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The Effect Of Inoculation with Arbuscular Mycorrhizal Fungi on The Yield of Woody Legumes and Cassava in Alley Cropping

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Abstract

A field investigation was carried out to assess the effect of inoculation with arbuscular mycorrhizal (AM) fungi on the yield of cassava (*Manihot esculenta* Crantz, *Gliricidia sepium (*Jacq.*)* Walp *Leucaena leucocephala* (Lam de Wit*)* and *Senna siamea* Irwin and Barneby in an alley cropping system. The soil was low in major nutrients. The plants were either uninoculated or inoculated with *Gomus deserticolum* Trappe, Bloss and Menge, but all plants were infected with indigenous AM fungi. The dry matter yield of roots of inoculated cassava was 43% greater than the uninoculated cassava. Apart from the positive effects on leaves of *Gliricidia*, mycorrhizzal inoculation did not influence the dry weights of stems and leaves of the hedgerow trees. These yield increases in cassava and *Gliricidia* were associated with increased P uptake and higher root colonization by AM fungi. The effect of inoculation with *Glomus deserticolum* was to increase nutrient uptake in *Senna* and improve extraction of soil water in *Leucaena*. The improved productivity of inoculated plants may be attributed in part to increased in stomatal conductance (gs), transpiration (E) and a stable xylem pressure potential (Ψxylem) resulting in greater extraction of soil water in the dry season.

Key words: Arbuscular mycorrhiza, *Manihot esculenta, Gliricidia sepium, Leucaena leucocephala*, stomatal conductance, transpiration, xylem pressure potential

Introduction

Substantial evidence exists that arbuscular mycorrhizas (AM) in symbiotic association with higher plants can increase the efficiency of nutrient and water uptake and this, in turn, may improve the growth of mycorrhizal plants, particularly in soils low in available phosphorus (P) (Abbott and Robson, 1982; Augé *et al.*, 2004; Harley and Smith, 1983; Reddel and Warren, 1986; Trappe, 1981)

In the humid and subhumid tropics, Alfisols are low in available P and other essential nutrients (Cobbina *et al.*, 1989; Kang and Wilson. 1987) such that crop productivity is limited by the deficiency of nutrients. In such soils, either chemical fertilizers have been applied to improve the fertility of the soil (Chosh *et al.*, 1989; Mittal and Singh, 1989; Njoku and Arene, 1980) or an alley cropping system has been used to improve nutrient flow in such soils. In this system, woody legumes and arable crops are interplanted respectively in hedgerows and alleys (Wilson and Kang, 1981). The woody legumes fix atmospheric nitrogen to enrich the soil and are pruned regularly to provide green manure and mulch to arable crops in the alleys. However, legumes require optimum P in the range of 8-15 mg kg-^{1} soil for nodulation, nitrogen fixation and growth (Bethlenfalvay *et al., 1985;* Marschner, 1986). One of the ways of achieving adequate P nutrition in leguminous trees and arable crops like cassava *(Manihot esculenta* Crantz) is through inoculation with AM mycorrhizas (Awotoye *et al.*, 1992; Dodd *et al.*,1991; Sieverding and Howeler, 1985).

In spite of the importance and natural occurrence of the arbuscular mycorrhizal fungi in tropical ecosystems (Miller, 1979), there is no study known to us that has demonstrated their use in an agroforestry system, particularly in alley cropping . It has been shown that inoculation of cassava with introduced AM fungi in addition to existing indigenous AM fungi may either increase (Dodd *et al.*, 1990; Howeler *et al.*, 1987) or not influence (Kang *et. al.*, 1980) crop yield. The use of efficient AM fungi for increasing crop productivity in infertile soils has been demonstrated in Colombia (Howeler *et al.*, 1987; Saif, 1987), but its usefulness is yet to be fully recognized in sub-Saharan Africa.

The purpose of our study was to investigate on an Alfisol in southwestern Nigeria if inoculation with AM fungi without fertilizer application could be used as a management tool to increase the above-ground biomass of hedgerow trees, *Senna siamea, Gliricidia sepium and Leucaena leucocephala* and the root yield of a cassava intercrop.

MATERIALS AND METHODS

Cultivation of Plants

The experiment was established on an Alfisol at a village (Latitude 7' 43^{o} N and Longitude 3' 9^{o} E) between the University of Ibadan and the International Institute of Tropical Agriculture (IITA) in Ibadan. The rainy season extends from April to October and the dry season from November to March. The site was chosen because it had been cropped continuously in the past five years and the soil had low nutrient availability (Table 1). The soil is of Balogun series (Rhodic Kandiustalf).

The experiment utilized three woody legumes, namely *Senna siamea* Irwin and Barneby, *Gliricidia sepium* (Jacql) *Walp and Leucaena leucocephala* (Lam) de Wit *as* hedgerow trees, which served as the main treatments; mycorrhizal inoculation and non-inoculation were sub-plot treatments. Cassava cv TMS 30572 (parentage 58308 x Branca de Santa Catarian) (Hahn *et al.,* 1973) was the sole intercrop for all the hedgerow tree treatments. The experimental design was a 3 x 2 factorial, replicated three times in a completely randomized complete block design. Cassava was intercropped with either *Senna siamea , Gliricidia sepium* or *Leucaena leucocephala* . The hedgerow trees and intercrop were either uninoculated or inoculated with the AM fungus *Glomus deserticolum* Trappe, Bloss and Menge. No attempt was made to destroy the indigenous AM fungi, which were mainly *Glomus mosseae* and three species of *Acaulospora*. Hence, the uninoculated trees and cassava were also infected with indigenous mycorrhizas.

The trees were planted in rows 4 m apart and 0.5 m within row while the cassava intercrop was planted in rows 1 m apart, and 1 m within rows to give a respective plant population density of 5000 hedgerow and 10,000 cassava plants ha-^{1} . Each sub-plot was 12 x 12 m and consisted of three lines of each hedgerow tree. Each external line of hedgerow trees in a sub-plot was surrounded by two rows of cassava to reduce the edge effects. Hedgerow trees were established from 4-week-old seedlings previously grown in nursery polyethylene bags (11.5 cm diameter, 15 cm depth) containing over-sterilized topsoil from the field site. Cassava plants were established with 0.2 m stem cuttings in the field. During establishment, the hedgerow trees were planted in the furrows between the ridges while the cassava stem cuttings were inclined in the ridges to avoid initial competition between the two plants.

Arbuscular mycorrhizal inoculation, consisting of spores and hyphae and infected root fragments was done with 10 g of crude inoculum of *G. deserticolum*. Hedgerow trees and cassava were inoculated by placing the crude inoculum 3 cm under either the seeds in the polyethylene bags or under the stem cuttings in the field at the time of planting.

Plant Physiology, Harvest and Analysis

Arbuscular mycorrhizal colonization was assessed by taking root samples of the trees and cassava at three random locations within each sub-plot. Roots were washed free of soil by hand and stored in 50 % ethanol. The root samples were cleared using 10 % KOH in an autoclave at 121 ^{o}C for 15 min. The pigmented roots of Seena *and Leucaena* were further bleached with alkaline H_20_2 before staining with Chlorazole Black E solution (Brundrett *et al*., 1984). The grid intersect method was used to evaluate the percentage of root colonization (Giovanetti and Mosse, 1980).

At 12 months after planting, the hedgerow trees were cut 50 cm above soil level, separated into leaves and stems, dried at 70 ^{o}C to constant weight and the weights recorded. At the same time, the cassava plants were harvested to determine the leaf, stem and root dry weights. All tuberous and fibrous roots were recovered by excavation as far as it is possible.

Leaf samples of harvested materials were digested by the microkjeldahl method and total N determined colorimetrically using a Technicon autoanalyser (IITA, 1982). Levels of K, Ca and Mg were determined by atomic absorption spectrometry after wet ashing in nitric-perchloric acid mixture. Phosphorus content was determined by the molybdenum blue method of Murphy and Riley (1962).

Soil moisture characteristics were determined for soil samples from 0 - 30, and 30 - 60 cm soil depths. The samples were dried for one day at 80 ^{o}C to obtain the soil moisture content. The soil water potential (Ψ soil) was inferred from a curve relating soil moisture content to Ψ soil using a pressure plate apparatus (Soil Moisture Instruments Co, Santa Barbara, CA, USA).

Water relations parameters, i.e. gs, E. Ψ xylem and Ψ soil were measured on hedgerow trees and cassava plants between 11.00 and 14.00 h on specific days in the dry and wet seasons. Three sets of measurements were taken during the dry and the wet seasons, but the results for only one season are shown, as trends for the other seasons were similar. The period between 11.00 - 14.00 h was chosen because preliminary observations showed this to be the period of maximum effects. Stomatal conductance and E were determined with a Li-1600 Steady State Porometer (Li-cor Inc. Lincoln, Nebraska, USA), In *Leucaena*, the gs and E could not be determined with the porometer owing to the small nature of its leaves. Xylem pressure potential was determined with a pressure chamber apparatus (Soil Moisture Instruments Co., Santa Barbara, CA, USA) using single shoots of trees and single leaves of cassava.

Introduced AM fungus contribution to nutrient content and yield of trees and cassava was estimated using the formula below (Kothari *et al.*, 1991).

$$\text{Introduced AM fungus contribution } (\%) = \frac{A - B}{A} \times 100\,,$$

where total uptake of P or N, or leaf or stem biomass or root yield by AM inoculated plants is denoted by A, and uninoculated plants by B. Total uptake or content of any mineral nutrient was calculated as the product of concentration and leaf dry weight.

Statistical Analysis

The data for inoculated (I) and uninoculated (C) plants of each species were subjected to analysis of variance, using LSD at $p<0.05$ for test of significance.

Results

Arbuscular mycorrhizal root colonization

Inoculated and uninoculated hedgerow trees, and cassava were colonized by AM (Table 2). The colonization level of inoculated and uninoculated plants did not differ significantly from each other except for *Gliricidia* and cassava. In *Gliricidia* and cassava, percentage root colonization in inoculated plants was about 1.5 - 3.5 times higher than those of uninoculated plants.

Plant biomass and cassava root yield

Only inoculated and uninoculated *Gliricidia* plants differed in leaf dry weight (Table 2). There were no significant differences in the cassava yields with the different hedgerow species and therefore, the data under all the hedgerow trees for inoculated or uninoculated cassava were combined (Table 2). Inoculation with *G. deserticolum* significantly increased cassava root yield, the yield of dry matter of roots of uninoculated control (C) was about 57% of the inoculated (I) plants (Table 2).

Data on water relations are presented in Table 3. Soil moisture extraction appeared to be evenly distributed between the topsoil (0 – 30 cm) and subsoil (30 – 60 cm) during the dry season (Table 3). However, inoculated plants (I) extracted more soil moisture (more negative Ψ soil) than the control (C). In the wet season, soil moisture extraction appeared to be mainly from the subsoil layer , indicating that the concentration of absorbing roots were in this layer.

Stomatal conductance (gs) and E were generally higher in the wet season than in the dry season (Table 3). While inoculated *Senna* and cassava plants showed higher gs and E than their uninoculated plants during the dry season, inoculated and uninoculated *Gliricidia* did not show any difference. In the wet season, there were no significant differences in the gs, E and Ψ xylem between inoculated and uninoculated plants. In spite of the differences in the soil water potential at 0 - 30 cm between the dry and wet seasons in *Leucaena* and cassava plants, the values of Ψ xylem in both seasons were similar. However, in *Senna* the lower Ψ soil at this soil depth in the dry season caused more negative Ψ xylem than in the wet season. However, there were no differences in the Ψ xylem values between inoculated and uninoculated plants in either the dry or wet season.

Nutrient concentrations, particularly P, were increased by AM inoculation in cassava and *Gliricidia* (Table 4). In the other hedgerow trees, there was a tendency for higher nutrient concentration in inoculated plants but the effects were not significant until nutrient uptake (in leaves) was considered. This is probably attributed to the dilution effect of greater biomass production in the inoculated plants. In spite of the lack of growth response in inoculated *Senna*, the total uptake of all nutrients in leaves was significantly increased with AM inoculation except for Na.

The contribution of mycorrhizal inoculation is as shown in Table 5. Phosphorus uptake corresponded with *Gliricidia* leaf biomass or cassava root yield, while for *Senna* and *Leucaena* the N uptake corresponded with their leaf biomass.

Discussion

In this study, the yields of inoculated hedgerow trees and cassava were similar to yields in other studies where fertilizer rather than inoculation with AM fungi had been used (Ghosh *et al.*, 1980; Mittal and Singh, 1989; Njoku and Arene, 1980; Sieverding and Howeler, 1985). Although in this cropping system, fertilizers were not applied, the data showed that in alley cropping systems efficient AM fungi may augment the deficiency of nutrients particularly P. This is evident in the data presented in Table 5. The agreements in mycorrhizal contribution between P uptake and leaf biomass for *Gliricidia*, and root yield for cassava highlight the role of AM fungi in increasing crop productivity in an alley cropping system and in tropical soils where P deficiency is common. These findings confirm that *G. deserticolum* is effective for Gliricidia *and* cassava by forming extensive and well distributed mycelium in the soil coupled with extensive colonization of newly formed roots for P uptake as reported for other AM fungi and plants (Abbott and Robson, 1982). Also, these results confirm the mycotrophic nature of these plants, as earlier suggested for cassava (Howeler *et al.*, 1982: 1987) and for *Gliricidia* (Kang and Mulongoy, 1987; Osonubi *et al.*, 1991).

The benefits to be derived from inoculation of a mycorrhizal dependent crop follow from the concentration of AM propagules that can be achieved and the efficiency of the native AM fungi population (Howeler *et al.*, 1987). Dominant indigenous arbuscular mycorrhiza (*G. mosseae) has been* shown to be highly effective for maize plants (Kothari *et al.*, 1991). In this study, the root yield from uninoculated cassava was about 57% of that from inoculated plants, and in *Gliricidia*, leaf biomass of uninoculated plants was about 58% of inoculated plants. These suggest that inoculation with AM fungi is still necessary for cassava and *Gliricidia* plants, to achieve optimum productivity despite the occurrence of indigenous mycorrhiza.
Although, the productivity of the crop is not necessarily related to root colonization by AM fungi, in this study, the root infection data appear to correspond with cassava root dry weight and hedgerow leaf dry weights (Table 2).

It is tempting to speculate that the non-significant effect of leaf biomass with the introduced AM mycorrhiza may be attributed to the available soil P, which may be lower than the threshold or critical level required for efficient functioning of mycorrhizas under *Leucaena* and *Senna* establishment. Similarly, at low soil P, AM isolates have been shown to be ineffective with cassava plants (Howeler *et al.*, 1987).

Except for *Gliricidia*, inoculation of *Senna* and cassava with *G. deserticolum* increased their gs and E during the dry season (Table 3). Although the gs and E of inoculated *Gliricidia* plants were similar to those of uninoculated plants, the sum total of these water relation parameters in the inoculated plants per hectare are bound to be greater than those of the uninoculated plants because of the greater leaf weight (hence greater leaf area) in the inoculated (see Tables 2 and 3). If higher E means higher photosynthesis (Allen and Allen, 1986) then the increased water relation parameters are likely responsible for the greater biomass and better root yield in inoculated plants than the uninoculated plants. In addition, the enhanced gs and E in the inoculated plants during the dry season may have caused the greater extraction of soil moisture (more negative Ψ soil) from the inoculated sub-plots than from the uninoculated sub-plots.

In the present study, there were no clear-cut indications that AM inoculation improved the water relations of both tree hedgerows and alley-cropped cassava except for a few cases during the dry season. Instead, responses to drought stress have been related to species differences. For example, *Gliricidia* and *Senna* came to their peak of gs and E earlier than cassava during the dry season. This might reflect a strategy on the part of the trees to enhance their uptake of CO_2 earlier in the day before drought-stress is at a maximum in the afternoon.

This observation is confirmed with hydraulic conductivity data which became negligible 1-2 hours after peak transpiration in inoculated and uninoculated trees whereas in cassava plants, it continued at similar rates in both inoculated and uninoculated plants throughout the day. Hence, the observed yield differences between AM inoculated and uninoculated tree hedgerows and alley-cropped cassava may not be due to improved water relations per se, but to differences in nutrient uptake observed for other cultivated crops (Nelson and Safir, 1982; Bolgiano *et al.*, 1983).

The similar Ψ xylem in inoculated and uninoculated plants with stomatal closure in the uninoculated without signs of visible wilting during the dry season (Table 3) clearly indicate that the uninoculated trees and cassava can maintain favourable internal water status through stomatal regulation. These results confirm earlier findings by Awotoye *et al.* (1992) and Osonubi *et al.* (1991) on adaptive strategy of preventing drought-stress by non-mycorrhizal *Gliricidia* and *Leucaena*.

Compared to inoculated *Gliricidia*, the plant biomass of *Senna* was not influenced by AM inoculation (Table 2), but total uptake of nutrient and water relation parameters were significantly improved (Tables 3 and 4). This lack of growth response but improved physiological response to AM inoculation is in agreement with previous reports (Awotoye *et al.*, 1992; Augé *et al., 2003;* Pacovsky *et al.*, 1986) and it shows the variation of benefits of AM fungi to plants.

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Table 1: Chemical and physical characteristics of the soil at the experimental sites

		CHEMICAL			EXCHANGEABLE CATIONS					PHYSICAL		
Dept (cm)	pH H_2O 1:1	% Organic C	Total N	Extractable P (mg kg^{-1})	Ca	Mg	Na	K	CEC	% Fine Sand	% Silt	% Clay
0 - 8	6.1	0.48	0.03	1.4	1.20	0.96	0.21	0.11	4.98	68	20	12
8 - 38	6.0	0.52	0.03	1.3	1.64	0.98	0.13	0.10	3.94	82	10	8
38 - 74	5.7	0.28	0.03	2.3	1.04	0.62	0.18	0.07	2.98	64	22	14
74 - 115	5.1	0.06	0.01	0.1	0.80	0.67	0.06	0.05	2.38	64	22	14
115 - 140	4.9	0.06	0.02	0.1	0.34	0.20	0.04	0.01	1.71	60	22	18

Table 2: Percent arbuscular mycorrhizal colonization, dry weights of roots of cassava and above ground biomass of hedgerow woody legumes, 12 months after planting

	Arbuscular mycorrhizal		**Dry weight**		
Plant species	**Treatment**	**Colonisation**	**Leaf**	**Stem**	**Cassava root**
		%	**(t/ha)**		
Cassava	I	76.8a	1.2	3.3	10.4a
	C	50.5b	1.0	2.4	5.9b
Senna	I	54.6a	3.6a	4.7	na
	C	46.8a	1.8ab	2.5	na
Gliricidia	I	70.6a	3.21	6.4	na
	C	19.8a	1.8b	4.3	na
Leucaena	I	57.3a	5.6a	5.9	na
	C	44.1ab	3.8a	2.8	na

I = Inoculated with *G. deserticolum*, C = Control uninoculated plants. Different letters within each plant species indicated significant difference at LSD $P < 0.05$. Absence of letters indicate non-significant difference.

Table 3: Water relations characteristics of cassava and hedgerow woody legumes during dry and wet seasons

Plant species	**Season**	**Inoculation Treatment**	**Ψ soil (Mpa)**		**gs (mmol)**	**E (mmol)**	**Ψ xylem (Mpa)**
			0-30cm	**31-60cm**	**m^{-2} s^{-1}**	**m^{-2} s^{-1}**	
Cassava	**Dry**	I	-0.6b	-1.0b	51a	2.0a	-0.5
		C	-0.4a	-0.4a	0b	0b	-0.7
	wet	I	-0.1*	-0.9	333*	9.4*	-0.5
		C	-0.2	-0.6	343*	6.2*	-0.5
Senna	**Dry**	I	-0.3	-0.4b	64a	0.9a	-2.2
		C	-0.4	-0.2	0b	0b	-2.2
	wet	I	-0.1*	-0.8*	575*	9.5*	-1.8*
		C	-0.1*	-0.8*	569*	7.7*	-1.9*
Gliricidia	**Dry**	I	-0.4	-1.4b	71	1.4	-1.6
		C	-0.4	-0.3a	95	1.7	-1.5
	wet	I	-0.1*	-0.4a	645*	9.7*	-1.7
		C	-0.1*	-0.8b	512	8.8*	-1.5
Leucaena	**Dry**	I	-0.3b	-0.3b	na	na	-0.5
		C	-0.1a	-0.1a	na	na	-0.5
	wet	I	-0.1*	-0.7*	na	na	-0.6
		C	-0.1	-0.7*	na	na	-0.6

I = Inoculated with *G. deserticolum*, C = Control uninoculated. Different letters within each plant species and within a season (dry or wet) indicate significant difference at LSD $P<0.05$. Absence of letters indicated non-significant difference also, means followed by *indicate significant difference between dry and wet seasons. Zero (0) values of gs and E indicated stamatal closure Inoculation.

Table 4: Nutrient concentrations and uptake in leaves of cassava and hedgerow woody legumes, 12 months after planting

Plant Species	Inoculation Treatment	Nutrient concentration (%)						Nutrient uptake in leaves (Mg Plant^{-1})					
		P	N	Ca	Mg	K	Na	P	N	Ca	Mg	K	Na
Cassava	I	0.29*	3.22	1.31	0.22	1.89	0.45	0.48*	4.16	1.66*	0.31	2.42*	0.64
	C	0.16	2.74	1.20	0.18	1.61	0.40	0.20	1.64	1.04	0.16	1.54	0.35
Senna	I	0.14	2.20	1.32*	0.16	0.83	0.15	1.01*	9.42*	15.60*	0.91 *	5.50*	1.15
	C	0.11	2.00	0.81	0.14	0.74	0.13	0.32	7.40	2.50	0.52	2.62	0.50
Gliricidia	I	0.21*	3.73	0.83	0.36	1.95	0.32	1.30*	22.70	5.11	2.10	12.10	2.31
	C	0.15	3.42	1.14	0.38	1.37	0.25	0.90	15.57	4.08	1.80	7.10	1.4
Leucaena	I	0.21	3.64	1.06	0.39	1.97	2.52	2.52	40.20	12.0	4.32*	21.10*	2.80
	C	0.10	3.30	1.01	0.23	1.35	0.77	0.77	25.70	7.81	1.50	9.90	1.90

I = Inoculated with *G. deserticolum*; C = Control uninoculated; * indicate significant difference between the means within a plant species at $P<0.05$.

Table 5: **Contributions of introduced arbuscular mycorrhiza fungus to the total uptake of phosphorus, nitrogen, leaf and stem biomass of the four hedgerow woody leguminous trees and root yield of cassava, 12 months after planting**

	Contribution %				
Plant species	**P**	**N**	**Leaf**	**Stem**	**Cassava Root yield**
Cassava	45	15	16	27	43
Senna	21	9	50	47	na
Gliricidia	29	8	44	33	na
Leucaena	52	9	32	52	na