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VESICULAR ARBUSCULAR *MYCORRHIZAE* IN THE CARIBBEAN - PAST, PRESENT AND FUTURE

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ABSTRACT

Mycorrhizae formed between plant roots and zygomycetous fungi are ubiquitous and can improve the productivity of most crop plants. The use of effective vesicular-arbuscular (VA) mycorrhizal fungi could enable the development of sustainable agricultural systems with reduced input of high cost fertilizers. This paper reviews the limited knowledge of the VA mycorrhizal fungal species present in Caribbean agriculture and highlights the need to determine which species/strains are present in Caribbean soils and also their effectiveness. The potential of surrogate plant transformation via genetic engineering of VA mycorrhizal fungi is considered and the possible consequences of releasing genetically modified VA mycorrhizal fungi are discussed.

INTRODUCTION

Vesicular-arbuscular (VA) mycorrhizal fungi are obligate endophytes that generally lack specificity for host plants. They are found in almost all ecosystems, forming mutualistic associations with members of a very wide range of plant families. These associations, VA mycorrhizae, are found predominantly in angiosperms but their presence in bryophytes, pteridophytes and gymnosperms have also been reported. Among the angiosperms, VA mycorrhizae may be absent in members of the Brassicaceae, Chenopodiaceae, and Cyperaceae (Hirrel *et al.*, 1978) and in ecosystems where ectomycorrhizae and/or ericoid mycorrhizae are prevalent. It is postulated that the VA mycorrhizal association occurred early in plant evolution and co-evolution of plants and VA mycorrhizal fungi followed (Nicolson, 1975).

VA mycorrhizal fungi are Zygomycetes and are classified into six genera, *Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* (Schenck and Perez, 1988; Morton and Benny, 1990). This classification of VA mycorrhizal fungi is based on characteristics of the asexual spores, such as their gross morphology, ontogeny and spore wall murographs, as no sexual spores of these fungi have been identified conclusively (Siquiera *et al.*, 1985; Morton and Benny, 1990).

In the soil, fungal colonisation starts when extraradical hyphae or germtubes come in contact with host roots. The fungal hyphae then differentiate and appressoria are produced followed by penetration into the epidermal and subepidermal layers of the root. Extensive inter- and intracellular hyphal growth follows and eventually arbuscules are formed by invagination of root cortical cells. Intraradical vesicles may be produced depending on the genera of VA mycorrhizal fungi involved in the symbiosis (intraradical vesicles are not produced by *Scutellospora* and *Gigaspora*) and extraradical hyphae grow into the soil to continue the process. The same root can be colonised several times by extraradical hyphae. When suitable environmental conditions are available the fungus sporulates, the asexual spores are usually borne on the extraradical hyphae. *Gl. intraradix* has the unique feature of producing spores within host roots.

The positive affect of VA mycorrhiza formation on the uptake of minerals by the host plant, especially phosphorus in P-limited soils, has been reported repeatedly. The physiological effects on plants as a result of VA mycorrhiza formation are similar to those of phosphorus additions to soils low in this mineral. Conversely, soils with high P levels do not promote mycorrhizal development

(Menge, 1978; de Miranda *et al.*, 1989). It is not uncommon for P depletion zones to develop around roots especially in soils with low concentrations. VA mycorrhizal fungi extend the surface area of the roots thereby enabling the mycorrhizal root system to explore a greater volume of soil for phosphorus, usually beyond the depletion zones. The smaller diameters of the hyphae also allow the fungus to explore smaller pore spaces than roots and roots hairs. Some studies have suggested that VA mycorrhizal fungi can alter unusable forms of phosphorus (i.e. adsorbed and organic phosphates) and make them available to the plant (Brundrett, 1990).

VA mycorrhizal fungi exert other effects on the physiology of colonised plants. VA mycorrhizae affect the water relations of plants (Allen *et al.*, 1981), the carbon economy, and may influence plant community structure by regulating nutrients and water among plants (Allen and Allen, 1986). VA mycorrhizae also enhance plant growth (Smith, 1980; Nielsen, 1990), plant disease resistance (Morandi *et al.*, 1984; Caron, 1989; Feldmann *et al.*, 1989), and plant reproduction (Koide *et al.*, 1988) and reduce competition between large plants and seedlings (Eissenstat and Newman, 1990). VA mycorrhizal fungi enhance plant micronutrient uptake when low levels of these micronutrients are present in the soil and provide protection to the plant when these micronutrients are available in phytotoxic amounts (Persad-Chinnery and Chinnery, 1994).

A better understanding of VA mycorrhizae and the VA mycorrhizal fungus lifecycle could hasten their eventual exploitation in agroecosystems. In recent years, as a result of agricutural diversification and intensification, fertilizer use has been increased. Although Barbados spent about US$2 million dollars per year between 1982 and 1991 on imported fertilizers, the quantity has increased (Barbados Customs Statistics). Usually more than half of an applied phosphorus fertilizer is unavailable to the plant. In order to compensate for this loss, phosphorus fertilizer is added in amounts greater than necessary for plant growth. Since VA mycorrhizae can extract nutrients more efficiently from the soil than non-mycorrhizal roots, it is thought that VA mycorrhizal fungi could be used as "biotic fertilizers" and that VA mycorrhizal fungi may substitute for a substantial part of fertilizer input in the field. For the crops they were working with, Medina *et al.* (1990) found that field inoculations with *Gl. intraradix* and *Gl. etunicatum* resulted in a minimum of 30% decrease in the amount of phosphorus fertilizer (40 kg ha^{-1}) required for maximum yields. It is predicted that marginal soils low in nutrients, for example soils slashed and burnt, that, due to the high fertilizer input required, are too expensive to cultivate, could be used for agriculture once the soil is inoculated with a suitable species of VA mycorrhizal fungus.

Since VA mycorrhizal fungi occur in association with most cropped plants, their potential exploitation in agriculture is significant. Thus, an immediate, and coordinated, effort should be made to identify the species of VA mycorrhizal fungi present in Caribbean soils and to evaluate the effectiveness of the various species/strains present with each of our crop plants. This information is necessary before large scale field inoculation can be considered. Phytosanitary considerations may limit use to indigenous species.

Under field conditions successful inoculation of soil with VA mycorrhizal fungi will depend on several factors:

(1) the native VA mycorrhizal fungal species present,
(2) the VA mycorrhizal fungal species introduced and its competitive advantage over the native species,
(3) the type of inoculum, that is whether spores or root pieces are used,
(4) interplant connection that may result in donors and recipients of nutrients,
(5) grazing, since fungi are a food source for many soil organisms,
(6) root longevity,
(7) the seasonality of spores, and
(8) the interaction between VA mycorrhizal fungi and other soil microor ganisms.

Traditional agronomic practices must also be examined to determine their effects on VA mycorrhizal fungi (Persad-Chinnery *et al.*, 1992). For example, since tilling tends to create environments more conducive to the growth of bacteria, untilled soils support greater mycorrhizal fungal colonisation of roots and sporulation (Hendrix *et al.*, 1986). Pesticides and inorganic fertilizers may also have deleterious effects on populations of beneficial soil organisms including VA mycorrhizal fungi and inter-cropping may be better than monoculte since VA mycorrhizal fungal diversity tends to increase with host diversity (Rabatin and Stinner, 1989).

Since all the factors stated above for successful field application need to be evaluated for each soil type, it is likely that evaluation would require a long time before the potential of field inoculation is fully realised. However, there are aspects of Caribbean agriculture where VA mycorrhizal fungi can be utilised almost immediately to enhance plant growth and survival. For example, the survival rate of tissue cultured plants during the hardening and outgrowing process can be increased significantly if the potting soil is inoculated with the appropriate species of VA mycorrhizal fungus. Another area of immediate utilisation is in plant/tree nurseries. VA mycorrhizal fungi have been shown to increase the survival rate of transplanted seedlings. Michelini and Nemec (1988) used two *Glomus* spp. and an unidenified local inoculum, in a Barbadian nursery, to improve the survival of transplanted citrus.

VA MYCORRHIZAE IN THE CARIBBEAN - PAST

Several studies conducted in the tropics have shown that VA mycorrhizal fungi are prevalent in tropical soils and form mycorrhizae with plants of agricultural importance. A selection of of these studies, restricted to those in which the fungal endophyte(s) was identified, is presented in Table 1.

Based on a recent search of the Dialog[(R)] databases and our own extensive mycorrhizal literature collections, knowledge of VA mycorrhizae in the Caribbean is limited. In 1968, Kreisel published a survey of the fungus flora of Cuba, however, there was no documentation of VA mycorrhizal fungi. Only species involved in the more noticeable ectomycorrhizal type of mycorrhiza were included. The first reports on VA mycorrhizae in the Caribbean were those of Pyke (1935), who reported the presence of mycorrhizae in *Theobroma cacoa*, and Laycock (1945), who also reported the benefits of mycorrhizae in cocoa. The first study to report the prevalence of VA mycorrhizae in Caribbean soils and the positive effect of VA mycorrhizae on plant growth was conducted by Johnston (1949). This study was conducted in two parts. In the first, plants representative of the flora of Trinidad were collected from various habitats and classified as weeds, secondary bush (species regenerating waste land), forest trees, orchard savannah species and crop plant species. About 87% of the crop plants and savannah species, about 77% of weeds and 79% secondary bush were VA mycorrhizal. Interestingly, 100% of the forest trees examined were VA mycorrhizal and colonisation was well established. In the other plant categories colonization was moderately established In the second part of the study the effect of different soil types on colonisation and growth of sea island cotton (*Gossypium barbadense* L.) was investigated. It was found that root colonisation by VA mycorrhizal fungi was greatest, about 90%, and plant growth was improved in nutrient poor soil, low in organic matter, nitrogen and deficient in phosphorus and potasium. Improving the soil fertility by the addition of pen manure did not alter the level of colonisation or the benefits. However, when the soil was amended with inorganic fertilisers the percentage VA mycorrhizal colonisation was reduced by just over 20%. No species identifications of the VA mycorrhizal fungi observed were made. This lack of identifications in older papers on VA mycorrhizae is not uncommon since, as stated above, identifications are made on spore characteristics and spores are at times difficult to obtain.

A study was conducted by Chinnery *et al.*, (1987) to determine the influence of VA mycorrhizal fungi on the growth of 16 clones of sugarcane (*Saccharum* hybrids) in Barbados. It was noticed that sugarcane yields, in Barbados, were maintained despite continuous growth on the same fields without the addition of phosphorus fertilisers. Roots were collected and analysed in December, January and February. VA mycorrhizal colonisation of 70-75% was recorded in December and February, and a lower colonisation rate of 56% was measured in January. These high levels of colonisation are thought to contribute to consistent sugarcane yields in Barbados. It was postulated that the reduction in colonisation in January may have been due to the change in soil moisture that is characteristic of the start of the dry season and that the increased colonisation in February may have been due to the dominance of another species of VA mycorrhizal fungi, possibly one more tolerant of dryness. No species identifications were attempted in this study.

The influence VA mycorrhizal fungi have on the growth of mycorrhizal roots was investigated in two independent studies (Inniss, 1989; Persad-Chinnery, unpublished). Both found that VA mycorrhizal fungi affect meristematic cell division in the roots of host plants such that the root mitotic indices of VA mycorrhizal plants were lower than non-mycorrizal plants.

Michelini *et al.* (1989) examined the effect of paclobutrazol on the VA mycorrhizae of alemow (*Citrus macrophylla* Wester) in Barbados. Paclobutrazol, that has limited fungicidal properties and is a plant growth regulator, was added at concentrations of 0.03, 0.06, 0.125, 0.5 and 1.0g active ingredient to soil in containers planted with alemow. Mycorrhizal colonisation by *Glomus* spp. was greatest at 1.0 g paclobutrazol, possibly due to greater mycorrhizal dependence of the plant as its root became stunted as a result of treatment. Paclobutrazol did not appear to have any fungicidal effects on these mycorrhizal species.

In Jamaica, species of *Gl. pallidium*, *Gl. aggregatum*, and *S. microcarpa* were selectively used with species of *Rhizobium* to determine the best combination for optimum growth of cowpea (*Vigna unguiculata* L.) planted in Jamaican soils (Ames *et al.*, 1991). Their study concluded that VA mycorrhizal fungi and *Rhizobium* functioned synergisticly to improve plant growth when compared to either alone. Also in Jamaica, Coates-Beckford and Pereira (1992) examined the microorganisms associated with breadfruit (*Artocarpus altilis*) roots in an attempt to identify the causative agent of "decline", characterised by premature fruit drop, leaf chlorosis, flower abscission and branch dieback. *Gl. tenuis* was found associated with roots of both healthy plants and those exhibiting decline.

Persad-Chinnery *et al.* (1992) developed a consistent and reliable method of *in vitro* spore germination for spores of *Gigaspora* using a commercial preparation of cellulase. These spores were recalcitrant to germination by standard methods.

In another study by Michelini *et al.* (1993), citrus roots were examined in Barbados, Dominica, Grenada and St. Lucia. They were able to show that environmental factors such as rainfall, pH, altitude, organic matter and micronutrients influenced the level of VA mycorrhizal colonisation. The healthiest plants were the most highly colonised. Most of the VA mycorrhizal fungi identified from soils around the citrus roots were *Glomus* spp. A few were *Sclerocystis*, *Gigaspora* and *Scutellospora*.

Extensive research has been conducted on the sand dunes on the east coast of Barbados. *Gigaspora margarita* and *Scutellospora gregaria* have been identified as the two species present in the sand dunes. L.D. Waterman (unpublished) has not been able to find any seasonality trends based on spore abundance in the sand dunes after several years of monitoring. A study conducted by Kirton (1993) in the same area showed that spore abunduance was greatest in the higher, older and vegetated parts of the sand dunes. The intensity of infection in this region of the dune was much lower than the areas that were newer and vegetated. The findings of this study may be incorporated into strategies to conserve and stabilise the dunes.

VA MYCORRHIZAE IN THE CARIBBEAN - PRESENT

Although research on VA mycorrhizae in the Caribbean is limited, a significant portion of the published literature originate from U.W.I., Cave Hill and research in this area is ongoing. Presently, L.D. Waterman (unpublished) is developing species specific primers that could be used in PCR (polymerase chain reaction) to identify the species of VA mycorrhizal fungus present in a mycorrhiza or in a mixed population of fungi. The usefulness of this development goes beyond the Caribbean. The present method of identification is based on spore characteristics and it can take several months before spores are available. This novel method will enable the researcher to know almost immediately the genus and species of VA mycorrhizal fungus involved in the mycorrhiza.

Studies on the nuclear cytology during the precolonisation and colonisation phases of the VA mycorrhizal fungal lifecycle, on drug resistance of *Gigaspora* spores and on the construction of a VA mycorrhizal fungal genomic library are continuing (Persad-Chinnery, unpublished). These studies are aimed at developing a transformation system for *Gigaspora* that would enable genetic manipulation of the VA mycorrhiza for improved effectiveness.

Another active area of research is to determine the influence VA mycorrhizal colonisation may have on alleviating the symptoms of bunchy top disease in Papaya (A. Waterman, unpublished).

VA MYCORRHIZAE IN THE CARIBBEAN - FUTURE

The future of VA mycorrhizal research in the Caribbean is likely to progress from two aspects. Firstly, VA mycorrhizal fungi could become incorporated into the tissue culture and nursery operations, as the relevent persons realise the potential of these fungi. Secondly, there is likely to be greater attempts at manipulating the VA mycorrhizal association through genetic engineering to enhance effectiveness. Genetic engineering refers to the use of laboratory or industrial techniques to modify an organism's genetic material. One possible method of manipulating VA mycorrhizal fungi is via transformation. This method normally involves introducing DNA encoding desirable genes into the organism by protoplast isolation and regeneration, biolistics or electroporation This introduced DNA may then become incorporated into the fungus' DNA and function as fungal DNA.

As there are no known sexual stages for VA mycorrhizal fungi and since they are obligate symbionts the development of a transformation system could enable genetic studies such as the organization of their eukaryotic genome, the structure of mycorrhizal genes and the regulation of different molecular processes, to be studied. All of this information is necessary to fully comprehend the VA mycorrhizal association in the soil.

The development of a transformation system for *Gigaspora*, currently being pursued at U.W.I., Barbados, will allow the introduction of genes from both plant and microbial sources to be introduced into the fungus that may lead to a better understanding of the VA mycorrhizal symbiosis at a molecular level. There are several candidate genes that could be introduced into the fungus, should a transformation system be developed. Firstly, genes encoding the production of phytohormones that stimulate root and mycorrhizal development. Harley and Smith (1983) reported that IAA (indoleacetic acid) promotes root growth and enhances mycorrhizal symbiosis. The introduction of the IAA gene into VA mycorrhizal fungi may lead to further understanding the role of phytohormones in mycorrhizal formation and function.

Secondly, genes for substances repellent to soil insects or nematodes could be introduced into VA mycorrhizal fungi. These transformed fungi could be used to reduce the cost of pesticide input in agriculture. Similarly, genes for more efficient absorption and translocation of nutrients from the soil to the plant would greatly increase the importance of VA mycorrhizal fungi as biofertilisers. For example, genes encoding enzymes such as phosphatase or nitrate reductase, could influence nutrient availability or alleviate various edaphic stress factors. Additional genes of agricultural importance are those encoding for metabolites that foster plant osmotolerance, plant disease resistance and plant resistance to pesticides. The advantage of introducing these genes into the fungus is that the plants are likely to benefit without being subjected to bioengineering. Trangenic VA mycorrhizal fungal inocula may effectively substitute for fertilizers and/or pesticide inputs in agriculture. The ultimate goal is to develop improved fungal strains for use as inocula in agriculture.

Another advantage of introducing genes into VA mycorrhizal fungi is that genes for root related functions can be restricted to expression by the fungus. Thus the plant benefits from the gene product without the gene being present in the plant genome and the plant is free from any genetic manipulations. This is obviously a more desirable situation since in many parts of the world genetic manipulation of food plants is viewed with suspicion by most consumers.

There seem to be an inexhaustible scope for research should a transformation system for VA mycorrhizal fungi develop. However, in considering strategies to improve symbiosis it will be important to avoid non-target effects that could disrupt the balance among rhizosphere components. The general intention of the research should be to optimise the plant-fungus symbiosis through genetic manipulation of the fungal component without impairment of the components of the rhizosphere ecology that may be supportive to that symbiosis.

The process of genetic engineering is similar to the activities of traditional plant breeders and, as such, should offer no additonal risks. However, since the techniques used in molecular biology are not natural, genetically engineered organisms are viewed as things to fear. The release of transgenic VA mycorrhizal fungi in the field could pose two potential concerns. Firstly, the introduced gene, although providing a benefit to a particular crop plant, could be passed on to other plants, possibly weeds since the host range of these fungi is diverse. Secondly, the transgenic VA mycorrhizal fungus may behave as an invasive species, possibly leading to the reduction or extinction of native species of VA mycorrhizal fungi. Both concerns can only be evaluated to determine the risks when transgenic VA mycorrhizal inoculum can be taken into the field for regulated testing following the proper procedure defined by an objective governing body. Possibly the best course of action to users of this technology is to evaluate the risks and the benefits involved. As with all modern inventions, if the benefits are greater than the risks involved then the technology can be classified as useful. If improved plant growth results from transgenic VA mycorrhizal fungal inoculum, such that the plant product of economic importance is increased significantly, then the threat of improving the growth of weeds or reducing native populations of VA mycorrhizal fungi become acceptable risks.

CONCLUSION

At least 80% of the angiosperms that inhabit the earth are VA mycorrhizal, implying that many plants are always VA mycorrhizal, however these fungi tend to be considered as part of the root anatomy and not as separate entities and have received little attention in the Caribbean. Among plant physiologists these fungi and other microbial mutualists are seldom considered as contributors to the nutritional status of the plant. The literature published on VA mycorrhizal fungi show that these organisms can greatly improve plant health. The VA mycorrhizal association is too prevalent to be ignored and sustainable utilisation should be the goal of all plant growers. Sustainable agriculture which is as much a process as an end point can incorporate VA mycorrhizal fungi as an important component of the process. Beyond the Caribbean region there are examples of plant nurseries and tissue culture laboratories that utilise VA mycorrhizal fungi without any adverse effects when the plants are introduced into the field. It is important that we are aware of the species present in Caribbean soils if field inoculation is to achieve any success in the future. Interaction between these fungi and particular crop plants also need to be evaluated for this purpose. This information will also prove useful in evaluating the risks of using transgenic VA mycorrhizal fungi should they become a reality in the future.

Table 1: Reported associations between agricultural crops and VA mycorrhizal fungi in the tropics.

CROP SPECIES	VA MYCORRHIZAL SPECIES	REFERENCES
Chilli (*Capsicum annuum*)	*G. calospora* *G. margarita* *Gl. fasiculatum* *Gl. intraradix*	Hari Babu *et al.* (1988)
Corn (*Zea mays*)	*A. caulospora* *Glomus* spp. *Scutellospora* spp.	Berch (1989)
Onion (*Allium cepa*)	*Gl. fasciculatum* *Gl. mosseae*	Alexander *et al.* (1989) Afek *et al.*, (1990)
Bean (*Phaseolus vulgaris*)	*Gl. fasciculatum*	Alexander *et al.* (1989)
Tomato (*Lycopersicon esculentum*)	*Gl. fasciculatum* *Gl. etunicatum* *Gl. intraradix*	Alexander *et al.* (1989) Bryla & Koide (1990) Caron et *al.* (1985)
Cotton (*Gossypium hirsutum*)	*Gl. deserticola*	Afek *et al.*, (1990)
Citrange (*Poncirus x Citrus*)	*Gl. intraradix*	Johnson & Hummel (1985)
Sour orange (*Citrus aurantium*)	*Gl. intraradix*	Graham & Syvertsen (1989) Nemec, (1992)
Sweet orange (*Citrus sinensis*)	*Gl. intraradix*	Graham & Syvertsen (1989)
Avocado (*Persea americana*)	*Sclerocystis sinuosa* *Gl. macrocarpum* *Gl. fasciculatum* *Acaulospora* spp. *Glomus* spp.	Haas & Menge (1990)
Sweet potato (*Ipomoea batatas*)	*Gl. clarum*	Paula *et al.*, (1992)
Coffee (*Coffea arabica* var. Catturra)	*A. longula* *A. myriocarpa* *E. columbiana* *Scutellospora heterogama* *Gl. fasciculatum* *Gl. manihitis* *Gl. occultum*	Siverding & Toro (1990)

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