Current Status of Mycorrhizal Spore Numbers and Root Colonization of Hevea Saplings as Affected By Seasonal Variations in Plantations of Rubber Research Institute of Nigeria, Iyanomo.

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Abstract: Monoclonal *Hevea* seedlings, RRIM 600, Tjir1, and GT1, at 17 months old, were evaluated for varying levels of mycorrhizal spore soil content and root colonization at three months interval for one year in 2001, under influence of season from March and December. Results showed that significant differences in spore numbers existed and were evidently higher in the dry season from December to March, but were reduced during the rainy season compared to lack of marked differences in the root colonization occurring through out the year and ranged from 40-64% among the test clones. Tjir1 exhibited greater degree of root colonization than RRIM 600 and GT1. Seedlings harvested in June had large crop of fresh internal roots with numerous ultimate rootlets. The present study was aimed at elucidating factors implicated in the results presented.

[Omorusi, V.I., Igeleke. C.L., Ogbebor, N.O., Evueh, G.A., Omo-Ikerodah, E.E. Current Status of Mycorrhizal Spore Numbers and Root Colonization of Hevea Saplings as Affected By Seasonal Variations in Plantations of Rubber Research Institute of Nigeria, Iyanomo. Researcher. 2012;4(1):37-41]. (ISSN: 1553-9865). http://www.sciencepub.net.

Key words: *Hevea* clones, mycorrhizal spores, root colonization seasonal variation.

1. Introduction

Arbuscular-mycorrhizas(AM) are naturally occurring soil fungi belonging to a recently new ascribed phylum, Glomeromycota with a presumed origin at least 460 million years ago (1,2). Mycorrhizal fungi exhibit an ancient association with roots of virtually all terrestrial plants, and due to this ancient association, they have lost their ability to live and complete their life cycle in the absence of a green plant, hence they are obligate symbionts forming symbiotic association with roots of terrestrial plant communities.

This host plant – fungi relationship is naturally mutualistic. The benefits of this mutualism is related to the fungus deriving carbon from the host and the plants, in turn obtains numerous potential benefits (3), mostly enhanced uptake and transport of relatively immobile soil nutrients (especially phosphorus), improved water relations, reduced pathogenic infections, greater volume of permeated soil, decrease transplant injuring, help plants withstand high temperatures and promote establishment of plants in wastelands (4,5).

The distribution and functionality of AM in natural ecosystems are not clearly understood, but information in their prevalence and importance in natural ecosystem is limited and often contradictory (5). Previous studies have shown the development and seasonal fluctuations in AM colonization in most mycorrhizal-dependent plant species (6,7,8), and these studies failed to provide consistent seasonal patterns of VAM development (5). Edaphic factors or soil nutrient status are claimed to be implicated in the patterns and timing of the development of AM fungi (9,10). Conditions of the soil moisture is known to affect root development and AM colonization (11). Plants generally in natural habitats are said to experience fluctuations in soil moisture levels due to rainfall and evapotranspiration. Reduction in soil moisture may lead to reduced nutrient availability and may be favourable for AM colonization patterns in mycorrhizal dependent species are closely adapted to the growth stages of the host plants (10).

In Nigeria, no reports are available on the seasonal variations in AM development in the rubber crop (*Hevea brasiliensis*), as a result, this study was aimed at investigating the effects of seasons on AM root colonization and soil spore concentrations in *Hevea* seedlings within one year.

2. Materials and methods

2.1. Site of study

An experimental field investigation on the development of arbuscular mycorrhizal (AM) fungi under the influence of seasonal fluctuations was carried out at the Rubber Research Institute of Nigeria main station, Iyanomo in the 2001 planting season. *Hevea* seedling clones, GT1, RRIM 600, and Tjir1 were planted out in an old farmland at a spacing of 30 x 30 m in a complete randomized block design of three replicates of each test clone per plot of 15 x 9 m. Root colonization and spore load of AM fungi were evaluated at 17 months of growth and at three months intervals and no fertilizer applications were instituted.

2.2 Assessment of Am root colonization and spore numbers

Thirty seedlings with intact lateral roots were randomly sampled and harvested from each plot. Harvested roots were washed under tap water and fine lateral roots were cut into 1 cm pieces and fixed in Formo Acetic Acid (FAA). Fixed root pieces were then washed free of FAA, cleared in 70% KOH and stained in 0.05% trypan blue-lactophenol for mycorrhizal colonization determination (12). AM fungal colonization was quantified by the grid-line slide intersection method (13), under a compound-light microscope at 100 x magnification. One hundred root pieces per replicate sample were observed for the presence or absence of the colonization (Insections between roots and the vertical eyepiece crosshair). The presence of arbuscle, non-septate hyphae and vesicles were recorded and expressed as percentage colonization (14).

Spores of AM fungi in soil samples collected from ten sub plots were recovered by the wet-sieving and decantation technique (15) and enumerated. Spore density was expressed as spores per 100 g of dry soil. Only healthy spores retained on the 45 μm sieve after passing through 425 μm sieve were counted under a stereomicroscope at 50 x magnification (Willd-Hambrugg, Germany).

2.3 Statistical Analysis

Analysis of variance (ANOVA) was performed to determine patterns of mycorrhizal colonization, spore numbers.

3. Results

Mycorrhizal association was observed in all test clones and throughout the growing season in the field plots. Presence of inter-and intra-cellular hyphae, vesicles and arbuscles characterized AM colonization. The degree of root colonization was not significantly different among the three test clones at sampling times, except in June when the root samples of Tjir1 contained significantly higher fungal level of colonization than those from roots of RRIM 600 and GT1 (fig 1). No significant variation in mycorrhizal root colonization in each clone throughout the growing season was obtained. Generally, root colonization ranged between 40-64%.

Assessment of mycorrhizal spore load in soils with the three test clones indicated that no significant differences existed at each sampling time. However, spore numbers were significantly lower in June and September during the rainy season ($P<0.05$), than those obtained during the dry season (March and December). The highest spore load (numbers) occurred in December (Fig. 2).

Seasonal variations showed that root colonization increased gradually from March to December, while spore population decreased from march to September, thereafter, it increased greatly and reach a peak in December (Fig. 3).

In June, seedlings harvested had a large crop of fresh internal roots with numerous ultimate rootlets, whereas seedlings harvested at other sampling times produced fewer fresh root growths. In September, observations revealed that lateral root growth was indeed slow and that ultimate rootlets were few or absent.

4. Discussion

This survey obviously showed the occurrence of AM fungi within the roots of the three test clones. The fact that there were no marked differences recorded in the root colonization of clonal rubber seedlings throughout the year, the mean percentage colonization increased from March to December, which corroborates previous studies in other crops such as cassava (16, 17).

Seasonal changes affected AM spore numbers which were observed to be higher in the dry season and lower during the rainy season. The level of spore numbers is known to be affected by soil moisture content of the soil (18). High precipation may be implicated to be detrimental to the development of AM spores in soils which accounted for low spore population obtained during the rainy season especially in September, following long period of rainfall. Lowest spore counts observed in September were related to *Hevea* lateral root growth which was observed to be every slow and the ultimate rootlets were either few or absent. This report is in credence to the findings of (19). Several workers (18, 20, 21) reported increase in spore numbers during seasons of slow or intermittent root growth. AM spores are survival or storage structures which are known to be resistant to adverse environmental conditions (20), it is expected that more spores will be found in soils under such unfavourable climatic conditions as elucidiated in this study, which were less favourable for fungal and plant root development.

It is asserted that results obtained in this study indicated that the effects of season, the rainy season from April to October, and the dry season from November to March produced little effects on AM root colonization of the test seedlings contrary to variations in spore numbers as affected by the season. It is suggested therefore that spore load in soils had no direct influence on the intensity of mycorrhizal root colonization of the *Hevea* saplings (Fig 1). This non-relationship between rhizospheric spore numbers and the intensity of root colonization has been observed frequently by (3).

This minimal investigation is intended to form the basis for an elaborate study of the influence of seasons on the AM soil spore numbers and root colonization of *Hevea* saplings.

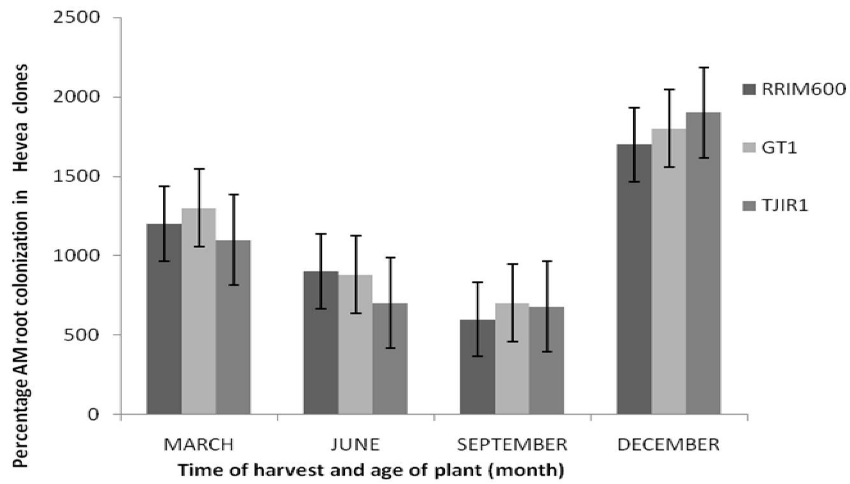

Fig. 1: Percentage mycorrhizal root colonization in *Hevea* clonal seedlings (100 root segments examined per clonal replicate + S.E)

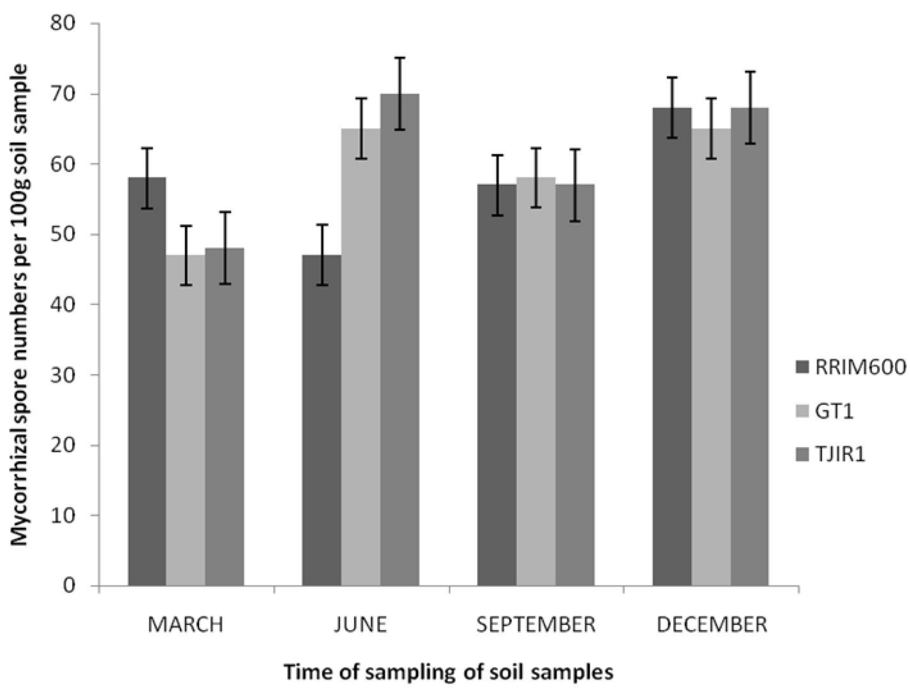

Fig. 2: Mycorrhizal spore numbers per 100 g soil sample under *Hevea* clonal seedlings

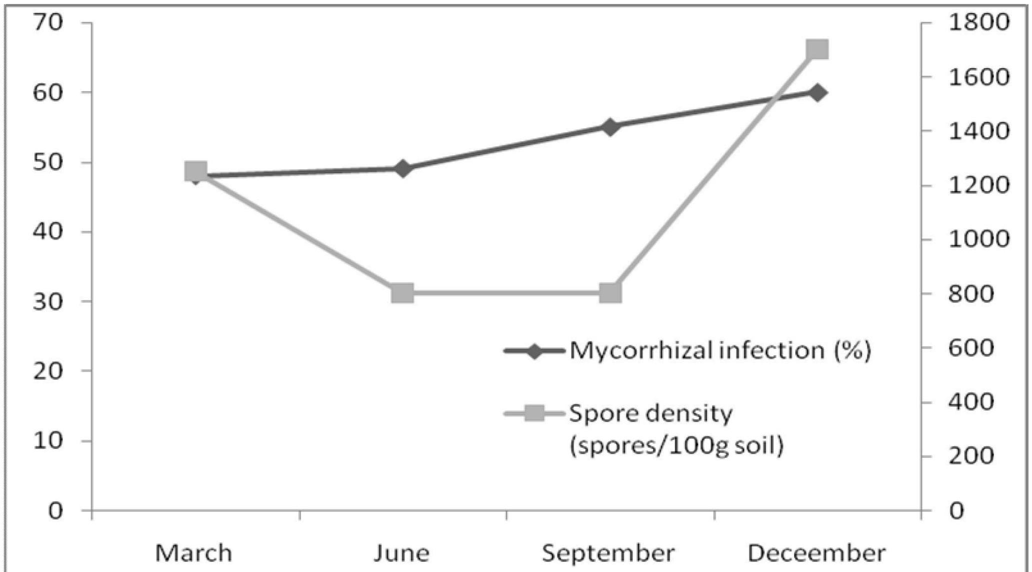

Fig. 3: Seasonal variation in mean mycorrhizal root infection and spore numbers of AM fungi associated with Hevea clonal seedlings.

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27/12/2011