrophospora* at all sites except at Chabwino (fallow soil) and Thorn Park (sorghum) where *Acaulospora* < *Scutellospora and Sclerocystis* > *Scutellospora* = *Acaulospora*. Relative proportions of the different VAM genera were similar across soils but population sizes varied with soil management. There was no clear relationship between VAM spore numbers and cropping history although among the cultivated plots, soils that had legumes as the previous or current crop generally had higher VAM populations than under sorghum. Of the soil parameters measured, only percent organic carbon was significantly correlated with VAM numbers and infective propagules.

Keywords: Infective propagules, MPN, precrops, spore counts, vesicular arbuscular mycorrhizae.

Introduction

Vesicular arbuscular mycorrhizae (VAM) fungi are characterised by microscopic structures called vesicles and arbuscules through which they exchange materials with the plant (Morton, 1987; Medina *et al.*, 1988a). Hyphae of VAM grow outwards from the root cortex beyond the root hair and mineral depletion zone and can take up poorly mobile nutrients particularly phosphorus (P) and zinc (Zn) several centimetres away from the root surface (Jackson, *et al.* 1972; Medina *et al.*, 1988a; Bell *et al.*, 1989; Lee and Wani, 1991; Thompson, 1991). Vesicular arbuscular mycorrhizae have also been shown to improve plant growth by acting as physiological sinks for photosynthate (Medina *et al.*, 1988b), increase drought tolerance (Michelsen and Rosendahl, 1990b), enhance disease resistance, improve soil structure and increase plant species diversity (Miller and Jastrow, 1992). Therefore there is potential to exploit VAM for yield improvement of cultivated crops.

Development of VAM inoculant technology has been limited by the inability to culture for use in field situations (Dodd *et al.*, 1990a). Between 1980 and 1986, an EEC-funded CIAT-based project identified and isolated indigenous VAM fungi from different edapho-climatic regions to establish a large 'germplasm' bank for greenhouse, field and on-farm trials (Howeller *et al.*, 1987). The problem of growing VAM axenically could be addressed through growing appropriate precrops to boost the inoculum potential of indigenous VAM in the soil (Dodd *et al.*, 1990b). This option may not be feasible if the ecology and diversity of VAM in the target soils are not understood.

According to Morton (1987), there are 126 identified species of VAM in six genera. Microscopic examination of fungal spores and propagules for characteristic morphological features has been the traditional method of identifying VAM fungi (Gerdemann and Nicholson, 1963; Phillips and Hayman, 1970). Different taxa of VAM can be distinguished microscopically after propagules are extracted by density gradient centrifugation (Daniels and Skipper, 1982). Identification is achieved by comparing individual spores with preserved specimens, species descriptions, illustrations and keys (Morton, 1990).

In Zimbabwe, however, little research has been conducted on VAM and their interactions with crop plants. While the effect of VAM on nematode infection in roots of pepper (*Capsicum annum*) has been assessed (Dube, 1993), no information is available on population levels and diversity of VAM fungi in local soils. Vesicular arbuscular mycorrhizal inoculant technology could represent a low-cost input management option for communal farmers. A better understanding of the ecology of indigenous VAM is essential for successful exploitation of this technology and managing the productivity of local soils. We report on the prevalence and diversity of VAM populations in a range of field environments in Zimbabwe.

Materials and Methods

Soil Sampling and Analysis

Sites were chosen to represent different agro-ecological regions, soil type and management. Composite soil samples were collected from the top zero to 20 cm and transported to the laboratory in ice-cooled chests. Soils were sieved to pass through a 2 mm sieve. A sub-sample was used for recovery of spores and determination of most probable number of infective propagules by the plant infection method. The other portion was air-dried and analysed for mineral nutrients, pH, percent organic carbon, cation exchange capacity (CEC) (Page *et al*, 1982). Particle size analysis was achieved by mechanically dispersing soil and estimating silt and clay fractions using a Bouyoucos hydrometer.

Recovery of spores from the soil

Spores were recovered using sucrose/water density gradient separation (Gerdemann and Nicholson, 1963). The spore suspension was carefully decanted

onto the surface of a 70 percent sucrose solution and centrifuged for three minutes at 1700 rpm. Spores removed from the surface layer were rinsed on a fine sieve (53µm) with a strong stream of water to remove the sucrose and quantitatively transferred into a known volume of water.

Quantification and Identification of Spores

Spores were counted on a nematode counter. Identification was achieved by examining spores microscopically. Different genera were distinguished by colour, size and wall ornamentations. Separated spores were also crushed and wall structure observed for possible identification and delineation of species (Morton, 1987).

Estimating infective propagules

Soil collected from individual sites was used as the inocula. The inocula were mixed with an autoclaved (2 x 2 hr at 120 °C) Mhondoro granitic sand at serial dilutions of 10^{-1} to 10^{-5} (Daniels and Skipper, 1979). Phosphorus (P) and Nitrogen (N) were added at rates equivalent to 40 and 60 kg/ha respectively to ensure these were not limiting. Soil inocula and autoclaved soil (total = 250g) and the fertilizer amendment were thoroughly mixed. The soils were placed in 300 ml plastic pots, wetted to field capacity and four sorghum seeds (var. DC75) planted per pot. Plants were thinned to one per pot after seedling emergence and watered regularly with sterile distilled water. Four replicates were allowed per soil at each dilution level. Plants grown in non-inoculated autoclaved soil acted as controls.

At 14 days after seedling emergence, plants were carefully harvested to ensure that sufficient terminal feeder roots on which VAM develop came out with the plant. The roots were washed clean of soil and weighed. Representative samples were obtained from four different portions of the root systems and combined to make up 30 percent of total fresh root weight. Roots that could not be analysed immediately were preserved in a killing and fixing agent which was prepared by mixing formalin: acetone: alcohol = 90:5:5 by volume.

Root specimens were cleared by heating with 10 percent potassium hydroxide (KOH), bleaching with fresh alkaline hydrogen peroxide (H_2O_2) and acidifying with 1 percent hydrochloric acid (HCl) prior to staining with acid fuchsin (Daniels and Skipper, 1979). The roots were then destained in lactic acid and microscopically examined for VAM infection. The VAM were identified on the basis that they are thick and rough hyphal strands with no septa (Daniels and Skipper, 1979). Stained roots were observed under a microscope at low power magnification. Vertical and horizontal gridlines were scanned for the presence or absence of infection points per field of view and colonisation was recorded at each point where a fungal strand made a point of entry into the root. Five fields of view were scored per sample. The most probable number of infective propagules was estimated by the MPNES computer program based on presence or absence of infection units per dilution level (Bennett *et al.*, 1990).

Results

Soil characteristics

Soils used were generally acid and low in nutrient levels with pHs ranging from 4.1 to 6.1 (Table 1). Except for Crop Science and Thorn Park sites, percent organic carbon, CEC, P, Ca, Mg and K were very low. The soils were of three contrasting types i.e. medium / coarse grained sand for Musana, Mhondoro and Gokwe, sandy clay loam for Chabwino and clay for Thorn Park and Msiiwa.

VAM Fungal Populations

Spore numbers ranged from 5 x 10^4 in University of Zimbabwe Crop Science plot to 53 x 10^4 / 100g in Chabwino fallow under *Eucalyptus* (Table 2). Spore numbers were highest in fallow soils of Musana and Chabwino. There appeared to be no clear trend of the relationship between VAM spore numbers and cropping history although among the cultivated plots, soils that had legumes as the previous or current crop generally had higher counts of VAM than under hybrid sorghum (Table 2). Three sites Gokwe, Mhondoro and Crop Science had less than 20 x 10^4 spores / 100g soil.

The genera represented in the study sites were: *Acaulospora, Scutellospora, Gigaspora, Glomus, Sclerocystis and Entrophospora* (Table 3). The relative abundance was *Glomus* > *Acaulospora* > *Scutellospora* > *Sclerocystis* > *Gigaspora* > *Entrophospora* at all sites except at Chabwino (fallow soil) and Thorn Park (sorghum) where *Acaulospora* < *Scutellospora and Sclerocystis* > *Scutellospora* = *Acaulospora*. Relative proportions of VAM genera were more or less constant across eight of the ten sites (Table 3).

Most Probable Number (MPN) of infective propagules

Crop Science plot had the lowest number of infective propagules (7 x 10^4) and Chabwino fallow the highest (69 x 10^4)(Table 2). Of the cultivated soils, Musiiwa had the highest number of infective propagules (49 x 10^4). Infective propagule numbers were generally higher than total numbers of spores (Table 2). Vesicular arbuscular mycorrhizal fungal spore numbers were correlated to VAM infective propagules at all ten test sites. There was no significant correlation between most soil parameters and VAM numbers and infective propagules, except percent organic carbon (r^2 = 0.62; p = 0.05) (Table 4).

Discussion

Relative abundances of *Acaulospora* and *Scutellospora* were lower at Chabwino and Thorn Park compared to other eight sites, while that of *Glomus* higher. Differences in relative abundances of VAM at Chabwino and Thorn Park compared to other sites is not unusual and could be attributed to differences in cropping history or natural vegetation growing at a particular site which may favour proliferation of certain species more than others.

Table 1: Soil properties at locations sampled in the mycorrhizae population study

Location	Soil Family	Soil Texture	pH	% Organic Carbon	Available P (µg/g)	Exchangeable Cations --------meq/100g ---------			
						K	Ca	Mg	CEC
Musana (C)	6G	mg/sand	4.10	0.72	15.80	0.17	1.69	0.15	3.80
Musana (F)	6G	mg/sand	4.80	0.66	10.20	0.09	1.15	0.26	2.80
Chabwino (F)	6G	Loam sand	4.90	0.77	11.10	0.22	1.02	0.41	3.20
Chabwino (C)	6G	Loam sand	5.40	0.83	36.80	0.30	1.58	0.26	3.60
Msiiwa (C)	5E	Clay	4.90	0.77	3.20	0.29	0.66	0.49	2.10
Mhondoro (C)	6G	mg/sand	4.10	0.12	5.40	0.07	0.30	0.03	1.00
Gokwe (F)	1K	cg/sand	5.20	0.48	5.60	0.04	0.49	0.14	2.30
Crop Science (C)	5E	Clay	5.20	1.67	5.30	0.40	6.00	2.28	15.30
Thornpark 1 (C)(M)	5E	Clay	5.80	1.31	22.30	0.32	5.92	1.92	15.10
Thornpark 2 (C)(S)	5E	Clay	6.10	1.43	18.80	0.37	6.98	2.19	15.50

C = cultivated soil; F = fallow soil; M = maize; S = sorghum

1K = Regosols. Deep sands with <10% silt + clay and poorly developed genetic horizons. Mainly Kalahari sands.

5E = Fersialitic 2:1 mineral lattice, moderately deep to deep reddish brown soils on mafic rocks.
6G = Paraferralitic group on granitic rocks. Moderately deep to deep greyish brown, coarse grained sands over pale sands.
cg = coarse grained; mg = medium grained; CEC = cation exchange capacity

Table 2: Mycorrhizal spore and infective propagule numbers at 10 sites in Zimbabwe.

Site	Natural Region (NR)	Management system	Soil Texture	MPN Infective propagules x10^4/100g soil	Total Spore soil Number x10^4/100g	Cropping History
Musana (C)	II	Communal low-input	mg/Sand	32.8	25.6	Soyabean after maize
Musana (F)	II	Communal low-input	mg/Sand	48.8	38.2	*Eucalyptus* and wild legumes
Chabwino (C)	II	Commercial high-input	Loamy Sand	21.6	19.3	Tobacco after groundnuts
Chabwino (F)	II	Commercial high-input	Loamy Sand	68.8	52.5	*Eucalyptus* and wild legumes
Msiiwa (C)	II	Communal low-input	Clay	49.2	42.5	Soyabean after sunflower
Mhondoro (C)	III	Communal low-input	mg/Sand	6.9	5.4	Maize after maize
Gokwe (F)	IV	Communal low-input	cg/Sand	21.3	15.1	Sparse miombo
Crop Science (C)	II	Commercial high-input	Clay	6.9	5.0	Beans after cowpea + regular soil fumigation
Thornpark 1 (M)	II	Commercial high-input	Clay	38.3	34.6	Maize after soyabean
Thornpark 2 (S)	II	Commercial high-input	Clay	32.8	27.6	Sorghum after soyabean

C = cultivated; F = fallow; M = maize; S = sorghum
NR I >1000mm; NR II = 750 to 1000mm; NR III = 680 to 800mm; NR IV = 450 to 650mm; NR V < 650mm mean annual rainfall (MAR)
cg = coarse grained; mg = medium grained;

Table 3: Vesicular arbuscular mycorrhizae fungal spore distribution at 10 sites in Zimbabwe.

Site	% of total VAM population					
	Acaulospora	Enterophospora	Gigaspora	Glomus	Scutellospora	Sclerocystis
Musana (C)	18	2	3	52	17	8
Musana (F)	19	2	3	50	17	8
Chabwino (C)	18	3	4	51	16	8
Chabwino (F)	10	2	4	59	16	9
Msiiwa (C)	18	2	4	51	17	9
Mhondoro (C)	18	2	4	51	16	8
Gokwe (F)	19	3	3	48	18	9
Crop Science (C)	18	2	4	50	18	8
Thornpark 1 (M)	18	2	4	51	9	16
Thornpark 2 (S)	11	1	2	46	12	29

C = cultivated; F = fallow; M = maize; S = sorghum

***Table 4*: Correlation between VAM spore numbers and soil properties at 10 sites in Zimbabwe.**

Factor	Sites	Trap Host	Sample Size	Correlation Coeff. r^2
pH	All	Sorghum	10	0.12 ns
N	All	Sorghum	10	ND
P	All	Sorghum	10	0.43 ns
K	All	Sorghum	10	0.38 ns
Ca	All	Sorghum	10	0.15 ns
Mg	All	Sorghum	10	0.46 ns
CEC	All	Sorghum	10	0.26 ns
% Moisture	All	Sorghum	10	ND
Texture	All	Sorghum	10	0.10 ns
% OC	All	Sorghum	10	0.62*

* = significant (p = 0.05); ns = not significant (p = 0.05); ND = not done
OC = organic carbon

Fallow soils of Musana and Chabwino had higher VAM fungal populations compared to cultivated soils in the same area (Table 2). The fallow soils were under canopy of *Eucalyptus spp.* plantations while the adjacent cultivated soils had no cover at the time of sampling. Michelsen and Rosendahl, 1988, noted that vegetation cover increases VAM propagule density. This may explain the high spore numbers in these fallow soils. The results however are in contrast to a finding by Abbott and Robson (1977) who showed that agricultural soils were the most infected with VAM. Our soils had low levels of organic carbon (OC), barely exceeding one

percent (Table 1). There was a significant correlation between VAM spore numbers and percent OC ($r^2 = 0.62$; $p = 0.05$) indicating that this may be one of the key parameters affecting spore numbers. Abbott and Robson used soils which had a higher organic matter content compared to local soils.

There was no clear relationship between VAM spore numbers and cropping history although among the cultivated plots, soils that had legumes as the previous or current crop generally had higher VAM populations than under sorghum (Table 2). Soils with soyabean after sunflower had the highest counts and those with beans after cowpea the lowest (Table 3). Cropping pattern may be one of the factors influencing VAM populations and infective propagule counts since pre-crops differ in their ability to stimulate VAM growth and multiplication. Although sorghum has been generally held to be the most appropriate pre-crop for stimulating VAM (Dodd *et al.*, 1990b), our results do not seem to confirm this, judging by the low spore numbers in the sorghum plot. Since a hybrid sorghum variety was used, it is possible that its compatibility with indigenous VAM was low. Further investigations using different cultivars are required to confirm whether sorghum is the best pre-crop for boosting VAM populations in local soils.

Numbers of VAM fungal spores recorded in this study were generally higher (5×10^4 to 53×10^4) than those reported elsewhere (Howeller *et al.*, 1987; Michelsen and Rosendahl, 1990). Seasonal variations in VAM numbers have been observed (Dodd *et al.*, 1990b). Counts in this study were made at the beginning of the dry season, when most VAM would be sporulating with the onset of moisture stress. This could explain the unusually high spore counts. Seasonal variation in spore numbers is likely.

Gokwe recorded the lowest VAM numbers compared to the other eight sites. Possible reasons include the fact that the ground was bare and there was evidence of a forest fire having swept the area prior to sampling. Vesicular arbuscular mycorrhizae are not heat tolerant. Heating for 30 minutes at 50°C reduced colonisation of wheat and heating to 60°C effectively eliminated it (Thompson, 1990). Spore numbers in the upper soil layer are probably reduced due to desiccation during the dry season. Bush fires and high ground temperatures in excess of 50°C that frequently occur during the hot season could seriously reduce VAM numbers in soils with little or no cover.

Low numbers in Crop Science plot were not unexpected given the particular field's history of fumigation with broad spectrum agents for example methyl bromide. Fungicides can eliminate VAM resulting in poor growth of mycorrhizal dependent crops (Thompson, 1990). Jakobsen (1987) also observed reduced VAM numbers in plots where broad spectrum fumigants 'Dazomet' and 'Atrazine' had been applied in some Danish soils. Further research is needed on the effects of pesticides and fungicides on local VAM populations.

Mhondoro and Gokwe sites had highly infertile soils (Table 1), and may therefore have the highest potential for VAM technology application. Dodd *et al.*, (1990b) suggested the use of precrops as a way of producing inoculum using

resident spores as primary infective propagules. The soils from Mhondoro and Gokwe had a low inoculum potential given the low spore numbers recorded hence pre-crops might not significantly boost resident VAM populations. However, these soils would have the best potential for crop response to inoculation because introduced VAM strains would meet little competition from low indigenous populations.

The MPN's of infective propagules were positively correlated with spore numbers, an indication that VAM spore numbers are a good indicator of inoculum potential. The number of infective propagules counted by the MPN method were generally higher than total numbers of spores per site (Table 2). Root density and rooting volume affect number of infection units (Wamocho, 1992). The high numbers of infective propagules may be due to multiple infection whereby one fungal hypha meets the root several times. Plants were grown in pots only 300 ml in volume, a situation which may result in multiple infections from one germinated spore. Secondly, the plant infection method counts all infective propagules including hyphal fragments and spores. Total spore counts accounted for only a fraction of the infective propagules. This may therefore partly explain why MPN counts were generally higher than the actual spore counts. To minimise these problems of over-estimation of inoculum potential, plants need to be grown in large soil volumes for shorter periods.

Our research is the first documented study that we are aware of to look at types and population levels of indigenous VAM fungi in Zimbabwean soils. The study showed that all the main VAM genera are represented in local soils. Different VAM genera occurred in constant relative proportions in all soils studied but population sizes varied with soil management.

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