AN ASSESSMENT AND ENUMERATIONS OF VESICULAR-ARBUSCULAR MYCORRHIZA PROPAGULES IN SOME FOREST SITES OF JENGKA

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NORANI, A. 1996. An assessment and enumerations of vesicular-arbuscular mycorrhiza propagules in some forest sites of Jengka. Vesicular-arbuscular mycorrhizal spore numbers found in three representative forest areas in Jengka were low and well below those recorded in other tropical soils. However, levels of root infection in soil cores were comparatively high. Evidence showed that current forest logging practices influenced significant changes in vesicular-arbuscular mycorrhizal propagules. Propagules were reduced by 30-50 % when forest soils were severely disturbed through heavy soil mechanical compaction, exposure and erosion.

Key words: Vesicular-arbuscular mycorrhiza propagules - mechanical compaction - exposure - erosion

NORANI, A. 1996. Taksiran dan pengiraan propagul mikoriza vesicular-arbuscular di beberapa kawasan hutan di Jengka. Bilangan spora mikoriza vesikular-arbuscular di tiga kawasan hutan di Jengka rendah dan jauh lebih rendah daripada yang direkodkan di tanah-tanah tropika yang lain. Bagaimanapun, paras serangan ke atas akar pada teras tanah secara perbandingannya tinggi. Bukti menunjukkan bahawa amalan penebangan hutan kini mempengaruhi perubahan ketara di dalam propagul mikoriza vesicular-arbuscular. Propagul berkurang sebanyak 30-50% apabila tanah-tanah hutan diganggui dengan teruk melalui pemadatan tanah oleh mesin berat, pendedahan dan hakisan.

Introduction

Indication of the vesicular-arbuscular mycorrhiza (VAM) fungal population in soil has often been based on spore enumerations which have been taken as an estimate of the number of VAM propagules (Mosse & Bowen 1968, Hayman 1970, Sutton & Baron 1972, Hayman *et al.* 1975, Black & Tinker 1977). Other than from spores, infection can also takes place from infected roots or vegetative hyphae.

In the study by Norani (1989), the levels of VAM infection in specific plant species from the three study sites in Jengka were found to be different. Several possible reasons responsible for this were: changes in plant species composition, changes in soil properties and a reduction in mycorrhizal propagules as a result of land disturbance. Possible related factors are changes in the number and type of VAM spores in the soil. In addition the root material surveyed in Norani (1989)

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came from specific species and there is no doubt that the large woody vegetation was under-represented in those samples. Therefore as spores were extracted from soil cores, opportunity was also taken to assess overall levels of VAM infection in randomly sampled roots.

Table 1. Spore numbers in some tropical soils

Location	Vegetation type	No. of spores ($100g^{-1}$ air dried soil or * fresh soil)	Reference
Malaysia (Jengka)	Undisturbed forest	25	See Table 2.
	Disturbed forest	32	
	Very disturbed forest	7	
Malaysia	Sandy soil under cashew	8 261	Azizah Chulan *et al.* (1983)
	Sand tailings under *Imperata cylindrica*	726*	Azizah Chulan (1986)
	Clayey soil under cocoa	73*	Azizah Chulan & Ragu (1986)
	Organic soil:		
	Coffee	5 900*	
	Oil palm	45 860*	
	Secondary forest	1 000	
	Virgin forest	3 000	
Nigeria	Lowland rain forest	48 100 (100 cm^{-3})	Redhead (1977)
	Semi-deciduous forest	66 (100 cm^{-3})	
	Savanna (grass, shrubs and trees)	83 (100 cm^{-3})	
	Savanna (closed canopy, no grass)	110 (100 cm^{-3})	
	Savanna (open with grass)	257 (100 cm^{-3})	
	Sudan savanna	530 (100 cm^{-3})	Redhead (1977)
	Sahel savanna	889 (100 cm^{-3})	
Pakistan	Cultivated	792	Sheikh *et al.* (1975)
Pakistan (Islamabad)	Cultivated	*c.*555	Saif *et al.* (1975)
Sri Lanka	Rubber growing soils	22 350	Jayaratne & Waidyanatha (1982)
Singapore	Lowland rain forest		Louis & Lim (1987)
Taiwan	Rice-soybean system	) cm^{-3})*	Wong & Tschanz (1982)

Several methods have been used to extract and determine mycorrhizal spore populations. Prior to the actual assessment, preliminary work to test the feasibility of these methods was carried out using soils from the study sites. A combination between wet sieving and the sucrose-centrifugation technique of Walker *et al.* (1982) was found to be most suitable for extracting spores from the Jengka soil, while the method of Daft and Nicolson (1972) was found to be most suitable for scoring percentage colonisation of VAM in randomly selected roots.

Many qualitative and quantitative surveys on numbers of spores in soil have been attempted, e.g. Mosse & Bowen (1968), Hayman (1970), Powell (1977), Hayman & Stovold (1979), Sward *et al.* (1978). Quantitative reports on spore enumeration and distribution from Asian soils are still lacking except for work reported by Azizah Chulan (1986), Azizah Chulan *et al.* (1983), and Louis and Lim (1987). There are a number of unpublished data and reports from other tropical areas (see Table 1). A survey of spores in forest soils in Jengka was therefore of added interest.

Materials and methods

In this investigation, the study sites chosen were an undisturbed forest (Site A), a semi-disturbed secondary forest (Site B) and an area of logged-over forest (Site C).

Soil assessment for mycorrhizal roots and spores was carried out in January 1985 and again in July/August 1985. These correspond with the maximum and minimum rainfall periods of Jengka (Figure 1).

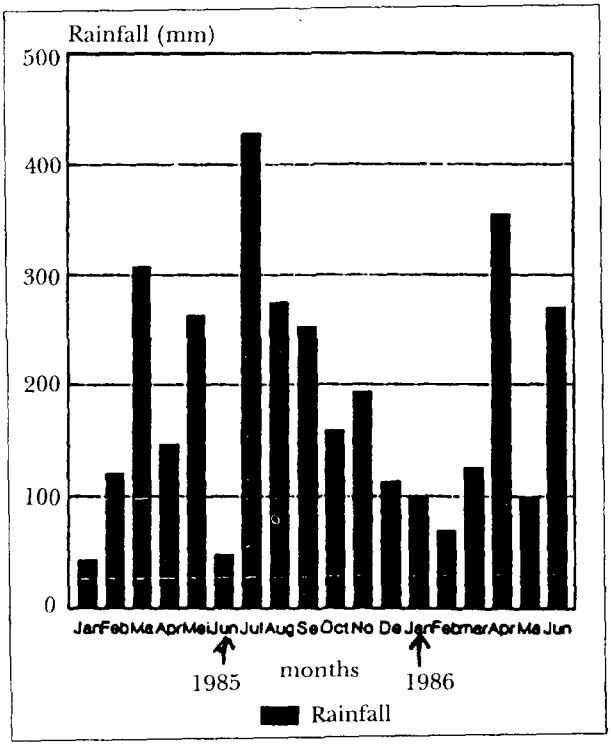

Figure 1. Rainfall pattern in Jengka Forest Reserve between January 1985 and June 1986

Soil samples were obtained from sites A, B, and C. Each sample comprised five bulked cores from the top 15 cm of rhizosphere soils collected at random points within each study site. The soil core was of 4 cm diameter and 15 cm depth. A total of 30 composite samples were collected from each study site. The samples were air-dried and thoroughly mixed before subsamples were removed and examined for VA endophyte spores and mycorrhizal infections.

A hundred gram soil was removed from each sample and mixed thoroughly in a bucket of water. Lumps of soil were carefully broken and the suspension was stirred vigorously, allowed to settle for 15 seconds, and decanted through a 600 µm sieve into another bucket. Root fragments retained on this sieve were subjected to infection assessment.

The suspension in the second bucket was swirled vigorously and again allowed to settle for 15 seconds, and the supernatant poured through a 250 µm sieve. The sievings retained on this sieve were placed in Petri dishes and spores counted under a dissecting microscope at 60x magnification. Spores were subsequently removed from the dishes to prevent double counting. Non-viable spores, devoid of cell contents were discarded.

Fractions retained on 106 and 63 µm sieves were further processed using the centrifugation technique of Tommerup and Kidby (1979). In this technique, the sievings were first shaken with a suspension of finely ground kaolin which was then spun at approximately 2000 rpm for 5 min. The supernatant containing remaining soil particles and spores was dispersed in 50% sucrose solution. The solution was again spun at 2000 rpm for 45 seconds. The supernatant was quickly poured onto a 43 µm sieve and sluiced with tap water to remove the sucrose. The material retained on the sieve, consisting mostly of endogonaceous spores, some soil contaminants and sclerotia, was washed into a Petri dish and spores counted under a dissecting microscope. Spore number was expressed as number of spores $100g^{-1}$ of air dry soil.

Pooled roots retained previously were cut into 1 cm segments, cleared in hot 10% KOH, and stained in trypan-blue lacto-phenol (Phillips & Hayman 1970) and mounted on slides in lacto-phenol. Assessment of infection was made based on direct observation of whole mounts. Sufficient root material was selected to give a total of 20 to 30 cm root per slide, with four slides per soil sample. The levels of infection in these roots were determined by the presence or absence of endophytes. All roots with hyphae plus vesicles, or hyphae minus arbuscules, or hyphae plus hyphal coils , or any combination of these structures were recorded as positive colonisation, and infection was scored as percentage of segments colonised.

Results

Spore numbers

Spore numbers in the soil sampled from all the three sites were very low (Table 2). Maximum spore number was recorded in the disturbed site B, with least spore numbers recorded from the very disturbed site C. The difference in mean spore

numbers in 100 g air dried soils was significantly different ($p < 0.05$) between sites A, B and C (Table 2). However, a slight fluctuation in spore number was observed between the two sampling times at maximum and minimum rainfall periods.

Table 2. Number of VAM spores recovered and percentage mycorrhizal infections in roots from the undisturbed site A, the disturbed site B and the very disturbed site C

Study site	Sampling time	Spores recovered $100g^{-1}$ dry soils)	Mycorrhizal infection (%)
Undisturbed	January 1985	25.30 ± 2.00*a	77.65 ± 2.68d
site A	August 1985	24.53 ± 1.79a	72.85 ± 2.60d
Disturbed	January 1985	31.90 ± 3.38b	77.50 ± 2.60d
site B	August 1985	31.50 ± 3.04b	76.10 ± 2.52d
Very disturbed	January 1985	6.13 ± 0.62c	42.50 ± 1.52e
site C	August 1986	8.37 ± 0.78c	46.80 ± 1.27e

Mean ± standard error
* Within the same column, values not sharing a common letter differ significantly at $p < 0.05$, using Duncan's Multiple Range Test.

Spore types

Spores of varying morphology were found from the three study sites but most fell into the distinct bulbous vacuolate group of *Gigaspora* species and the honey colored sessile group of *Acaulospora* species (Figures 2 & 3 respectively). The spores of *Gigaspora* ranged between 200 and 400 µm in diameter while those of *Acaulospora* were approximately 300-420 µm in diameter. The majority of the spores occurred singly in soils or in loose or dense hyphal networks (Figures 4a & 4b). These later were often retained on the 250 µm and 600 µm sieves. Sporocarps of *Sclerocystis* sp. with spores orderly arranged within the sporocarps were not frequently recovered. Intraradical spores found single or clustered together (Figure 5) were also occasionally isolated on the 106 µm and 250 µm mesh sieves.

A large proportion of the spores recovered from site C were found to be non-viable and devoid of contents and hence were discarded.

Mycorrhizal colonisation

Percentage mycorrhizal colonisation of roots from sites A and B which ranged between 73.15 and 76.67% was not significantly different ($p \leq 05$). However, in the logged-over forest (site C) less than 38.22 % mycorrhizal colonisation was recorded which is significantly lower ($p<0.01$) than in sites A and B.

A majority of the root samples examined from above were heavily colonised by VAM structures. Intracellular hyphae, coils and vesicles formation were also seen inside the cortical cells of the roots. Arbuscules, however, were uncommon.

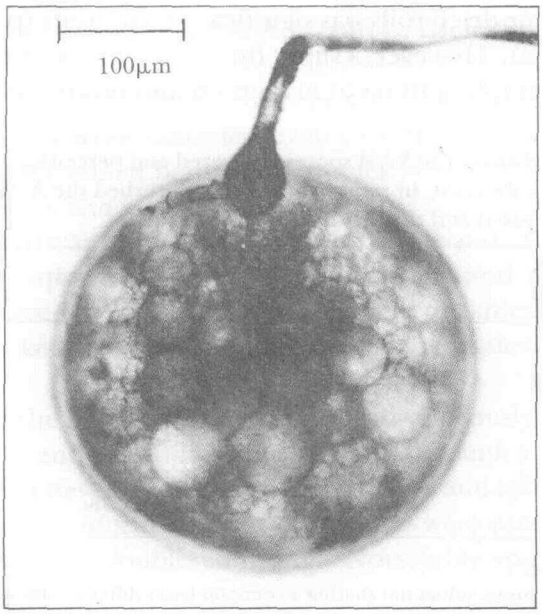

Figure 2. *Gigaspora* spp. whole spore with bulbous-shaped hyphal attachment and oil globules

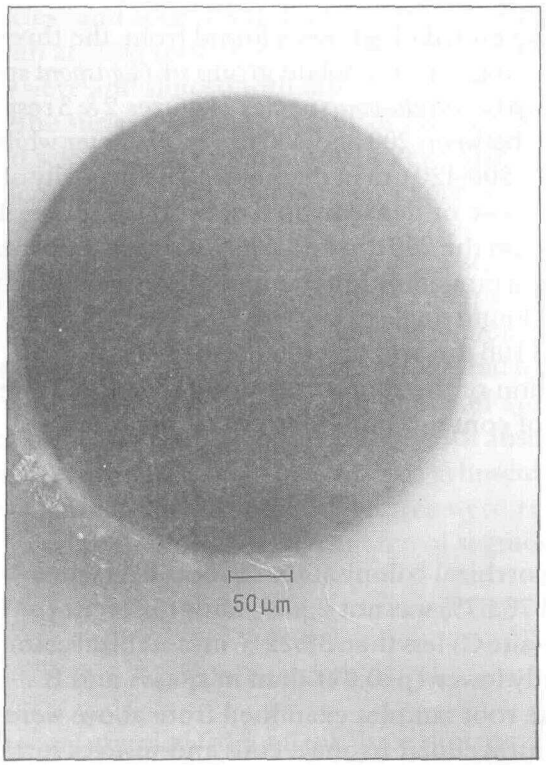

Figure 3. Honey coloured sessile resting spore of *Aculospora* spp.

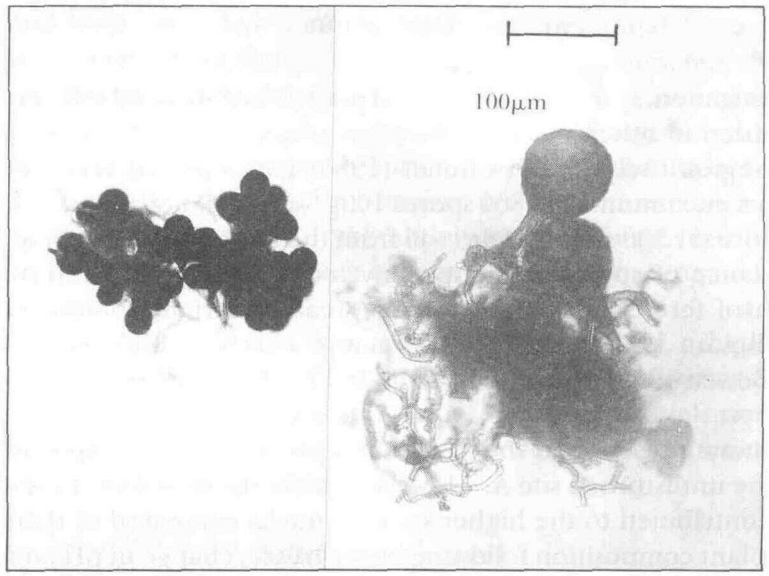

Figure 4a & 4b. Clustered spores in loose hyphal networks

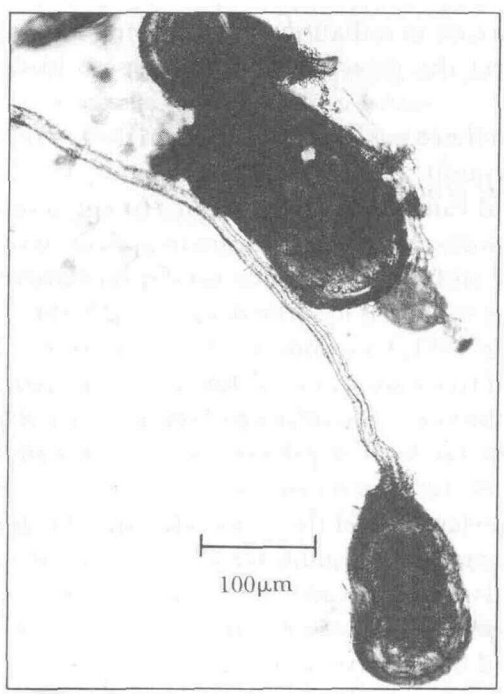

Figure 5. Intraradical spore found single or clustered together in root fragments

Discussion

In this investigation, spore numbers found per 100 g of air dried soil were well below those recorded in other studies from other tropical soils (Table 1). Within the Malaysian region itself, Azizah Chulan (1986) had reported very high counts of spores with a maximum of 45 860 spores $100g^{-1}$ soil under oil palm. Louis and Lim (1987) reported *c.*500 spores $100g^{-1}$ soil from the lowland rain forest of Singapore. The abundance of spores in many cultivated soils may be connected with the application of fertilisers and the usually greater nutrient content of such soils (Mosse & Bowen 1968). However, few native soils have high spore populations (Mosse & Bowen 1968, Abbott & Robson 1977). As suggested by Baylis (1969), because of year-round adequate soil moisture and temperature, actively growing roots are always present and the VAM fungi present need not sporulate. This is typical of the undisturbed site A. However, in the disturbed site B, several factors may have contributed to the higher spore count as compared to that of site A. A change in plant composition following disturbance, change in pH and root death may induce sporulation. In situations where a forest is cleared, a greater proportion of the top soil is removed together with root fragments and the decomposed leaf litter. This contributes to the direct reduction effect on the mycorrhizal propagules. Site disturbance also reduces the number of host plants in the area which not only decrease the number of VAM propagules but also the potential for infection of new hosts. The abrupt increase in radiation heat following logging and land clearance may also directly affect the growth of germ tubes to host roots thus lowering infections .

In this investigation there was little difference in the spore numbers with respect to rainfall peak and trough. This contrasts with Louis and Lim's (1987) observations from the lowland rain forest of Singapore or the temperate environments where seasonal variations cause fluctuations in spore numbers (Hayman 1970, Sutton & Baron 1972) and in the maritime sand dunes where season and stage of host development cause variations in spore density and VAM colonisation (Nicolson & Johnson 1979, Koske 1981, Giovannetti 1983, Giovannetti 1985). The microclimate under the closed tree canopy of the lowland Malaysian forest remains fairly constant throughout the year. According to Abdul Rahim *et al.* (1986) the highest mean soil temperature (at 5 cm depth) recorded under an open forest in Jengka was 33.8 °C while under the closed canopy it was 24.9 °C. Also, under the close forest canopy of the lowland forest there is fairly continuous cover of leaf litter or floor vegetation which maintains a stable microclimate at the soil surface. There are also no marked dry and wet seasons and the soil moisture rarely falls to wilting point. These explain the more consistent mycorrhizal spore population and infection levels of VAM observed between years.

In contrast to the low spore numbers recorded from sites A and B, levels of root infection were high. This contrasts with the result of Daft and Nicolson (1972) where spore abundance in soil of potted plants was a fairly accurate index of degree of infection. However, from the native perennial vegetation in New Zealand (Mosse & Bowen 1968) and in U.S.A (Gerdeman & Trappe 1974), low spore

numbers have been recorded for heavily infected perennial hosts. It follows then that spores may not be the principal source of inoculum, but that infected plant roots or mycelium in soil might be a more obvious source of mycorrhizal propagules (Read *et al.* 1976). Furthermore, different mycorrhizal fungi are difficult to distinguish morphologically inside root tissue, and hence it is difficult to tell how many (if any) non-sporing strains are present in the soil assessed. When non-sporing strains are the dominant mycorrhizal fungi, infection of seedling roots must be by hyphae which have grown out from fragments of roots already in the soil.

In this study, by using the random core sampling method, a higher level of infection (by 10-30%) was recorded than that found by excavating individuals Norani (1989). In this instance, more roots of woody perennials were sampled rather than just roots of seedlings, shrubs and herbaceous species. This may account for the increase in infection level.

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