Ghana J. Sci. **31-36** (1991-96), 81-86

PRELIMINARY STUDIES ON THE OCCURRENCE AND INFECTIVITY OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI IN SOME GHANAIAN SOILS

A. E. ASMAH
Department of Soil Science, University of Cape Coast, Cape Coast, Ghana

Summary

The occurrence and infectivity of vesicular-arbuscular mycorrhizal (VAM) fungi in three soils from the rainforest zone and the coastal savanna zone of Ghana was investigated. Wet-sieving and density gradient centrifugation methods were used to isolate and recover the propagules of the fungi in the soils. Fungal spores were examined microscopically and classified into the known genera. The infectivity of the VAM fungi present in the soils was determined by the most probable number (MPN) method using a two fold dilution series with onion as the test plant. Spores of VAM fungi in the genera *Glomus* and *Gigaspora* were found in all the soils. The infectivity of fungi present ranged from 0.4 to 4.4 propagules per gram of soil. The presence of VAM fungi which belonged to the same taxa in the different soil environments was indicative of some degree of adaptation by the fungi mycobionts.

Introduction

The term 'mycorrhiza' was first coined by Frank (1985) to describe the association between plant roots and fungi. The fungi involved span a broad range in the Zygomycetes, the Ascomycetes and the Basidiomycetes (Harley & Smith, 1983). It is generally believed that the mycorrhizal association is mutualistic, although it has also been described by Hacskaylo (1972) as a physiologically well-balanced parasitism. Zygomycetous endomycorrhizas are the most abundant and widespread mycorrhizas forming in the roots of many Angiosperms, Gymnosperms, Pteridophytes and Thallophytes (Mosse, Stribley & Tacon, 1981). In recent years, these associations have been referred to as 'vesicular-arbuscular mycorrhizas' (VAM) even though some of the fungi do not produce intraradical vesicles (Morton, 1988).

Early investigations on mycorrhizas in the tropics can be traced back to the 19th century when Treub (1895) recorded VAM in sugar cane in Java. A survey of mycorrhizal associations in bryophytes, vascular cryptogams, gymnosperms and some species of monocots and woody dicots was also carried out by Janse (1896).

A greater proportion of the work done on VA mycorrhizas has been on the effect of the mutualistic association on the phosphorus nutrition of plants (e.g., Hayman, 1980; Owusu-Bennoah & Wild, 1979; Krishna *et al.*, 1985). Other non-nutritional effects of mycorrhizas on crop plants such as enhanced production of auxins, improved water relations and increased resistance of plants to disease have been reported (Dehne, 1982; McGraw & Schenck, 1981).

In recent years, the development of sustainable agricultural production systems has been a subject of much concern and many strategies and guidelines have been proposed to achieve this (e.g., BIFAD, 1988; CGIAR/TAC, 1988; FAO, 1986). A combination of elements of traditional crop production systems and their component technologies that make maximum use of biological inputs available locally, combined with affordable elements of modern high input systems appears to be the way forward.

Expenditure on fertilizers in crop production systems in developing countries is a major financial constraint to farmers with limited capital. Vesicular-arbuscular mycorrhizas could be used to increase the utilization of current fertilizer applications as well as some residual nutrients from

previous applications.

Although not host-specific, VAM fungi have been known to exhibit host preferences which have been attributed in part to differences among species and among the genera due to differing abilities to form mycorrhizas rapidly and extensively. The differences found in soil environments in Ghana with regard to temperature, moisture, nutrient status and soil reaction could, therefore, result in the existence of diverse strains, species or genera of VAM fungi adapted to the varied environments.

To date, over 126 species of VAM fungi in six genera have been reported in the literature (Schenck & Perez, 1990), but a dearth of information exists regarding the presence of these fungi in soils of Ghana. The wide variation in the ability of VAM fungi to stimulate nutrient uptake and plant growth demands a knowledge of the species or genera involved in order to optimize the benefits to be derived from the association.

A preliminary investigation was conducted to determine the diversity of the indigenous mycorrhizal fungal population in some soils from the rainforest and savanna ecological zones of Ghana.

Experimental

Site description and soils

Two sites located in the rainforest and savanna agroecological zones were chosen for the sampling of soils used int he investigation. Site 1 was located at Cape Coast in the coastal thicket zone of Ghana and the soil belonged to the Udu Series, an Aquick Paleustalf. Site 2 was located at Aiyinase in the high rainforest zone of southwestern Ghana and the two soils at the site were Basachia (Typic Haplustox) and Tikobo series (Ustox). Surface soils (0-20 cm) from 2-h areas were sampled with polypropylene corers. At each location, 20 samples were taken and mixed; composite subsamples were obtained and stored at room temperature.

Soil analysis

Analysis was carried out on sieved (2 mm) subsamples of the soils. Soil *pH* was determined in a 1:2.5 soil to $CaCl_2$ ratio using a glass-calomel electrode. Effective cation exchange capacity was determined by the method of Gillman (1979). Available phosphorus was determined by anion exchange resin (Biorad AG 21k 16-20 mesh) in sealed nylon mesh bags were placed in 100 ml centrifuge tubes with 2 g soil in 70 ml deionised water, the tubes were shaken for 24 h at room temp (24 °C), the bags were then suspended in 60 ml 0.1 *M* HCl, after washing with distilled water to remove soil particles. Phosphate concentration in the acid extractant was determined colorimetrically by the method of Murphy & Riley (1962). Available nitrogen (NH_4-N and NO_3-N) was determined by the method of Keeney & Nelson (1982) and organic carbon by the Walkley-Black procedure (Page, Miller & Keeney, 1982). Exchangeable aluminium was determined by shaking 10 g soil with 50 ml neutral 1 *M* KCl for 15 s and the suspension filtered. The residue on the filter paper were then leached with 10 ml portions of the extracting solution. Aluminium in the filtrate was determined by the colorimetric method of Pritchard (1967) using xylenol orange as the colour-developing reagent. Mechanical analysis was carried out using the International Pipette Method.

Isolation and recovery of VAM propagules

Spores and other propagules of VAM fungi in the soils were isolated by a modified wet sieving and decantation method (McKenney & Lindsey, 1987). Spores were recovered from debris by density gradient centrifugation (Ohms, 1957) and examined microscopically. Spores were identified and tentatively placed in taxonomic classes with the aid of an identification manual (Schenck & Perez, 1990) and taxonomic keys of Hall & Fish (1979) and Trappe (1982).

Bioassay of mycorrhizal fungal propagules

Infectivity of VAM fungi was determined by the most probable number method using a two-

fold dilution series (2^{-1} to 2^{-7}). Gamma irradiated (2.5 Mrad) samples of the soils were used as diluents and onion as a test plant. After 6 weeks of growth, the roots were washed and cut into 1 cm pieces, cleared in 10 per cent potassium hydroxide solution (Phillips & Hayman, 1970) and acidified after thorough rinsing in distilled water. Roots were stained with a solution of 0.05 per cent trypan blue in acidic glycerol (Koske & Gemma, 1989). Root pieces were placed on glass slides and examined under a compound microscope for the presence or absence of mycorrhizal fungal structures (vesicles, arbuscules and hyphae) at each dilution.

The most probable number (MPN) of infective mycorrhizal propagules was calculated from the number of pots with infection by an approximation of the maximum likelihood method (Mather, 1949) as described by Fisher & Yates (1963).

Statistical analysis

Confidence limits (95%) were calculated for the MPN of infective propagules using the tables of Fisher & Yates (1963).

Results

Soil properties

The physical and chemical properties of Basachia, Udu and Tiboko soils are presented in Table 1. The soils were strong to moderate acidity as indicated by their *p*H. The effective cation exchange capacities of the soils were below 5 *c*mol per kg soil. Available P concentrations in the soil were low and Al saturation of the exchange complex was below 20 per cent.

Spore characteristics

Different spore types were isolated and recovered from the soils. The density of spores ranged between 55 and 70 per 10 g of soil and consisted of different sizes with variations in colour from brown to white or hyaline. Spore size ranges in the different soils are presented in Table 2. From morphological features, spores isolated from Udu soil were tentatively identified as *Glomus occultum, Glomus mosseae, Gigaspora magarita* and *Gigaspora gigantea.* Spores belonging to the genus formerly known as *Sclerocytis (Sclerocytis clavispora)* now included in the genus *Glomus*, were found only in Udu soil. Other *Glomus* species were present in all the three soils. The densities of spores in Basachia and Tikobo soils were 70 and 55 spores per 10 g soil respectively. In these soils, spores were identified by morphological features and placed in the genera of *Glomus* spp. and *Gigaspora* spp.

Infectivity of mycorrhizal fungal propagules

The MPN of infective propagules per gram of soil in the three soils are presented in Table 2. The MPN values ranged from 0.79 to 1.92.

TABLE 1.
Some physical and chemical properties of the soils

Soil series	*pH* ($CaCl_2$)	*Available P*	*Available N*	*Al*	*CEC*	*Al sat.*	*Org C*	*Clay*	*Silt*
		mg kg^{-1}		*cmol kg^{-1}*				*per cent*	
Basachia (Typic Haplustox)	4.65	4.85	52.55	0.32	3.27	9.79	2.31	18.56	17.23
Tikobo (Ustox)	4.31	4.14	52.76	0.52	2.01	19.92	2.24	22.23	18.25
Udu (Aquic Paleustalf)	5.31	8.78	61.36	0.11	4.36	2.52	2.22	30.14	20.22

TABLE 2
Number of spores by size ranges and infectivity of mycorrhizal propagules in the soils

Soil series	>75 m				75-210 μm				210-600μm				>600 μm		Infectivity	
	Spore types															
	A	B	C	D	A	B	C	D	A	B	C	D	D	Total	MPN	Confidence limits)*
Basachia	5	0	5	10	10	0	15	20	5	0	30	10	30	140	1.92	0.65 - 2.19
Tikobo	5	0	0	10	15	0	10	25	5	0	10	20	20	110	0.79	0.43 - 1.45
Udu	6	0	0	3	13	3	10	20	10	3	20	9	13	120	1.78	1.30 - 4.39

Spore types: A - *Glomus* spp. B - *Glomus (Sclerocystis)*, C - *Gigaspora* spp. D-Unidentified spores
MPN: Most Probable Number/g soil
* Confidence limits ($P \leq 0.05$) were calculated using average values of the variances of the 'mean infection' levels (Fisher & Yates, 1969).

Discussion

The low concentration of available P found in the soils is characteristic of most of the highly-weathered acid soils of the tropics, most of which are usually deficient in available P. This has been attributed to the low P reserves and the dominance of occluded forms of P over more active P forms (Dabin, 1980). The low available P in soils could provide a favourable environment among others for the proliferation of VAM fungi and colonization of plant.

The community of mycorrhizal fungi in a given soil has been shown to be rather complex and to be composed of a number of species, all of which are not necessarily active on a given plant host (Modjo, Hendrix & Nesmith, 1987; McGraw & Hendrix, 1984). The spore types isolated and recovered from Udu, Basachia and Tikobo soils indicated that the indigenous fungi present consisted of different species of the known genera of mycorrhizal fungi at different stages of maturation.

The composition of VAM fungal populations has been reported to vary depending on factors which include the management practices (McGraw & Hendrix, 1984; Schenck & Kinlock, 1980). The soils used in this study had different histories of management practice. The previous crop at the site where Udu soil was sampled had been maize while the standing crop at the site where Basachia and Tikobo soils were sampled was cassava, and this could possibly be the reason for the high spore numbers per gram of soil since the two crop plants are known to be mycotrophic.

Generally, VAM fungi are not limited in their host range but different VAM fungi are not always equally infective for one plant species (Hayman, 1987). The variation in their physiological interaction with different plants will in turn affect reproduction and survival of VAM fungi in the field and this would also affect the species composition of field populations.

Spore numbers generally do not reflect the viability or infectivity of mycorrhizal fungi present in soils since recovered spores may all not be viable. The density of propagules in the soils determined by the MPN showed that the three soils had different densities of viable propagules. These propagules may have consisted of root pieces with embedded spores or hyphal fragments, as well as spores and sporocarps. The MPN values were in the range of 0.4 to 4.4 per gram of soil. Some temperate soils have been reported to have MPN values in the range of 0.1 to 10 (Porter, 1979; Wilson, Trinick & Parker, 1983) as well as 0.07 to 1.58 (Gianinazzi - Pearson, Gianinazzi & Trouvelot, 1985).

The VAM fungi in Basachia, Udu and

Tikobo soils suggest a range of infectivity based on the MPN values found in this study which provides a measure of the potential of the VAM fungal populations. The occurrence of VAM fungi from different taxonomic groups in the soil environments investigated was also an indication of some degree of adaptation by the fungal mycobionts since the sites were characterized by different physico-chemical and climatic conditions. Further work on the influence of edaphic factors and agronomic practices on the root-fungus association may be necessary, if the potential of mycorrhizas for increased and sustained crop yields is to be properly harnessed.

References

BIFAD (Board for International Food and Agricultural Development) Task Force (1988) *Environment and Natural Resources: Strategies for Sustainable Agriculture.* Washington, DC: US Agency for International Development.

CGIAR/TAC (Consultative Group on International Agricultural Research/Technical Advisory Committee) (1988) *Sustainable Agricultural Production: Implications for International Agricultural Research.* Rome.

DABIN, B. (1980) Phosphorus deficiency in tropical soils as a constraint on agricultural output. In *Priorities for Alleviating Soil-Related Constraints to Food Production in the Tropics.* Los Banos, The Phillipines: IRRI.

FAO (1986) *African Agriculture: The Next 25 Years Annex II. The l & Resource Bases.* Rome.

FISHER, R. A. & YATES, F. (1963) *Statistical Tables for Biological, Agricultural & Medical Research.* Edinburgh: Oliver & Boyd.

FRANK, A. B. (1885) Uber die auf Wurzelsymbiose beruhende, *Ernahrung Berichte Deotochen Botanischen Gesellschaft* **3**, 128-145.

GILLMAN, G. P. (1979) A proposed method for the measurement of exchange properties of highly weathered soils. *Australian J. Soil Res.* **17**, 129-139.

GIANINAZZI-PEARSON, V., GIANINAZZI, S. & TROUVELOT, A. (1985) Determination of the infectivity and effectiveness of indigenous vesicular-arbuscular fungal populations in some agricultural soils in Burgundy. *Can. J. Bot.* **63**, 1521-1524.

HACSKAYLO, E. (1972) Mycorrhizas: The ultimate in reciprocal parasitism? *Bioscience* **22**, 577-583.

HALL, I. R. & FISH, B. J. (1979) A key to the Endogonaceae. *Trans. Br. mycol. Soc.* **73**, 261-270.

HARLEY, J. L. & SMITH, S. E. (1983) *Mycorrhizal Symbiosis.* London: Academic Press.

HAYMAN, D. S. (1980) Mycorrhiza and crop production. *Nature* **287**, 487-488.

HAYMAN, D. S. (1987) VA mycorrhizas in field crop systems. In *Ecophysiology of VA Mycorrhizas* (ed. G. R. Safir), pp. 171-192. Boca Raton, Florida: CRC Press.

JANES, J. M. (1896) Les endophytes radicaux de quelques plantes Javanais. *Anals Jard. bot. Buitenz.* **14**, 53-212.

KEENEY, D. R. & NELSON, D. W. (1982) Nitrogen - inorganic forms. In *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties:* (ed. A.L. Page, R. H. Miller and D. R. Keeney), pp. 643-709. Madison, Wisconson: ASA.

KOSKE, R. E. & GEMMA, J. N. (1989) A modified procedure for staining roots to detect VA mycorrhizas. *Mychol. Res.* **92**, 485-488.

KRISHNA, K. R., SHETTY, K. G., DART, P. J. & REWS, D. J. (1985) Genotype dependent variation in mycorrhizal colonization and response to inoculation of pearl millet. *Pl. Soil* **86**, 113-125.

MATHER, K. (1949) The analysis of extinction time data in bioassay. *Biometrics* **5**, 127-143.

McGraw, A. C. & Hendrix, J. W. (1984) Host and soil fumigation effects on spore population densities of species of endogonaceous mycorrhizal fungi. *Mycologia* **76**, 122-125.

MCGRAW, A. C. & SCHENCK, N. C. (1981) Effects of two species of vesicular-arbuscular mycorrhizal fungi on the development of *Fusarium* wilt of tomato, *Phytopathology* **79**, 894-956.

MCKENNEY, M. C. & LINDSEY, D. L. (1987) Improved method for quantifying endomycorrhizal fungal spores from soil. *Mycologia* **79**, 779-782.

MODJO, H. S., HENDRIX, J. W. & NESMITH, W. C. (1987) Mycorrhizal fungi in relation to the control of tobacco stunt disease with soil fumigants. *Soil Biol. Biochem.* **19**, 289-295.

MORTON, J. B. (1988) Taxonomy of VA mycorrhizal fungi. Nomenclature & Identification. *Mycotaxon* **32**, 267-324.

MOSSE, B., STRIBLEY, D. P. & LE TACON, F. (1981) The ecology of mycorrhizae and mycorrhizal fungi. In *Advances in Microbiology and Ecology* Vol. 5 (ed. M. Alexander), pp. 137-210. New York: Plenum

Press.

MURPHY, J. & RILEY, J. P. (1962) A modified single solution method for determination of phosphate in natural waters. *Analytica Chim. Acta.* **27**, 31-36.

OHMS, R. E. (1975) A flotation method for collecting spores of phycomycetous mycorrhizal parasite from soil. *Phytopathology* **47**, 751-752.

OWUSU-BENNOAH, E. & WILD, A. (1979) Effects of vesicular-arbuscular mycorrhiza on the size of the labile pool of soil phosphate. *Pl. Soil* **54**, 233-237.

PAGE, A. L., MILLER, R. H. & KEENEY, D. R. (1982) *Methods of Soil Analysis. Part 2: Chemical and microbiological properties.* Madison, Wisconson: ASA.

PHILLIPS, J. M. & HAYMAN, D. S. (1970) Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. mycol.* Soc. **55**, 158-161.

PORTER, W. M. (1970) The 'Most Probable Number' method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. *Aust. J. Soil Sci.* **17**, 515-519.

PRITCHARD, D. T. (1967) Spectrophometric determination of aluminium in soil extracts with xylenol orange. *Analyst* **92**, 103-106.

SCHENCK, N. C. & KINLOCH, R. A. (1980 Incidence of mycorrhizal fungi on six field crops in mono culture on a newly cleared woody site. *Mycologia* **72**, 445-453.

SCHENCK, N. C. & PEREZ, Y. (1990) *Manual for the Identification of VA Mycorrhizal Fungi,* p. 261. Gainesville, Florida: Synergistic Publications.

TRAPPE, J. M. (1982) Synoptic keys to the genera & species of zygomycetous mycorrhizal fungi. *Phytopathology* **72**, 102-114.

TREUB, M. (1885) Onderzoekingen over Serch-zick sunkerriet. *Onderzoekingen ober Sereh-zick Meded Planta Tuin Batavia.* II. n

WILSON, J. M., TRINICK, M. J. & PARKER, C. A. (1983) The identification of vesicular-arbuscular fungi using immunofluorescence. *Soil Biol. Biochem.* **15**, 439-445.

Received 10 Aug 93; revised 13 May 96.