Pertanika J. Trop. Agric. Sci. 17(1): 33-42(1994) ISSN: 0126-6128

Role of Edaphic Factors on VAM Fungal Colonization and Spore Populations in Certain Tropical Wild Legumes

T. MUTHUKUMAR, K. UDAIYAN and S. MANIAN
Microbiology Laboratory, Department of Botany
Bharathiar University, Coimbatore 641046
Tamil Nadu, India

Keywords: VAM fungi, nodulating legumes, root colonization, spore number, edaphic factors

ABSTRAK

Empat kekacang pembintilan tahunan tropika iaitu Alysicarpus monilifer, Desmodium triflorum, Indigofera linnaei *dan* Tephrosia purpurea *daripada tiga kawasan yang berbeza di Western Ghats ekosistem dikaji untuk menilai status mikoriza mereka. Tindakbalas pendudukan akar mikoriza vesikel-arbuskular (VAM) dan bilangan spora terhadap faktor-faktor edafi seperti kelembapan tanah, pH dan N serta P telah dikaji. Walaupun bilangan spora berbeza di dalam dan di antara kawasan, pendudukan akar yang setara didapati pada semua tumbuhan di dalam kajian ini. Bilangan spora yang direkodkan adalah tinggi, iaitu antara 15 hingga 165 spora* g^{-1} *tanah. Spora 16 spesis kulat VAM yang tergolong dalam keluarga Acaulospora, Glomus dan Scutellospora diasingkan daripada tanah rizosfera.*

Secara umum, kelembapan tanah mempunyai kesan positif terhadap pendudukan VAM dan pensporaan kecuali terhadap I. linnaei. *pH pula mempunyai perkaitan negatif dengan jangkitan akar pada* I. linnaei *dan* T. purperea, *tetapi ia tidak mempengaruhi dua spesis lain. Kesan 2-pH ke atas pensporaan berbeza berdasarkan spesis induk dan kawasan. Tiada perkaitan umum wujud di antara khasiat tanah, pendudukan akar dan bilangan spora tetapi pengaruh N dan P adalah kaunteraktif ke atas jangkitan VAM. Kajian ini menunjukkan tindakbalas pendudukan akar dan bilangan spora terhadap faktor-faktor edafi adalah lebih merupakan suatu fenomena bertumpu dan tidak menyeluruh.*

ABSTRACT

Four nodulating annual tropical wild legumes, viz., Alysicarpus monilifer, Desmodium triflorum, Indigofera linnaei and Tephrosia purpurea *from three different regions in the Western Ghats ecosystem were investigated to assess their mycorrhizal status. The response of vesicular-arbuscular mycorrhizal (VAM) root colonization and spore number to edaphic factors such as soil moisture, pH and available N and P was analysed. Though the spore number varied significantly both within and between sites, a uniformly high degree of root colonization was observed for all the plants in the present study. The spore number recorded was high, ranging from 15 to 165 spores* g^{-1} *soil. Spores of sixteen VAM fungal species belonging to* Acaulospora, Glomus *and* Scutellospora *were isolated from the rhizosphere soils.*

Soil moisture generally had a positive influence on VAM colonization and sporulation except in I. linnaei. *The pH correlated negatively with root infection in* I. linnaei *and* T. purpurea, *but had no influence in the other two species. The effect of 2-pH on sporulation varied with host species and sites. No general correlation existed between available soil nutrients, root colonization and spore number but the influence of N and P was counteractive on VAM infection. The present study indicates that the response of root colonization and spore number to edaphic factors is a localised rather than a generalised phenomenon.*

INTRODUCTION

Vesicular-arbuscular mycorrhizal (VAM) fungi are ubiquitous and are an important factor in regulating and cycling of nutrients in most natural ecosystems. Information is lacking on how the composition and distribution of these fungi are affected by climatic and edaphic conditions. Researches in the past were directed mainly to understanding the general phenomenon of the mycorrhizal symbiosis such as differences in

their distribution, effect on host plants and their ability to colonize roots and sporulate. Such studies have demonstrated the sensitivity of individual species/isolates of VAM fungi to soil and environmental conditions (Mosse *et al.* 1981).

Spore germination, colonization of host roots and the ability of VAM fungi to influence the growth and physiology of the host are affected by edaphic factors (Daniels Hetrick 1984). Most of these studies were conducted in pot experiments under simulated field conditions, which have a limited predictive value under natural conditions although they provide a basic understanding of the physiology and ecology of the VAM systems (Bethlenfalvay *el al.* 1982). The intricacy of various interacting factors in natural ecosystems makes it difficult to assess the relationship between the host plant and its VAM endophyte (Bowen and Bevege 1976; Ross and Gillam 1973). Even as the distribution and abundance of VAM fungi are known to vary with climatic and edaphic environments, the factors which control their actual distribution are poorly understood (Azizah Chulan and Omar 1991). Our ability to manipulate mycorrhizal symbiosis for agricultural benefits would be limited without a clear understanding of the role of edaphic factors on VAM fungal colonization and sporulation.

In the present study, four annual nodulating legumes from three different regions in the Western Ghats ecosystem were selected for assessment of their mycorrhizal status and the response of VAM system to edaphic conditions. Since the response of VAM fungi may vary with host age, season etc., it seemed worthwhile to determine the response of VAM fungi in these annual legumes which are available for a short period during the monsoon. The aims of the present study are to: 1) assess the mycorrhizal status of the legumes under investigation, 2) find out the relationship between edaphic factors and VAM fungal root colonization and spore density and 3) use the results from this study as a reference for the introduction of exotic VAM fungal species in future experimental studies.

MATERIALS AND METHODS

Study Area and Plants Investigated

The studies were conducted at three different regions in Western Ghats. Site A, an *Acacia* dominated jungle of Maruthamalai forest, is located at 76°93′N and 11°4′E. It is an offshoot of Western Ghats. Site B is *Cymbo pogoncaesius* dominated grassland at the foot of Maruthamalai hills. Site C, a *Teciona grandis* dominated dry deciduous forest at Siruvani, is a part of Nilgiri Biological Reserve, located at 76°37′N and 10°58′E.

Four annual nodulating legumes *Alysicarpus monilifer* DC., *Desmodium triflorum* W. & A., *Indigofera linnaei* Ali and *Tephrosia purpurea* Pers., were examined for VAM association and their rhizosphere soils examined for VAM spores. Soil samples were collected at 550m, 426.72m and 500m MSL from the respective study sites. Total annual rainfall during the study period was 134.6mm at sites A and B and 238mm at site C.

Collection of Samples

Roots and rhizosphere soil samples were collected from the three sites between August and October 1991, during the monsoon when the plants were plentiful. An average of five samples for each species were sampled. Plant roots were dug out, washed thoroughly to remove the adhering soil particles and fixed in FAA (5ml formalin, 5ml glacial acetic acid and 90ml 70% ethanol). Rhizosphere soil samples of the respective plant species (collected from different individuals) were thoroughly mixed to form a composite soil sample. The composite samples were packed individually in polyethylene bags for transport to the laboratory and stored at 4°C for future analyses.

Analysis of Soil Physiochemical Properties

The soil moisture (dried 24h at 105°C) and pH (1: 1, soil: water) were determined soon after the soil samples were brought to the laboratory. The available nutrients, nitrogen and phosphorus were analysed using standard procedures of Jackson (1958) and Misra (1968).

Determination of Mycorrhizal Status

Clearing and Staining of Roots

The root samples of each species were gently washed free of FAA and cut into approximately 1 cm long segments. The root segments of the individuals of a species were mixed to form a composite sample. The composite sample was cleared by boiling in 10% KOH and the boiling time varied according to the thickness of the root

segments. Cleared roots were acidified (5N HCI) and stained with 0.05% trypan blue in lactophenol (Phillips and Hayman 1970). Fifty root segments from each composite sample were examined for the presence of VAM structures. The percentage root colonization by VAM fungi was estimated using the root-slide technique of Read *el al.* (1976).

VAM Fungal Spore Enumeration

VAM fungal spores were recovered from soil by a slight modification of the wet sieving and decanting technique of Gerdemann and Nicolson (1963). One hundred grams of soil from each composite sample were dispersed in 500 ml of water to form a uniform suspension. The heavier soil particles were allowed to settle for 10-20 min. The suspension was then passed through a series of sieves ranging from 710-38m. The residues from the sieves were washed into beakers. After the settling of the heavier particles , the suspension was filtered through a Whatman No. I filter paper. To make spore count easier, lines at a distance of 2 mm were drawn on the filter paper and the intact spores were counted under appropriate magnification (x100).

Isolation and Identification of VAM Fungal Spores

Intact spores were picked up using a wet needle and mounted in lactophenol for identification. The species of VAM fungi were identified according to the sporocarpic and spore characters such as spore size, colour, spore walls, hyphal attachments and other morphological characters (Morton 1988; Schenck and Perez 1987).

Statistical Analyses

Two factor analysis of variance was used to compare the variation of root colonization and spore number between and within sites. Pearson's coefficient correlation was used to determine the degree of association between root colonization and spore number with edaphic factors. Data on root colonization and spore number were subjected to linear regression analysis (Zar 1984)

RESULTS

The soil at sites A and B was clayey loam and that at site C was sandy loam. The soils at all the study sites were low in available nutrients especially phosphorus(P) (Table 1). The soil pH ranged

TABLE 1
Soil characteristics of the sites surveyed in this study

	Soil moisture (%)	pH	Phosphorus ($mg\ kg^{-1}$)	Nitrogen ($mg\ kg^{-1}$)
Site A				
Aug.	16.86	8.3	0.60	9.80
Sept.	20.13	7.7	0.50	13.00
Oct.	27.85	7.3	0.60	3.40
Mean	21.61(1.66)	7.8(0.39)	0.57(0.04)	8.73(1.83)
Site B				
Aug.	17.28	7.4	1.30	7.40
Sept.	19.90	7.4	0.50	11.10
Oct.	27.20	8.0	0.40	11.60
Mean	21.46(2.42)	7.6(0.12)	0.73(0.22)	10.03(0.99)
Site C				
Aug.	20.75	8.0	2.3	12.60
Sept.	19.70	8.1	1.9	13.90
Oct.	31.27	7.3	0.9	12.60
Mean	23.91(3.01)	7.8(0.53)	1.7(0.47)	13.03(0.53)

SE in parantheses

from 7.3 to 8.3 and the soil moisture ranged between 16.86 to 31.27% (Table 1).

All the four legumes investigated showed a high degree of root colonization under varied edapho-climatic conditions (*Fig. 2*). Root colonization showed no variation both within and between sites except for *A. monilifer* which exhibited a significant variation ($P < 0.05$) between sites (Table 2). VAM structures mainly consisted of hyphae and vesicles with arbuscules restricted to younger regions of the root. The vesicles were terminal, spherical and/or oval (*Fig. 1*)

The spore number in the rhizosphere soil ranged from 15-165 spores g^{-1} dry soil (*Fig. 2*). The average spore number recorded were 109.78, 64.44 and 78.56 spores g^{-1} dry soil at sites A, B and C respectively. Spore density varied significantly both between ($P<0.01$) and within sites ($P<0.01$) except for *I. linnaei*, where the variation was significant ($P<0.05$) only between sites. No variation was observed in *T. purpurea* (Table 2).

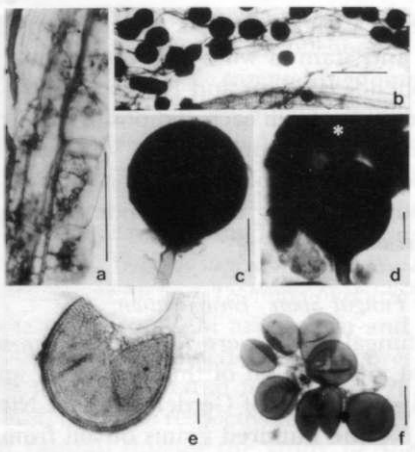


Fig. 1: (a) Arbuscules and distributive hyphae in Indigoferalinnaei*; (b) Vesicles in* Tephrosia purpurea*; pores of (c)* Glomus constirctum*; (d)* G. monporum *with peridial(asterisk) remains;(e)* Acauospora scrobiculata*; (f)* Glomu aggregatum. *Bars = 50*

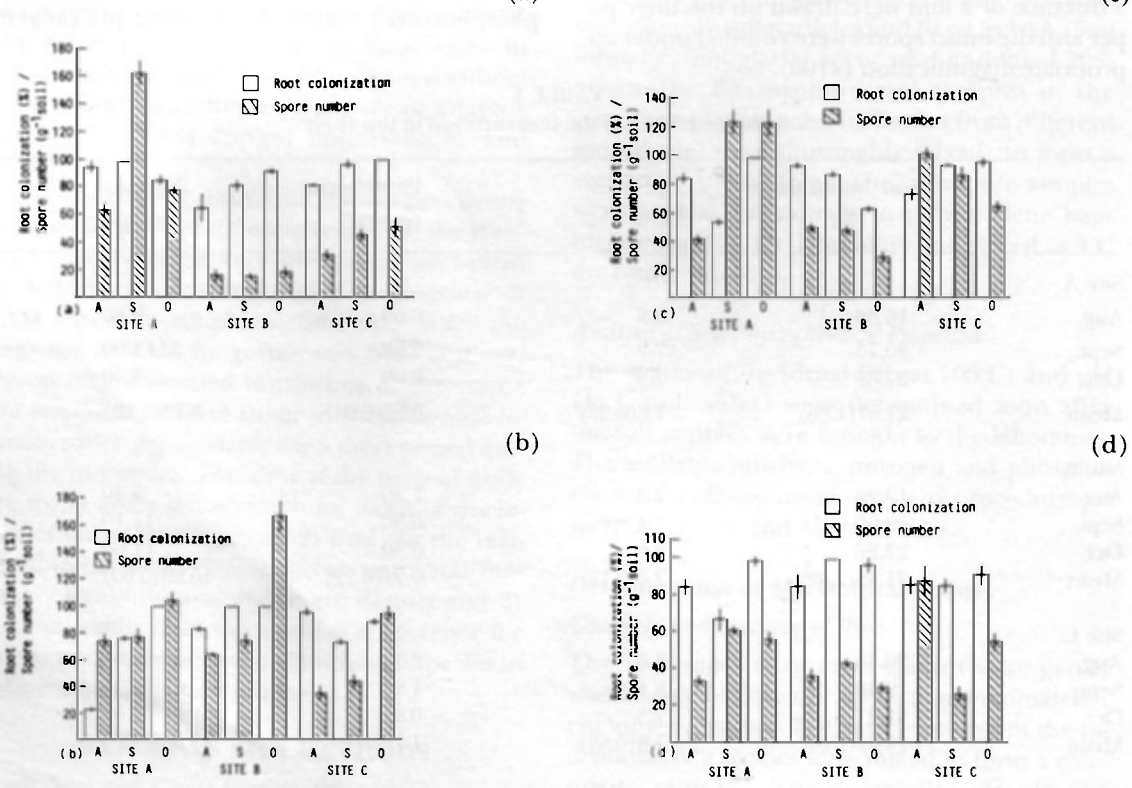


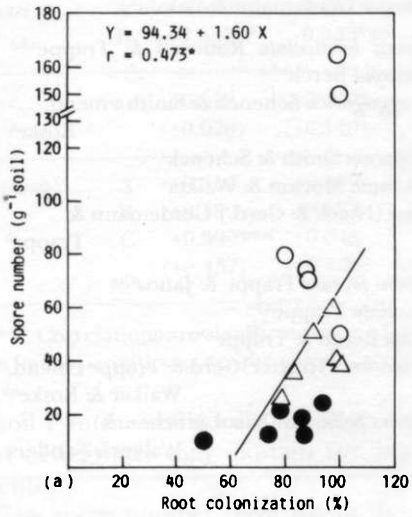
Fig. 2: Mean values for the spore number and VAM infection of A. monilifer *(a);* D. triflorm *(b);* I.linnaei *(c)* T. purpurea *(d) at different sites during the study period (A, August; S. September; O, October). Vertical lines indicate ± SE of the mean*

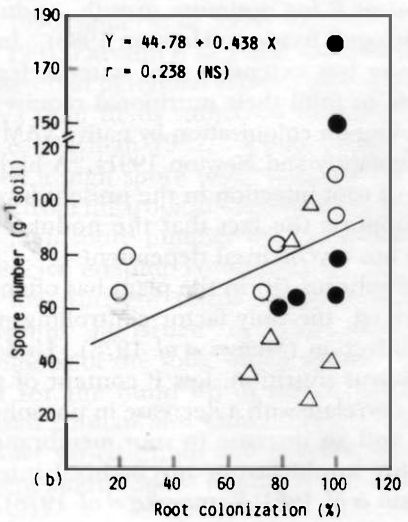
TABLE 2
F values from two-way analysis of variance on VAM root colonization and spore number both within and in between sites. A. monilifer (A.m); D. triflorum (D.t); L linnaei (1.1); T. purpurea (T.p).

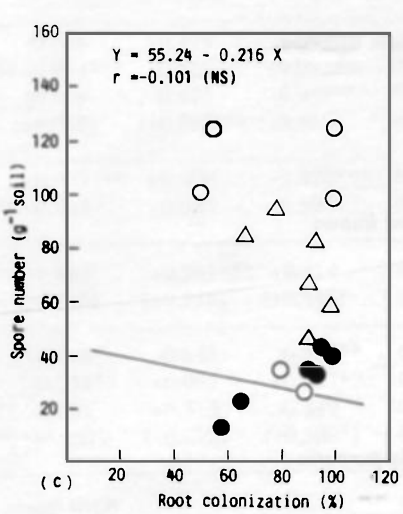
	Root colonization				Spore density			
	A.m	D.t	I.1	T.p	A.m	D.t	I.1	T.p
Among sites	3.54*	2.62	0.16	1.55	12.00**	6.91*	4.76*	1.33
Within sites	1.50	1.13	0.05	1.01	6.70**	7.00**	0.35	0.28

Significant at p=:0.05 and 0.01, respectively

A significant positive correlation (P<0.05) was established between spore number and root colonization in *A. monilifer* but not in the others. However, the degree and nature of correlation varied between plant species (*Fig. 3*).







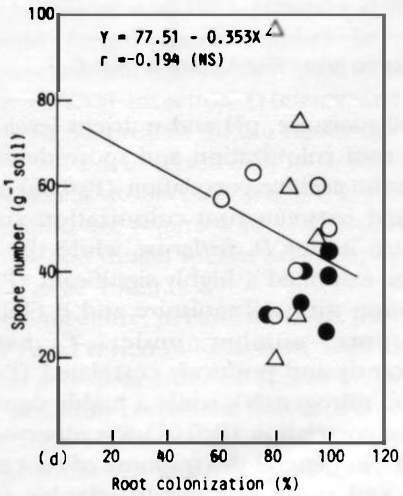


Fig. 3: Relationship between root colonization and spore number in A. monilifer *(a);* D. triflorm *(b)* I. linnaei *(c) and* T. purpurea *(d). Symbols represent the replicates for Site A* (o) *Site B* (o) *and Site C* (▲*). *Significant at P < 0.05, NS Not significant*

VAM spores belonging to 16 species assignable to Acaulospora (5 spp.), *Glomus* (10 spp.) and *Scutellospora* (I sp.) were isolated from the rhizosphere soils (*Fig.1*; Table 3).

TABLE 3
Vesicular arbuscular mycorrhizal fungal species isolated from the soils of study sites

Host plant	VAM fungal species*
Alysicarpus monilifer	*Acaulospora bireticulata* Rothwell & Trappe[a,b,c] *A. nicolsonii* Walker, Reed & Sanders[b] *Glomus ambisporum* Smith & Schenck[a] *G. microcarpum* Tul. & Tul.[a] *G. monosporum* Gerdemann & Trappe[a,b,c]
Desmodium triflorum	*Acaulospora bireliculata* Rothwell & Trappe[a,b,c] *Glomus constriclum* Trappe[a,b] *G. diaphanum* Morton & Walker[a,b] *G. fasciculatum*(Thaxter) Gerd. & Trappe emnd. Walker & Kos ke[b] *G. multicaule* Gerdemann & Bakshi[a]
Indigofera linnaei	*Acaulospora bireticulata* Rothwell & Trappe[a,b,c] *A. sporocarpia* Berch[a,b,c] *Glomus aggregatum* Schenck & Smith emend. Koske[a] *G. ambisporum* Smith & Schenck[a] *G. diaphanum* Morton & Walker[c] *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe[b]
Tephrosia purpurea	*Acaulospora foveata* Trappe & Janos[a,b,c] *A. scrobiculata* Trappe[a,b,c] *Glomus hoi* Berch & Trappe[a,b,c] *G. fasciculatum* (Thaxter)Gerd.& Trappe emend. Walker & Koske[a,b] *Scutellospora pellucida* (Nicol.& Schenck) Walker& Sanders[c]

* Collection area: [a]Site A; [b]Site B; [c]Site C.

Soil moisture, pH and nutrient levels influenced root colonization and spore density. A significant positive correlation ($P<0.05$) was established between root colonization and soil moisture under *D. triflorum*, while the spore number exhibited a highly significant ($P<0.01$) association with soil moisture and P (Table 4). The spore number under *T. purpurea* significantly and positively correlated ($P<0.05$) with soil nitrogen(N), while a highly significant negative correlation ($P<0.01$) was observed with soil pH. In general the response of root colonization and spore number to edaphic factors varied between sites and host species (Table 4).

DISCUSSION

Tropical soils are characterized by low available nutrients especially P (Kang and Wilson 1987). Nodulating legumes generally require a large amount of P for optimum growth, nodulation and nitrogen fixation (Hayman 1986). In spite of having less extensive root systems, legumes are able to fulfil their nutritional requirements depending on colonization by native VAM fungi (Bethlenfalvay and Newton 1991). A high incidence of root infection in the present investigation supports the fact that the nodulating legumes are mycorrhizal dependent.

Phosphorus (P) in the plant has often been considered the only factor controlling mycorrhizal infection (Menge *et al.* 1978). Under low phosphorus nutrition, low P content of plants could correlate with a decrease in phospholipid levels; and an increase in root membrane permeability would favour mycorrhizal infection (Graham *et al.* 1981; Ratnayake *el al.* 1978). The

TABLE 4
Pearson's correlation coefficient (r) for VAM fungal status (Root colonization - RC, Spore number - SN) and soil characteristics of the legumes at different sites (n = 4) and in general (n = 16).

	Site	Soil moisture		pH		Phosphorus		Nitrogen	
		RC	SN	RC	SN	RC	SN	RC	SN
A. monilifer	A	-0.781	-0.682	+0.525	+0.045	-0.785	-0.991***	+0.999***	+0.664
	B	+0.922**	+0.556	+0.792	+0.719	-0.958**	-0.287	+0.961**	+0.212
	C	+0.581	+0.874*	-0.549	+0.636	-0.832*	-0.885*	+0.339	0.240
		(+0.328)	(-0.010)	(+0.328)	(+0.008)	(-0.248)	(-0.306)	(+0.498)	(+0.126)
D. triflorum	A	+0.907*	+0.979***	+0.435	-0.853*	-0.248	+0.419	-0.447	-0.912*
	B	+0.704	+0.986***	+0.499	+0.996***	-0.994***	-0.655	+0.994**	+0.887*
	C	+0.396	+0.975***	-0.303	-0.967**	+0.468	-0.475	-0.945**	-0.372
		(+0.551*)	(+0.620**)	(-0.417)	(-0.122)	(+0.019)	(+0.620**)	(+0.453)	(-0.210)
I. linnaei	A	+0.529	+0.722	+0.447	-0.302	+0.945***	+0.509	-0.928***	-0.177
	B	-0.937**	-0.985***	-0.995***	-0.996***	-0.503	+0.653	-0.509	-0.659
	C	+0.512	-0.879*	-0.484	+0.863*	-0.684	+0.991***	+0.414	+0.109
		(+0.070)	(+0.140)	(+0.070)	(-0.189)	(-0.215)	(+0.346)	(+0.446)	(-0.420)
T.purpurea	A	+0.510	+0.599	-0.334	-0.834*	+0.898*	-0.643	-0.967**	-0.015
	B	+0.489	-0.674	+0.252	-0.834*	-0.932**	+0.057	+0.927**	-0.065
	C	+0.999***	+0.045	-0.850*	+0.078	-0.791	+0.313	-0.492	-0.258
		(+0.437)	(+0.140)	(+0.192)	(-0.647**)	(+0.257)	(+0.070)	(+0.509*)	(-0.046)

*,**,*** Correlations are significant at p = 0.05, 0.01 and .001 respectively
Given in the parentheses are the general response (combined response of all sites).

low soil P in the study soils and high P requirement of legumes may explain the high VAM infection.

The spore number recorded in the present study is many times greater than the previously reported numbers from tropics (Al-Garni and Deft 1990; Jasper *et al.* 1990; Khan 1974; Neeraj *et al.* 1991; Parvathi *et al.* 1984) which contradicts the view that perennial ecosystems contain fewer spores than fields subjected to annual disturbance (Hayman 1982). Louis (1988) also reported a high spore number of 64-160 spores g^{-1} soil from the tropical rain forests in Singapore. The high spore number may be either due to conducive edaphic conditions for sporulation like low nutrient status , high aeration and optimum moisture or the undisturbed conditions of the soils, which allowed sufficient time for the build up of mycorrhizal spores (Azizah Chulan and Omar 1991). The spore number varied within and between sites in the present study. Spore density is influenced by a wide range of soil, climatic, fungal and host factors (Anderson *et al.* 1983- Howerer *et al.* 1987).

VAM fungal spore number in natural ecosystems is not necessarily related to the abundance of VAM infection (Hetrick and Bloom 1986). In the present study a significant positive correlation was observed only in *A. monilifer*, but not in others. Root colonization and subsequent sporulation are reported to vary depending on the host and fungal species and edaphic factors (Khalil *et al.* 1992).

Soil moisture, pH and available nutrients (N and P) had varied influence on VAM fungal infection and spore number (Table 4). Soil moisture positively correlated with root colonization in *D. triflorum, A. monilifer* and *T. purpurea.* A similar influence was found on spore number. Generally VAM fungi are sensitive to soil moisture and the optimum moisture for plant growth is also suitable for VAM infection and sporulation (Redhead 1975). However, soil moisture had a negative influence on VAM root infection and

spore number in *I. linnaei*. It is known that species and strains of VAM fungi differ in their sensitivity to soil moisture due to their non-adaptability to changing O_2 tension (Mosse *el al.* 1981).

Soil pH had a negative influence on root colonization in *I. linnaei* and *T. purpurea* at sites B and C respectively, while its influence was not significant in others. This not in conformity with the earlier report that soil pH had no marked effects on mycorrhizal infection in natural vegetation (Abbott and Robson 1991). Varying soil pH may affect the development and functioning of VA mycorrhizas (Abbott and Robson 1985; Hayman and Tavares 1985) by altering the concentration of many nutrients and toxic ions in soil solutions as well as hydrogen ions (Russell 1973). The spore number on the other hand was both positively and negatively influenced by soil pH in *D. triflorum* and *I. linnaei* at different sites. However, they negatively correlated in *T. purpurea* and no correlation existed in *A. monilifer*. The response of VAM fungi to soil pH may depend on the species and strains constituting the indigenous VAM flora (Robson and Abbott 1989). The variation in response can also be attributed to the host mediated changes of the rhizosphere pH. The nitrate reduction process of the mycorrhizal host changes the pH of the root exudate, which in turn alters the rhizosphere pH, affecting pH sensitive micro-organisms including VAM fungi (Smith and Gianinazzi Pearson 1988). The species of *Glomus* constituted 63% of the total species recorded (Table 3). A neutral to alkaline soil has been reported to favour the predominance of *Glomus* species by early workers (Abbott and Robson 1977; Mosse *et al.* 1981).

No general correlation existed between the soil P on one hand and the root colonization and spore number on the other. Soil P had a negative influence on root infection and spore number in *A. monilifer* while it was positive in *I. linnaei*. In *D. triflorum* and *T. purpurea*, the soil P negatively correlated with root infection at site B, but a positive correlation was evident in the latter species at site A. However, soil P had no influence on the spore number in both the species. Previous studies have shown a negative association between the amount of extractable soil phosphate and the abundance of VAM infection (Bolgiano *et al.* 1983; Morita and Konishi 1989) and spore number (Sylvia and Neal 1990). Variations in the response of root colonization and spore number to soil P can be attributed to a number of factors such as

a) VAM fungal species colonizing the roots, since species and strains vary in their sensitivity to P (Trouvelot *et al.* 1987)
b) the varied host root growth response to changing P levels (Smith 1982) or
c) changes in the cell membrane permeability to varying cellular P concentrations, which affect the degree of mycorrhizal colonization and sporulation (Daniels Hetrick 1984).

The response of root colonization and spore number to soil N also varied. The influence of soil N on root colonization was positive and negative respectively in *A. monilifer* and *I. linnaei* but in *D. triflorum* and *T. purpurea*, the response varied from site to site. Generally, soil N had no influence on spore number of these wild legumes but in *D. triflorum* the spore number correlated positively with soil N at site A and negatively at site B . There are reports that N can stimulate or suppress root colonization and spore production through modifications of soil pH (Sylvia and Neal 1990, Thompson 1986). Results of our study also reflect the same trend (Table 4).

An interesting phenomenon observed in the present study was the counteractive influence of soil P and N on VAM colonization. When the soil P vs root colonization correlated negatively, it was positive for soil N vs root colonization and *vice versa* (Table 4). Thus, our results clearly indicate the decisive influence of soil P and N interaction on VAM colonization. It has already been reported that VAM fungal infection was not affected by increasing P when plants were N deficient, but when N was sufficient P additions suppressed root colonization (Sylvia and Neal 1990).

The findings of this study emphasize the need for an understanding of the ecology of VAM fungi in various ecosystems and soils for the successful selection and introduction of VAM fungal species and strains for a particular environment.

REFERENCES

ABBOTT, L.K and A. D. ROBSON. 1977. The distribution and abundance of vesicular arbuscular endophytesinsome Western Australian soils. *Aust. J. Bot.* **25**: 515-522.

ABBOTT, L. K and A. D. ROBSON. 1985. The effect of soil pH on the formation of VA mycorrhizas by two species of *Glomus*. *Aust. J. Soil Res.* **23**: 253-261.

ABBOTT, L. K and A. D. ROBSON. 1991. Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. *Agri. Ecosystems Environ.* **35**: 121-150.

AL-GARNI, S.M and M.J. DAFT. 1990. Occurrence and effectiveness of vesicular-arbuscular mycorrhizas in agricultural soils from Saudi Arabia. *Biol. Agric. Hortic.* **7**: 69-80.

ANDERSON, R.C., A.E. LIBERTA., L.A. DICKMAN and A. J. KATZ. 1983. Spatial variation in vesicular-arbuscular mycorrhiza spore density. *Bull. Torrey Bot. Club* **110**: 519-525.

AZIZAH CHULAN, H and M. OMAR. 1991. Incidence of VAM spores in some Malaysian soils. *Pertanika* **14**: 133-137.

BETHLENFALVAY, G. J and W. E. NEWTON. 1991. Agroecological aspects of the mycorrhizal, nitrogen-fixing legume symbiosis. In *The Rhizosphere and plant Growth*, ed. D.L. Keister and P.B.Cregan, p.349-354. Netherlands: Kluwer Academic Publishers.

BETHLENFALVAY, G. J., R. S. PACOVSKY., M. S. BROWN and G. FULLER. 1982. Mycotrophic growth and mutualistic development of host plant and fungal endophyte in an endomycorrhizal symbiosis. *Plant Soil* **68**: 43-54.

BOLGIANO, N.C., G.R. SAFIR and D.D. WARNCKE. 1983. Mycorrhizal infection and growth of onion in the field in relation to phosphorus and water availability. *J. Am. Soc. Hortic. Sci.* **108**: 819-825.

BOWEN, G. D and D. 1. BEVEGE. 1976. Micro-organisms, the third dimension in phosphate nutrition and ecology. In *Prospects for Improving Efficiency of Phosphorus Utilization*, ed. G.J. Blair, p.103-112. Australia: New England Press.

DANIELS HETRICK, B. 1984. Ecology of VA mycorrhizal fungi. In *VA Mycorrhiza*, ed. C. Li. Powell and D.J.Bagyaraj, p. 36-55. Boca Raton, Florida: CRC Press, Inc.

GERDEMANN, J. W. and T. H. NICOLSON. 1963. Spores of mycorrhizal *Endogone* extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **46**: 235-244.

GRAHAM, J.H., R.T. LEONARD and J.A. MENGE. 1981. Membranemediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. *Plant Physiol.* **68**: 549-552.

HAYMAN, D. S. 1982. Influence of soils and fertility on activity and survival of vesicular-arbuscular mycorrhizal fungi. *Phytopathology* **72**: 1119-1125.

HAYMAN, D. S. 1986. Mycorrhizae of nitrogen-fixing legumes. MIRCEN *J. Appl. Microbiol. Biotech.* **2**: 121-145.

HAYMAN, D.S and M. TAVARES 1985. Plant growth reponses to vesicular arbuscular mycorrhiza. XV. Influence of soil pH on the symbiotic efficiency of different endophytes. *New Phytol.* **100**: 367-377.

HETRICK, B.A.D and J. BLOOM. 1986. The influence of host plant on production and colonization ability of vesicular-arbuscular mycorrhizal spores. *Mycologia* **78**: 32-36.

HOWELER, R.H., E. SIEVERDING and S. SAIF. 1987. Practical aspects of mycorrhizal technology in some tropical crops and pastures. *Plant Soil* **100**: 249-283.

JACKSON, N. L. 1958. *Soil Chemical Analysis*. New Delhi: Prentice Hall of India Pvt. Ltd.

JASPER, D.A., L.K. ABBOTT and A.D. ROBSON. 1990. The effect of soil disturbance on vesicular-arbuscular mycorrhizal fungi in soils from different vegetation types. *New Phytol.* **118**: 417-476.

KANG, B. T and G. F. WILSON. 1987. The development of alley farming as a promising agroforestry technology. In *Agroforestry: A Decade of Development*, ed. H.A. Steppler and P.K.R. Nair, p. 227-243. Nairobi, Kenya: ICRAF.

KHALIL, S., T. E. LOYNACHAN and H. S. MCNABB.JR. 1992. Colonization of soybean by mycorrhizal fungi and spore populations in Iowa soils. *Agron. J.* **84**: 832-836.

KHAN, A.G. 1974. The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes, and of *Endogone* spores in adjacent soils. *J. Gen. Microbiol.* **81**: 7-14.

Louis, I. 1988. Status of mycorrhizal research in Singapore. In *Mycorrhizae for Green Asia. Proceedings of the First Asian Conference on mycorrhiza*, ed. A. Mahadevan, N. Raman and K. Natarajan, p.22-23. Madras, India.

MENGE, J.A., D. STERILE., D.J. BAGRAYAJ., E.L.V. JOHNSON and R.T. LEONARD. 1978. Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. *New Phytol.* **80**: 575-578.

MISRA, R. 1968. *Ecology Work Book*. India: Oxford and IBH publishing Co. Pvt. Ltd.