VASICULAR ARBUSCULAR MYCORRHIZAE - TREE ASSOCIATION OF VARIRATA NATIONAL PARK AND THE INFLUENCE OF VEGETATION TYPES

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ABSTRACT

A study to investigate the occurrence of Vasicular Arbuscular mycorrhizae among tree species of Varirata National Park was conducted at the University of Papua New Guinea. The study also investigated the influence of vegetation types, i.e., savanna, savanna-rain forest ecotone and rain forest on the VA mycorrhizae infection levels among the tree species. The infection levels were investigated using roots sampled from representative tree species. The roots were decolourized, stained and observed under the microscope to assess the levels of infections. The results of the study indicated that generally, all the tree species sampled were infected with the VA mycorrhizae. However, there was a high infection variation among the different tree species. A comparison of infection between the primary and secondary tree species indicated higher infection levels among the secondary tree species. With the highest infection observed among two secondary species, i.e., Leea indica *and* Rhus taitensis, *while the lowest were observed among the* Breynia cernua *samples. However, there was no significant difference within the same species across the three vegetation types. All the studied sites within the three vegetation types comprised of heavy clay acidic soils, with high organic carbon content ranging from 7.23% in the savanna to 11.91% in the savanna-rain forest. However, there was no correlation between the organic carbon levels and the infection levels. Overall, these results indicate that VA mycorrhizae infection levels vary with species and not vegetation types, and secondary tree species have a higher infection level in comparison to primary species.*

Key words: *VA Mycorrhiza, vegetation, rain forest, savanna, ecotone, infections, soils, carbon, Varirata National Park*

INTRODUCTION

Papua New Guinea possesses one of the last most valuable standing forests of the tropics. These forests are part of the circum Antarctica, South East Asia and endemic forest (Saulei 1993). Over 70% (36.2 X10^{6}ha) of the country's total land area is under forest cover ranging for swamps and lowland forests of coastal plains to the alpine and mossy forests of the highlands. However, due to shifting cultivation, logging and other developments, these forests are disappearing at an alarming rate (Saulei 1992). Thus efforts are being made to encourage reforestation through understanding some of the characteristics or factors involved in regrowth and regeneration of these forests. Some Fungi commonly known as mycorrhizae form symbiotic associations with many plants, ranging from the minute gametophytes of primitive non-vascular plants to trees. The association occurs among various types of terrestrial vegetations, from rain forests to savanna and to the regrowths of disturbed forests (Tinker 1980; Jackson and Mayson 1984). Generally mycorrhizae are believed to be associated with most trees. The association when in place results in increased uptake of nutrients such as Phosphorus, Zinc and Copper (Young *et al.* 1986). This association varies with tree species, climate, soils and topography.

Among the common tree families in the lowlands of Central Province are Lauraceae, Rubiaceae, Mrytaceae and Moraceae. The Lower montane include all of the above families and Fagaceae. Preliminary unreplicated studies on some of the above

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species of Varirata National Park in Central Province have shown that secondary tree species seemed to form a closer and much wider spread association with Vasicular arbuscular (VA) mycorrhizae than the primary tree species (Suruman 1996 unpublished). The park supports three different vegetation communities; rain forest, secondary forests and savanna woodland; the latter is now reverting back to natural forest. The southern portion of the Park is all rain forest while the northern part is covered with savanna woodland coupled with secondary regrowths.

Apart from the scanty unreplicated data, very little is known about the mycorrhizae-tree associations in Papua New Guinea. Thus, the objective of this study was to investigate the occurrence of vascular aburscular mycorrhizae (VAM) in dominant tree species of the Varirata National Park. The study also investigated variations in VAM populations between the different tree species among the different vegetation types. The study was carried out in two parts. The first part investigated the occurrence of mycorrhizae in both primary and secondary trees. The second aspect concentrated on the occurrence among secondary tree species, and the relationships with vegetation types.

MATERIALS AND METHODS

This study was conducted at Varirata National Park, 45 km southwest of Port Moresby (latitude 9° 26'S, and longitude 147(21'E). It is located on the Sogeri plateau, southeast of the Astrolabe Range (720 - 860 m above sea level). Several interconnecting creeks and tributaries dissect the Park and join the main Laloki river north of the area. The climate of Varirata National Park is very seasonal, receiving less than

Table 1. **Location and ecological classifications of the tree species sampled from the Park in the initial study.**

Family	Species	Ecological Classification	Location within Varirata National Park
Anacardiaceae	*Rhus taitensis*	Secondary	SE of Varirata Look out
Euphorbiaceae	*Bridelia macrocarpa*	Secondary	Picnic site 1, NE of main picnic
	Endospermum	Secondary	site SW of Rangers' camp
	Glochidion	Secondary	NE of Rangers' camp
	Macaranga	Secondary	Picnic Site 1, SE of Varirata lookout
	Mallotus	Secondary	Edge of savanna, NE of Rangers' camp
Leeaceae	*Leea indica*	Secondary	SE of Varirata Lookout & NE of Rangers' Camp
Melastomaceae	*Melastoma*	Primary	SE of Varirata Lookout & SW of Rangers' Camp
Rubiaceae	*Evodia*	Secondary	East of Rangers' camp
	Psychotria	Primary	SW of Rangers' camp
	Timonius timon	Secondary	NE & SE of Rangers' camp
	Wendlandia paniculata	Primary	SW of Rangers' camp

Table 2. Location and ecological classifications of the common secondary tree species sampled in the second study from the National Park.

Family	Species	Vegetation type	Height (m)	Location within the Park
Anacardiaceae	*Rhus taitensis*	Savanna	6	Varirata Lookout
	R. taitensis	Savanna	3	Picnic site 4
	R. taitensis	Savanna	4	Picnic site 3
	R. taitensis	Savanna/RF*	1	Picnic site 1
	R. taitensis	Savanna/RF*	1	East of Rangers' camp
Euphorbiacea	*Breynia cernua*	Savanna	2	Varirata lookout
	B. cernua	Savanna	4	" "
	B. cernua	Savanna	3	Picnic site 4
	B. cernua	Savanna/RF*	2	Picnic site 3
	B. cernua	Savanna/RF*	1	Picnic site 1
	B. cernua	Savanna/RF*	3	East of Rangers' camp. Tract to Varirata Lookout
	Glochidion	Savanna	7	Varirata Lookout
	"	"	2	" "
	"	"	2	Picnic site 4
	"	Savanna/RF*	<1	Picnic site 3
	"	" "	<1	Picnic site 1, East of Rangers' camp
	"	" "	3	New Rangers' camp
	"	" "	4	Tract to Varirata Lookout
	Macaranga	Savanna/RF*	3	Tract to Varirata Lookout, Picnic site 2
	"	" "	3	Picnic site 1, near Rangers' camp
	"	" "	3	Tract to Varirata Lookout
	"	" "	2	Tract to Varirata Lookout
	Mallotus	" "	3	Tract to Varirata Lookout
	"	" "	<1	East of Rangers' camp
Leeaceae	*Leea indica*	Savanna	3	Varirata Lookout point
	L. indica	Savanna	3	Varirata Lookout point
	L. indica	Savanna	2	Picnic site 4
	L. indica	Savanna/RF*	1	Picnic site 3
	L. indica	Savanna/RF*	1	Picnic site 1
Rubiaceae	*Evodia*	Savanna	5	East of Rangers' camp
	"	"	3	Varirata Look out
	"	"	2	Picnic site 4
	"	Savanna/RF*	10	Picnic site 3
	"	"	3	Picnic site 1, East of Rangers' camp
	"	"	5	Near Varirata Lookout
	"	"	15	Picnic site 2, near Rangers' camp
	"	"	10	Tract to Varirata Lookout
	"	"	12	Tract to Varirata Lookout

* RF - Rain forest

2000 mm of precipitation annually. The heaviest rainfalls occur from December to April, thus giving rise to a distinct wet season followed by a dry season. The index of seasonality was calculated to be 0.1 indicating strong seasonality (McAlpine *et al.* 1983). Temperatures oscillate from 19°C during the dry season to 30°C during the wet season, with a mean relative humidity of 80%.

Three representative sites from the vegetation types were selected for sampling. These were: savanna, savanna rain forest and the ecotone zone between savanna and rain forest. The savanna woodland vegetation is basically a secondary forest comprising trees and grasses with *Casaurina papuana*, *Evodia* spp. *Rhus taitensis, Macaranga,* spp., *Eucalyptus* (E. *papuana*, *E. tereticornis*) *Banksia dentata* and *Melaleuca leucadendron* being common. While *Imperata cylindria, Themeda australis* are common grasses. The rain forest found in the Park is that of lower montane which has a frequent occurrence of *Cryptocarya, Syzygium, Elaeocarpus, Sloanea, Castanopsis* and *Ficus.* The ecotone (transition zone) between Savanna and the rain forest is dominated by *Casaurina papuana, Rhus taitensis, Macaranga, Cryptocarya, Canthium, Evodia, Ficus* and *Aglaia.*

Root sampling

The dominant primary and secondary tree species of the National Park were selected for the first study are shown in Table 1. While the dominant tree families in the savanna, savanna - rain forest and the rain forest selected for the second study are given in Table 2. Three trees of each species were selected in each vegetation type. Approximately 5 g of fine (maximum thickness 5 mm) tree roots from the A horizon (top 30 cm) were obtained from each tree from each of the four cardinal directions. The 5 g samples from the four cardinal directions of the tree were mixed to yield the representative tree sample. In all, roots of three trees were sampled for each species per vegetation type. The roots were brought into the laboratory for staining. When it was not possible to stain the roots on arrival, the roots were rinsed under a gentle stream of water to remove excess soil and were then frozen and stored in small plastic freezer bags at -18°C until it was possible to stain them. The roots were only allowed to stay in their frozen state for a maximum of 2 weeks.

In order to aid in the understanding of the differences in the levels of mycorrhizal infections between species in the different vegetation types, soil samples from the A horizon of the three vegetation types were also collected. The soils were analysed for physical and chemical characteristics. The soil samples were air-dried and sieved to pass through a 2 mm sieve before measuring the characteristics. Soil pH was determined in 1:2 soil: water and 1:2 soil: 0.01 M calcium chloride ($CaCl_2$) suspensions. The pH was then measured using a pH meter. Particle size analyses of the soils were also carried out to determine the percentage of sand, silt and clay using the hydrometer method (Bouyoucos 1927). Textural classes were then determined using the textural triangle (Fitzatrick 1974). Soil organic matter levels were determined by oxidation of organic matter with a concentrated mixture of H_2SO_4 according to Walkley and Black procedure (Walkley 1974). The amount of organic carbon was then obtained by titration with $FeSO_4$ as out lined by Nelson and Somers (1982). Organic matter was calculated from organic Carbon by multiplying by 1.724 based on the assumption that organic matter contains 58% carbon (Nelson and Somers 1982).

Table 3. **Comparison of mycorrhizal infection in all primary and secondary tree species sampled**

Ecological Classification	Mean percentage of Infection (+ SD)
Primary	37.75+19.95
Secondary	46.94+21.69

Table 4. **Mean percentage* (+SD) of VAM infection levels on roots of different secondary tree species in the Variarata National Park.**

Species	Vegetation Type		
	Savanna	Savanna/Rain Forest	Rain Forest
Rhus taitensis	37.4+24.6	50.9+6.5	xxx
Leea indica	59.9+26.1	23.3+14.1	xxx
Glochidion sp.	10.3+1.9	20.6+11.3	17.4+11.1
Evodia sp.	16.6+12.0	9.6+0.6	3.9+2.8
Breynia cernua	8.0+3.3	34.5+13.7	xxx
Macaranga sp.	xxxx	0.33+0.1	7.56+0.7

*** Mean given is for three tree samples and each sample comprise of 3 slides/tree**
xxx Species not available in the vegetation type

Staining of roots

The presence of VA mycorrhizae on the roots was determined using the procedure described by Bevege (1968). The infection was measured by estimating the portion of the primary cortex occupied by the VA mycorrhizae. Approximately 1g of washed and thawed or fresh root sample was cut into approximately 1cm long segments. To remove the host cytoplasm and the nuclei, the roots were placed into 10% KOH and heated for 25 minutes without boiling. After which they were rinsed with sterile tap water to remove excess KOH. The roots were then soaked in 0.01N HCl to neutralize the remaining KOH. These roots were later stained by heating them in a staining solution consisting of 200 ml glycerol, 200 ml lactic acid, 200 ml distilled water and 0.30 g aniline blue for 10 - 15 minutes. The roots were then transferred to a 50% glycerol solution overnight to remove the excess stain.

Table 5. **Mean percentage of VAM infection levels in different vegetations at the Variarata National Park**

Vegetation type	Mean % VAM infection* +SD
Savanna	13.5+ 4.6
Savanna/Rain Forest	15.1+12.6
Rain Forest	10.7+ 4.0

***Mean VAM infection for two genera (Glochidion and Evodia) which occurred in all vegetation types for the 3 tree samples and three root slides per tree.**

Assessment of infection

Root pieces, each approximately 1 cm long, were selected at random from a stained sample and mounted on glass microscope slides in groups of 10. The roots were then observed under lower power microscope. Length of cortex infected was assessed in millimetres for each root piece, averaged for the ten pieces and expressed as means for the replicates. Each tree species replicate comprised of 3 slides each with 10 root pieces per slide. In situations where observations of the root samples could not be carried out immediately the stained roots were stored in tightly capped McCartney bottles at 4°C for periods not exceeding 10 days. The mean percentage of infection and standard deviation of each three replicate was calculated. An analysis of variance to determine the least significant difference between the vegetation types was also determined.

RESULTS

The initial study showed that most trees at the Park were infected. Although generally, secondary species showed a higher VAM infection levels than primary species (Table 3). The VAM infection levels on the roots of different tree species from the three

Table 6. **Soil characteristics of the sampled sites vegetation**

Vegetation	Soil pH H_2O	0.01M $CaCl_2$	Organic C (%)	Textural class
Rain forest	4.6	4.0	7.62	Clay
Savanna	4.7	4.6	7.23	Clay
Savanna/Rain forest	4.6	4.25	11.91	Clay

vegetation types found in the national park indicates that basically all trees sampled had some infections (Table 4). These infection levels ranged from very high (59.90%) in *Leea indica* in the savanna to very low (0.13%) in *Macaranga* species in the savanna-rain forest. Although the infection levels may be high, the standard deviations were also high, indicating high variations between the trees sampled within the same species.

Among the three vegetation types the VAM infection levels also showed some variations ranging from 10.64% in the rain forest to 15.09% in the savanna-rain forest transitional zone (Table 5). However, because of the very high standard deviations in the infection levels among the different tree species within the vegetation type, there was no significant statistical difference between the sample infection levels among the different secondary trees. In most cases, there was also no significant difference between the species. However, there was a significant difference in the infection levels between primary and secondary tree species (Table 3).

The soils are generally acidic with pH ranging from 4.6 to 4.7 in water and 4.0 to 4.6 in $CaCl_2$ (Table 6). The soils however, have high organic carbon levels ranging from 7.23 to 11.91 percent.

DISCUSSION

All the trees sampled at the Varirata National Park showed some infection with VA mycorrhizae. Infection levels ranged from the highest observed in *Leea indica* (Leeaceae) in the savanna to the lowest infection in *Macaranga* spp. in the savanna-rain forest site. The species in the savanna seemed to exhibit two levels of infection; the high levels observed among *Leea indica* and *Rhus taitensis* (i.e. 59.9% and 37.4% respectively); the second group which showed low infection were *Glochidion* sp., *Evodia* sp. and *Breynia cernua* with low infection levels of 10%, 16% and 8% respectively (Table 4).

The species *Rhus taitensis* and *Leea indica* are the dominant species in the eroded zones which are located in the Savanna-Rain forest. These species are also much heavier infected than the other species in these eroded zones. That is why we made the conclusion that their heavy infection may be the reason for their heavy dominance in the area. *Breynia cernua* is also very heavily infected with VA mycorrhizae, but it is not a dominant species in the eroded sites.

There were significant differences in infection levels among the different species within the same vegetation (Table 4). However, there were no significant differences within the same species across the three vegetation types (Table 4). This may indicate that vegetation variations may not be the main factor influencing infection level but rather by species variation.

Differences in infection levels among species in the same vegetation may be attributed to host physiology and perhaps age related changes in photosynthate allocation (Visser and Danielson 1989). This may be the case in this study as the there were differences in ages and sizes of the trees sampled. The *Leea indica* sampled in the savanna which showed higher infection levels were older than those sampled in the Savanna-Rain forest and the Rainforest. Species infection levels may also vary due to some species producing phenolic compounds which inhibits the growth of many fungi (Allen 1992).

High levels of phenolic compounds are mostly found in the seeds and leaves of plants. Under seed germination and/or leaf degradation (decomposition) such compounds could be released, thus preventing fungi from attacking the plant, especially the plant with high litter mass around its base.

In these studies, members of Euphorbiaceae have shown significantly lower infection levels when compared to the Anacardiaceae and Leeaceae families (Table 4). Similarly, Alexander and Hogberg (1986) reported low levels of infection among members of Euphorbiaceae under natural vegetation in comparison to Anacardiaceae and Leeaceae. While Molina *et al.* (1992) reported high mycorrhizae infection levels among members of the Rubiaceae and Anacardiaceae. The low level VAM infections observed in Euphorbiaceae could be attributed to high content of alkaloids found among members of family, which are used for protection.

In Variarata National Park *Leea indica* (Leeaceae) and *Rhus taitensis* (Anacardiaceae) sampled were found to have high VAM infections and also observed to be the dominant species on the partially eroded sites, i.e. savanna-rain forest. These high levels of infection with VA mycorrhizae may indicate the role mycorrhizae play in aiding plants to obtain the necessary nutrients in the eroded ecotones when part of the A1 horizon has been washed away.

Although soil physical and chemical characteristics may influence VA infection levels due to the decrease in nutrient availability with stand age (Visser and Danielson 1988), this may not have been the case here since all the soils had the same texture indicating a similar inherent cation exchange capacity due to their clay content (Table 6). These soils however, indicated high organic carbon content of up to 11.91% among the rainforest soils. These soils are part of the Kokoda Trail which have previously been documented to be very high in organic carbon (Bleeker 1983).

Although not always significant, generally, tree species in the savanna-rainforest had higher levels of VA infections in comparison to the established rainforest species (Table 5). The infection levels in the savanna-rainforest areas were also higher than the level among the savanna tree species, although they were non significant. This confirms the results observed by Reevese *et al.* (1979) and Janos (1980) which have indicated that the development of mycorrhizal association in disturbed tropical forests is based on the host's need for the fungus. The younger trees in the savanna-rainforest undergoing rapid growth require more nutrients and minerals, thus would be expected to have a higher demand for phosphorous and other cations. This may be the factor which has led to the higher infection levels among the species in the savanna-rainforest when compared to the other vegetation types.

When the levels of VA infection among primary and secondary tree species were compared, the secondary trees showed significantly higher levels of infections (Table 3). The secondary trees -VAM association increase the mycorrhizal spore population in soil. Secondary trees, due to their ability to grow on eroded sites are generally pioneers of bare lands. Thus, their mycorrhizal infection may be creating conducive growing conditions for the primary species that come in at later stages of succession (Kimminis 1987; Whitmore 1988). Similar studies in tropical Africa (Whitmore 1988) which compared the colonisation rate of pioneer trees of *Leea* spp., *Macaranga* spp. and *Trema* spp. to other tree species have shown high mycorrhizal association among these pioneers. These studies indicate that the secondary trees may have unique adaptive features conducive to high mycorrhizal infection which the primary species do not possess.

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REFERENCES

ALEXANDER, I.J. and HOGBER, P. (1986). Ectomycorrhizas in tropical angiosperm trees. *New Phytologist* 102:541-549.

ALLEN, F.M. (1992). Mycorrhizal Function; an integrative fungal process. Chapman & Hall Inc. New York, USA.

BLEEKER, P. (1983). Soils of Papua New Guinea. Commonwealth Scientific and Industrial Research Organisation & Australian National University Press, Canberra.

BEVEGE, D.I. (1968). A rapid technique for clearing tannins and staining intact roots for detection of mycorrhizae caused by *Endogone* spp., and some records of infection in Australian plants. *Trans. of the British Mycological Society* 51:808-810.

BOUYOUCOS, G.L. (1927). The new hydrometer method for mechanical analysis of soils. *Soil Science* 23:267-272.

FITZPATRICK, E.A. (1974). An introduction to Soil Science. English Language Book Society/Longman.

GIOVANNETTI, M. and MOSSE, B. (1990). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologists* 84:489-500.

JACKSON, R.M. and P.A. MAYSON (1984). Mycorrhizae. The Institute of Biology studies No. 159. Edward Arnold, Ltd, London.

JANOS, D.P. (1980). Mycorrhizae influence on tropical succession. *Biotropica* 12:56-64.

KIMMINIS, J.P. (1987). Forest Ecology. MacMillian Publishing Co., New York.

McALPINE, J.R., G. KEIG and R. FALLS (1983). Climates of Papua New Guinea. Australian National University Press, Canberra.

MOLINA, R., H. MASSICOTTE and J.M. TRAPPE (1992). Specificity phenomena. In:Mycorrhizal Symbiosis. Chapman & Hall Inc. New York.

NELSON, D.W. and L.E. SOMMER (1982). Total carbon, organic carbon and organic matter. In: Methods of Soil Analysis Part 2. Chemical and Microbiological Properties, pp. 821-830. Page A.L., R.H. Miller and D.R.Keeney (ed.) American Society of Agronomy Inc., Madison, USA

REEVES, F.B., D.W.WAGNER, T. Moorman and J. KIEL (1979). The role of endomycorrhizae in vegetation practices in semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed versus natural environments. *American Journal of Botany* 66:6-13.

SAULEI, S.M. (1993). Management of tropical forest in Papua New Guinea. In: Ecological Economics in Relation to Forest Conservation and Management. Tamin, N. M., pp. 29-47. Published by Syarikat Datar Raya Sdn. Bhd. Kuala Lumpur.

SAULEI, S.M. (1990). Forest Research and Development in Papua New Guinea. *Ambio* 19(8):379-382.

SEE, L.S. (1992). The mycorrhizal association of Dipterocarpaceae in the tropical rainforest of Malaysia. *Ambio* 19(8):383-385.

SURUMAN, B. (1995). Enumeration of Mycorrhizal-tree association of Varirata National Park. Unpublished Special Topic Project Report, Biology Department, University of Papua New Guinea.

TINKER, P.B. (1980). Role of rhizosphere microorganisms in phosphorus uptake by plants. pp. 617-655. In: F.E. Khasawaneh, E.C. Sample and E.J. Kamprett (eds.). The Role of Phosphorus in Agriculture. *American Society of Agronomy*, Madison, Wisc. USA.

VISSER, S. and R.M. DANIELSON (1988). Acid-forming emissions from oil processing plants in northern Alberta and soil biological chemical properties of Jack pine-lichen woodlands. Final Report submitted to Research Management Division, Alberta Environment, Canada.

WALKLEY, A. (1947). A critical examination of a rapid method for determining organic carbon in soils: Effect of various digestion conditions and inorganic soil constituents. *Soil Science* 63: 251-263.

WHITMORE, T.C. (1988). Tropical Rainforests of the Far East (2nd Ed.) Oxford Science Publications, Clarendon Press Oxford.

YOUNG, C.C., JUANG, T.C. and GUO, H.Y. (1986). The effect of inoculation with vesicular arbuscular mycorrhizae fungi on soybean yield and mineral phosphorus utilization in subtropical - tropical soils. *Plant and Soil* 95:245-253.