Soil factors limiting growth and establishment of pigeon pea (*Cajanus cajan* (L.) Millsp. in farmers' fields in the derived savannah of the Benin

K. Aihou[1], N. Sanginga[2], B. Vanlauwe[2], J. Diels[3], R. Merckx[4] and O. Van Cleemput[5]

Abstract

During the last 25 years, there has been a shift of paradigm in soil fertility management in West Africa: the use of agroforestry systems (alley cropping) and herbaceous legumes (e.a. exemplifies the most dominant low input systems, *Mucuna pruriens*). However, once these technologies are exposed to farmers, they do not pass the test of adoption because of lack of an economical product such as grain. Therefore, these technologies are being replaced or combined with optimum rates of mineral fertilizers. Systems including dual-purpose grain such as soybean, cowpea and pigeonpea (*Cajanus cajan* l. millsp.) are currently of interest to farmers because of their ability to restore soil fertility while providing nutritive grains for food and cash. This has a potential in Benin, but it is often limited by different soil factors including nutrient deficiency and soil borne diseases. in order to see the impact of biological fixation, diseases as well as N and P content, a pot experiment in a greenhouse at Ibadan, IITA, was conducted with sterilized (ss) as well as no sterilized (nss) soil collected from different farmers' fields in two villages [Zouzouvou (1°41'E, 6°53'N) and Eglimé (1°40'E, 7°05'N)] in the derived savannah of Benin. Growth of *Cajanus cajan* differs soils from different farmers' fields (ranging from 3.36 to 9.90 g/plant) and was on average higher on the Zouzouvou soils than on the Eglimé soils. Shoot dry weight per plant in no sterilized soil was higher than in sterilized soil. Three types of soils from farmers' fields could be identified according to the response of *Cajanus cajan* to soil sterilization: (i) 42 to 58 % of soils from Eglimé and Zouzouvou had the shoot dry weight higher in the nss than in the ss, (ii) 33 % of soils had a shoot dry weight higher in the ss than in the nss, and (iii) 25 to 42 % of soils showed a non significant difference of the shoot dry weight between the nss and the ss. These results imply that either the symbiotic properties or the root soil borne diseases affected the growth of the plants in the different soils. The role of n and p on plant growth and their interactions with the symbiotic parameters such as nodulation and the arbuscular mycorrhizal fungi (amf) supports the suppression or stimulative effect of *Cajanus cajan* grown in sterilized or no sterilized soils.

Key words: amf root infection, sterilized soils, derived savannah, *Cajanus cajan*, nitrogen, phosphorus, soil-borne diseases.

Facteurs du sol limitant la croissance et l'établissement du pois d'Angole (*Cajanus cajan* (L.) Millsp.) dans les champs des producteurs dans la savane dérivée du Bénin

Résumé

Pendant les 25 dernières années, il y a eu un changement de paradigme dans la gestion de la fertilité de sol en Afrique occidentale : l'utilisation des systèmes agroforestiers (cultures en couloir) et des légumineuses herbacées (par exemple des systèmes avec des apports les plus bas en intrants dominants, *Mucuna pruriens*). Cependant, une fois que ces technologies sont portées à la connaissance des fermiers, ils ne passent pas à l'essai d'adoption à cause du manque d'un produit économique comme les semences. Par conséquent, ces technologies sont remplacées ou combinées avec les taux optimums d'engrais minéraux. Les systèmes incluant les semences à double usage comme le soja, le niébé et le pois d'angole (*Cajanus cajan* l. millsp.) sont actuellement d'un intérêt aux fermiers à cause de leur capacité de restaurer la fertilité du sol tout en procurant des grains nutritifs pour l'alimentation et un revenu monétaire. Cela est un potentiel au Bénin mais c'est souvent limité par différents facteurs incluant la carence en éléments nutritifs et les maladies du sol. Pour mesurer l'impact de la fixation biologique, des maladies aussi bien le taux de N que celui de P, une expérience

[1] Institut National des Recherches AGRICOles du Bénin, B.P. 884 RP, Cotonou, Bénin, E-mail : k_aihou@yahoo.fr

[2] Tropical Soil Biology and Fertility Institute of the International Center for Tropical Agriculture (CIAT), PO BOX 30677, Nairobi, Kenya N.sanginga@cgiar.org; B.vanlauwe@cgiar.org.

[3] International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria J.diels@cgiar.org

[4] Laboratory of Soil Fertility and Soil Biology, Faculty of Agricultural and Applied Biological Sciences, K.U. Leuven, Kasteelpark, Arenberg 20, 3001 Heverlee, Belgium

[5] Laboratory of Physical Chemistry, Faculty of Agricultural and Applied Biological Sciences, Gent University, Coupure 653, B-9000 Gent, Belgium. Oswaldvancleemput@ugent.be;

en pots dans une serre à la station de l'IITA d'Ibadan, a été conduite avec du sol stérilisé (ss) et au sol non stérilisé (nss) collectés dans différents champs des paysans dans deux villages [Zouzouvou (1°41'E, 6°53'N) et Eglimé (1°40'E, 7°05'N)] dans la savane dérivée du Bénin. La croissance de *Cajanus cajan* diffère selon les divers types de sols des paysans (allant de 3,36 à 9,90 g/plant) et présente une valeur moyenne sur les sols de Zouzouvou supérieure à celle des sols d'Eglimé. Le poids sec des pousses par plant sur le sol non stérilisé était supérieur à celui obtenu sur le sol stérilisé. Trois groupes de sols des champs des fermiers peuvent être identifiés selon la réponse de *Cajanus cajan* sur sol stérilisé : (i) 42 à 58 % des sols d'Eglimé et de Zouzouvou ont produit le poids sec de pousses plus élevé dans le nss que dans le ss, (ii) 33 % des sols avaient un poids sec de pousses plus élevé dans le ss que dans le nss et (iii) 25 à 42 % des sols ont un poids sec de pousses avec une différence significative entre le nss et le ss. Ces résultats montrent que les propriétés symbiotiques ou les maladies des racines des plantes portées ont affecté la croissance des plantes dans les différents sols. Le rôle de N et P sur la croissance de la plante et leurs interactions avec les paramètres symbiotiques comme la nodulation et les champignons mycorrhiziens arbusculars ou moisissures (amf) soutient la suppression ou l'effet stimulateur de *Cajanus cajan* cultivé dans des sols stérilisés ou non stérilisés.

Mots clés : Infection racinaire Amf, sols stérilisés, savane dérivée, *Cajanus cajan*, azote, phosphore, maladies des sols.

Introduction

Low-input cropping systems in the 1980-90's have been practiced in marginal soils of the derived savanna zones of the Benin Republic where woody legumes (*Acacia auriculiformis*), alley cropping and herbaceous legumes such as Mucuna (*Mucuna pruriens*) play a role as sources of N to the succeeding or associated cereal crops (Versteeg and Koudokpon, 1993; Kang and Shannon, 2001). However, the transfer of planted fallow technologies to farmers has, overall, not been very successful. Farmers have been willing to invest into these technologies only under very specific circumstances. One example is the “Mucuna technology” in *Imperata cylindrica* infested areas in the derived savanna in the Benin Republic (Manyong and Degand, 1997). However, beside the suppression of weed, this technology has not been widely adopted because of lack of an economical product and loss of one food crop-planting season due to Mucuna.

One of the problem facing farmers in moist savanna in West Africa is the small capacity of their soils to supply the quantity of N required for food production. It was reported that available soil N declined from 0.214 % (0.5 cm depth) to 0.038 % after 4 years of maize cropping systems without Leucaena leucocephala hedgerows. The situation could be alleviated by the use of N fertilizers. But, these are often inaccessible to most resource-poor farmers because of their high cost and inadequate supplies. There is an urgent need for a cheaper and more available alternative source of N of which biological nitrogen fixation by leguminous plants currently presents the best potential way of providing a significant contribution to the maintenance of soil fertility levels (Sanginga *et al*., 1996).

Many researchers have extensively reviewed the importance of N_2 fixation in food, green manure and tree legumes in low input farming systems. Research experiments indicate that potential N_2 fixation is about 200-400 kg $N.ha^{-1}.year^{-1}$. However, studies on farmers' Fields indicate that N_2 fixation is typically well below this potential (20-200 $kg.ha^{-1}.year^{-1}$) because of limitations of nutrients, drought, pests or diseases (Ledgard, 2001). Soil is a limited resource and its health is critical for any sustainable development. Many anthropogenic practices have resulted in soil degradation, and the case of agricultural soils, in the progressive loss of soil fertility. The quality of soils depends not only of its physical or chemical properties, but also on the diversity and activity of its biota (Schreiner and Bethlenfalvay, 1997). Despite the recent activity of various researchers in the moist savanna agro ecological zones, there remain an enormous number of legumes whose capacity to nodulate and fix nitrogen has not been determined (Sanginga *et al.*, 1996). Therefore, there is the need for more detailed studies of N_2 fixation by herbaceous and shrub legumes so as to expand the number of leguminous species, not only those which contribute to soil fertility but also those used as forage for livestock feed and human consumption.

Cajanus cajan is one of the perennial shrub legumes known by farmers as a simultaneous fallow component in their cropping systems. Being a shrubby grain legume it is a source of protein (21 %), and a source of nitrogen in maize-based cropping systems. But like other resource management technologies, the rate of adoption is very low, only 3 % (Honlonkou *et al.*, 1999) because the ability of N_2-fixing plants to establish their symbioses and achieve their potential rates of N_2-fixation is often restricted by environmental constraints (Giller and Cadisch, 1995).

A number of management practices such as soil fumigation and flooding have been employed in efforts to control diseases and weeds. To gain insight into the nature of suppression of diseases, it was sought to describe the changes that these soil treatments induce in the microbial community in order to determine if particular microbial components could be associated with the suppression. To investigate the role of microbial community composition in the suppression of soil borne diseases, it was examined the response of microbial communities in soil treated (Kowalchuk *et al.*, 2003). For comparison, in the present study sterilized and nonsterilized soils were used.

To support and to enhance efforts for the introduction of *Cajanus cajan* into the characteristically low-input smallholder cropping systems and to evaluate the variability in biomass production across farmers' fields, a greenhouse study was conducted. The factors limiting *Cajanus cajan* biomass production were studied.

Material and methods

Site description

Soils used for the greenhouse experiment were collected from twelve (12) farmers' fields in each of the two (2) villages [Zouzouvou (1°41'E, 6°53'N) and Eglimé (1°40'E, 7°05'N)] belonging to the derived savanna ecoregional benchmark area.

The Zouzouvou soils are dominated by 'Terre de barre', which is a red soil, characterized by clay (kaolinite) increasing with depth. These soils are formed on Oligocene/sea-terrace (parental material is sedimentary origin-continental terminal). They are highly weathered up to 10 m deep and well drained, rich in sexquioxide mainly kaolinitic, and characterized by small textural changes within the soil profile. The soil of the site is sandy over sandy clay loam, classified as a Rhodic Ferralsol (FAO, 1991). Soil survey carried out at Zouzouvou classified this soil as Ferralitic Nitisol, according to the World Reference Base for Soil Resources (FAO, ISRIC, ISSS, 1998). The 'Terre de barre' zone covers 5322 km^2, i.e 26.3 % of the derived savanna benchmark area of the Benin Republic.

The Eglimé village is underlain by crystalline basement rocks consisting mainly of granite and gneiss, which give rise to a complex association of Acrisols, Lixisols, Luvisols and Leptosols with inclusions of Vertisols and Cambisols (Faure and Volkoff, 1998). In the basement rock area, the saprolite is often found at shallow depth and the clay fraction contains kaolinite and swelling (2:1) clays in varying proportions depending on parent rock and drainage conditions (Volkoff, 1976; Volkoff and Willaine, 1976). The Eglimé village is located in the 'savane arborée'. This zone covers 9728 km^2, representing 48 % of the derived savanna of the Benin Republic and 20 % of the land in the Mono-Couffo Department. Major crops in both villages are maize, cowpea, groundnut and cotton. A credit scheme for fertilizers and pesticides, and a government-regulated market for selling the produce support cotton production.

Soil sampling and characteristics

The soils of the two villages differed in their physical and chemical characteristics. The principal differences between the Zouzouvou and Eglimé soils were soil organic matter, total phosphorus, calcium and effective cation exchange capacity (ECEC). Their average contents were higher at Eglimé than at Zouzouvou. Soil samples were collected from 12 fields in each village, Zouzouvou and Eglimé, at a depth between 0 and 15 cm. Selected chemical characteristics of these two soils were measured according to IITA analytical procedures (IITA, 1982) and are given in Table 1.

Table 1. Selected soil physical and chemical parameters (0-15 cm) of 12 farmers' fields at Zouzouvou and 12 farmers' fields at Eglimé, derived Savanna of the Southern Benin Republic

	Locations			
	Zouzouvou		Eglimé	
Parameters	Means	Range	Means	Range
Sand (%)	72	51-88	69	50-84
Silt (%)	6	4-8	13	4-20
Clay (%)	22	7-45	17	6-34
pH Water	6.7	6.2-7.1	6.7	6.0-7.2
Total carbon (%)	0.79	0.57-1.03	1.06	0.07-1.93
Total nitrogen (%)	0.06	0.04-0.09	0.08	0.05-0.13
Olsen P (%)	8.06	1.38-22.21	13.30	8.65-22.21
Ca (cmol kg^{-1})	2.80	1.70-4.70	6.78	2.60-14.10
Mg (cmol kg^{-1})	0.94	0.70-1.70	1.65	0.90-3.40
K (cmol kg^{-1})	0.15	0.10-0.30	0.38	0.20-0.60
ECEC (cmol kg^{-1})	4.61	3.40-6.80	9.38	3.80-14.30

Pot experiment

The soil was sterilized by placing 3.1 ml of methylbromide in a cellophane bag containing 5 kg soil. The bag was tightly tied to prevent leakage and kept for 3 days after which it was aerated for one week.

The greenhouse experiment was arranged in a randomised complete block (RCB) design with pots containing 1 kg of non sterilized (NSS) or sterilized soil (SS), collected from previous plots having received 0 or 90 kg $P_2O_5.ha^{-1}$ as rock phosphate. Nitrogen was added as KNO_3 at two levels: 0 mg and 75 mg KNO_3-$N.kg^{-1}$ to pots before planting in 3 replications.

The seeds of *Cajanus cajan* were surface sterilized by rinsing in 95 % ethanol for 10s to remove debris, followed by agitating in 3 % hydrogen peroxide for 3-5 mn after which they were rinsed six times with sterile distilled water. Four seeds were planted and thinned to two seedlings per pot 1 week after emergence.

Plant sampling

Harvesting was done 8 weeks after planting. Shoots were cut at soil level in each pot and weighed. The shoots were sun dried for one week. The roots were placed on a sieve and carefully washed in a gentle stream of tap water before drying in an oven at 80 °C for two days to determine the dry weight. Nodules were detached from the roots, placed in 'Whirl Park' and preserved at 4 °C. Nodules were counted and freshly weighed. About 1 g of roots was taken from each root sample and placed in glass vials for clearing and staining for mycorrhizal colonization rating (Phillips and Hayman, 1970; Giovanetti and Mosse, 1980).

Statistical analysis

Statistical analysis was performed on data collected at harvest using the General Linear Model (GLM) procedure of the SAS system (SAS Institute, 1992). Means were estimated with the Lsmeans option because of the unbalanced data.

Results

Shoot dry weight

On average, for the Zouzouvou soils, the mean shoot dry weight per plant was 22 % higher in the NSS than in the SS (Table 2), but ranged between 5.35 and 9.90 g per plant for the NSS and between 4.08 and 9.40 g per plant for the SS. For the Eglimé soils, the mean shoot dry weight was 4.50 % higher in the NSS than in the SS. It ranged between 4.29 and 6.86 g per plant for the NSS and between 3.36 and 7.99 g per plant for the SS (Table 2).

Two types of plant growth responses were obtained as a result of sterilization. For the Zouzouvou soils, plants on 7 out of the 12 soils had a higher shoot dry weight in the NSS than in the SS, while in the other 5 soils they were not affected by the soil treatment. For the Eglimé soils, three types of plant growth responses were obtained as a result of sterilization. Plants on 5 out of the 12 soils had a higher shoot dry weight in the NSS than in the SS, plants on 4 out of the 12 soils had a higher shoot dry weight in the SS than in the NSS, and plants on 3 out of the 12 soils did not show a significant difference between the NSS and the SS (Table 2).

With N application, the shoot dry weight for the Zouzouvou soils ranged between 5.46 and 9.33 g per plant and between 5.22 and 9.30 g per plant without N application. The mean shoot dry weight increased only slightly (0.87%) compared to the mean shoot dry weight without N application (Table 3). For the Eglimé soils, where N was applied, the shoot dry weight ranged between 4.23 and 6.73 g per plant and between 3.91 and 6.80 g per plant without N. The mean shoot dry weight decreased (1.45 %) compared to the mean shoot dry weight without N application (Table 3).

Table 2. Mean effect of nonsterilized (NSS) and sterilized (SS) soils on Cajanus cajan L. Millsp. growth parameters, 8 weeks after planting: shoot dry weight (sdwt) g/plant; root dry weight (rdwt) g/plant; nodule fresh weight (ndfw) g/plant; nodule number (ndn0)/plant and percentage arbuscular mycorrhizal fungi (% AMF)/plant in 12 soils from farmers' fields at Zouzouvou and at Eglimé

Soil	Locations							
	Zouzouvou				Eglime			
	Non sterilized Soil (NSS)		Sterilized Soil (SS)		Non sterilized Soil (NSS)		Sterilized Soils (SS)	
Parameters	Mean	SE	Mean	SE	Mean	SE	Mean	SE
sdwt	7.64 (5.35-9.90)	0.36	6.28 (4.08-9.40)	0.36	5.60 (4.29-6.86)	0.41	5.36 (3.36-7.99)	0.41
rdwt	1.82 (1.08-2.46)	0.16	1.23 (0.76-1.60)	0.16	0.80 (0.60-1.51)	0.16	1.19 (0.88-1.48)	0.16
ndfw	1.55 (0.70-2.75)	0.19	1.55 (0.48-3.08)	0.19	1.20 (0.56-1.95)	0.16	0.90 (0.13-1.95)	0.16
ndn0	42 (20-95)	4.84	28 (9-41)	4.84	37 (25-55)	10.48	28 (9-48)	11.29
% AMF	27 (22-31)	2.01	11 (9-15)	2.77	30 (25-37)	5.29	14 (10-20)	2.77

Values between brackets are ranges (minimum and maximum)

Table 3. Mean effect of nitrogen (0 mg KNO_3-N.kg$_{-1}$ and 75 mg KNO_3-N.kg^{-1}) on Cajanus cajan L. Millsp. growth parameters, 8 weeks after planting: shoot dry weight (sdwt) g/plant; root dry weight (rdwt) g/plant; nodule fresh weight (ndfw) g/plant; nodules number (ndn0)/plant and percentage arbuscular mycorrhizal fungi (% AMF) per plant in 12 soils from farmers' fields at Zouzouvou and at Eglimé

Nitrogen	Locations							
	Zouzouvou				Eglime			
	0 mg KNO_3-Nkg^{-1}		75 mg KNO_3-N kg^{-1}		0 mg KNO_3-N kg^{-1}		75 mg KNO_3-N kg^{-1}	
Parameters	Mean	SE	Mean	SE	Mean	SE	Mean	SE
sdwt	6.93 (5.22-9.30)	0.39	6.99 (5.46-9.33)	0.32	5.52 (3.91-6.80)	0.45	5.44 (4.23-6.73)	0.73
rdwt	1.41 (0.92-1.88)	0.18	1.63 (1.10-2.21)	0.15	0.94 (0.64-1.64)	0.17	1.04 (0.68-1.52)	0.14
ndfw	1.70 (0.70-3.02)	0.21	1.40 (0.41-2.60)	0.17	1.22 (0.64-2.08)	0.17	0.88 (0.43-1.49)	0.14
ndn0	37 (20-72)	5.31	32 (18-56)	4.33	37 (19-47)	5.80	28 (17-35)	4.73
% AMF	19 (15-25)	2.21	19 (16-21)	1.80	18 (16-25)	3.04	25 (19-33)	2.48

Values between brackets are ranges (minimum and maximum)

The effect of residual rock phosphate on the mean of shoot dry weight per plant showed an increase of 7.3% with the soils of Zouzouvou, while with the Eglimé soils it decreased by 11.8% compared to the soils without residual rock phosphate (Table 4).

With the soils from Zouzouvou, the mean shoot dry weight per plant was significantly related to the mean root dry weight per plant (r = 0.73), to the mean nodule fresh weight per plant (r = 0.84), the mean nodule number per plant (r = 0.87) and the mean residual rock phosphate (r = 0.70). With the soils from Eglimé, the mean shoot dry weight per plant was significantly related to the mean nodule fresh weight per plant (r = 0.90), the mean nodule number per plant (r = 0.74) and the mean residual rock phosphate (r = 0.84) (Table 5).

Table 4: Mean effect of residual rock phosphate (rRP) 0 (RP0) and 90 (RP90) kg $P_2O_5.ha^{-1}$ on Cajanus cajan L. Millsp. growth parameters, 8 weeks after planting: shoot dry weight (sdwt) g/plant; root dry weight (rdwt) g/plant; nodule fresh weight (ndfw) g/plant; nodule number (ndn0)/plant and percentage arbuscular mycorrhizal fungi (%AMF)/plant in 12 soil from farmers' fields at Zouzouvou and at Eglimé

	Locations							
	Zouzouvou				Eglimé			
RRp	rRP0 (0 kg $P_2O_5.ha^{-1}$)		rRP90 (90 kg $P_2O_5.ha^{-1}$)		rRP0 (0 kg $P_2O_5.ha^{-1}$)		rRP90 (90 kg $P_2O_5.ha^{-1}$)	
Parameters	Mean	SE	Mean	SE	Mean	SE	Mean	SE
sdwt	6.86 (4.45-9.49)	0.36	7.36 (5.35-9.31)	0.36	5.82 (4.1-7.66)	0.41	5.13 (3.8-6.23)	0.41
rdwt	1.53 (1.01-1.98)	0.16	1.52 (1.04-2.41)	0.36	0.96 (0.50-1.57)	0.16	1.03 (0.68-1.57)	0.16
ndfw	1.68 (0.73-3.22)	0.19	1.43 (0.51-2.41)	0.19	1.15 (0.56-2.13)	0.18	0.96 (0.55-1.52)	0.16
ndn0	39 (23-72)	4.84	30 (13-57)	4.84	34 (13-48)	5.29	31 (23-45)	5.29
%AMF	16 (13-20)	2.01	22 (16-25)	2.01	24 (19-28)	2.77	21 (17-26)	2.77

rRP: residual rock phosphate **Values between brackets are ranges (minimum and maximum)**

Table 5. Correlation coefficients between shoot dry weight (sdwt), root dry weight (rdwt), nodule fresh weight (ndfw), nodule number (ndn0) and percentage arbuscular mycorrhizal fungi (% AMF) on the growth of Cajanus cajan L. Millsp. on soils from farmers' fields at Zouzouvou (n=12) and Eglimé (n=12)

Locations	Zouzouvou					Eglimé				
	% AMF	sdwt	rdwt	ndfw	ndn0	% AMF	sdwt	rdwt	ndfw	ndn0
% AMF	-	ns	ns	ns	ns	-	ns	ns	ns	ns
sdwt	ns	-	0.73**	0.84**	0.87**	ns	-	ns	0.90**	0.74**
rdwt	ns	0.73**	-	0.64*	ns	ns	ns	-	ns	ns
C	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P	ns	0.70*	0.58*	0.72*	ns	ns	0.84**	ns	0.80**	0.67**
pH	ns	ns	ns	ns	ns	ns	ns	ns	Ns	ns

*** significant at p < 0.05;** **** significant at p < 0.01;** **ns: non significant**

Root dry weight

The mean root dry weight per plant was 48 % higher in the NSS than in the SS with the Zouzouvou soils and ranged between 1.08 to 2.46 g per plant in the NSS and between 0.76 to 1.60 g per plant in the SS. However, with the Eglimé soils, the mean root dry weight was 49 % higher in the SS than in the NSS. It ranged between 0.60 and 1.51 g per plant in the NSS and between 0.88 and 1.48 g per plant in the SS (Table 2). With N application on the Zouzouvou and Eglimé soils, the mean root dry weight per plant increased by 16 % and 11 %, respectively (Table 3). In the treatment with residual rock phosphate no significant difference was observed between the mean root dry weight of the P treated and non-treated Zouzouvou soils. The mean root dry weight increased by 7.30 % by the residual rock phosphate in the Eglimé soils (Table 4).

Nodulation

The mean nodule fresh weight per plant ranged between 0.70 and 2.75 g in the NSS and between 0.48 and 3.08 g in the SS of Zouzouvou. No significant difference was observed between the NSS and the SS. With the Eglimé soils, it ranged between 0.56 and 1.95 g in NSS and between 0.13 and 1.95 g in the SS. The mean value was higher in the NSS than in the SS (Table 2). The nodule number was clearly higher by non-sterilization than by sterilization in both the Zouzouvou and Eglimé soils.

When N was applied, it was observed that the mean nodule fresh weight and the nodule number decreased in both the Zouzouvou as the Eglimé soils (Table 3). The residual rock phosphate decreased slightly the nodule fresh weight and the nodule number per plant in the soils at both locations (Table 4).

The means of nodule fresh weight per plant were significantly related to the residual rock phosphate, respectively for the soils from

Zouzouvou (r = 0.72) and those from Eglimé (r = 0.80) (Table 5).

Arbuscular Mycorrhizal Fungi (AMF)

In the soils from Zouzouvou, the AMF decreased by 59 % due to sterilization. On average, it ranged between 22 and 31 % in the NSS and between 9 and 15 % in the SS. In the soils from Eglimé, the AMF decreased by 53 % in the SS compared to the NSS and it ranged between 25 and 37 % in the NSS and between 10 and 20 % in the SS (Table 2). In the soils from Zouzouvou, the AMF response to N application was not significant. While the mean of AMF increased by 39 % compared to the control in the Eglime soils (Table 3). The AMF response to residual rock phosphate in the soils of Zouzouvou was significant and increased by 38 % compared to the control. In the soils of Eglimé, the mean AMF decreased by 12.5 % compared to the control (Table 4).

Discussion

The shoot dry weight per plant was 4.5 % higher in the NSS than in the SS for the soils from Eglimé, and 22 % for the soils from Zouzouvou. The results also showed that with Eglimé soils, the root dry weight was higher in the SS than NSS. The arbuscular mycorrhizal fungi (AMF) were clearly higher in the NSS than in the SS of both locations. This implies that in the SS the symbiotic microorganisms were suppressed, inhibiting the development of plant growth. On the other hand, in some farmers' fields growth in the SS was higher than in the NSS. This could be due to the suppression of root soil borne diseases in some of these soils. Sanginga et al. (1996) reported that large amounts of N_2 fixation were found when conditions are favorable for growth and biomass production. He pointed out that this should be the first criteria for maximizing N_2 fixation in most savanna cropping systems. Gibson *et al.* (1977) have reported that the deleterious effect of viral, fungal and bacterial pathogens with insects and nematodes may interfere with plant growth and its ability to fix nitrogen. With *Cajanus cajan* extensive damage to root nodules caused by *Rivellia angulata* has been reported by Nene et al. (1990). They reported also that the extent of nodule damage is greater in *Cajanus cajan* grown in Vertisols than in Alfisols. This finding confirms the results obtained with the Eglimé soils, where the mean nodule fresh weight per plant was 25% lower in the SS than in the NSS.

N application had no significant effect on shoot dry weight. However, the mean root dry weight showed a substantial increase. The arbuscular mycorrhizal fungi (AMF) increased with N application on the Eglimé soils and decreased with the Zouzouvou soils. Buwalda and Goh (1982) reported that N fertilization reduced the infection of AMF from 56 % (with no N applied) to 36 % with NH^{4+}-fed plants and to 38 % with NO^{3-}-fed plants. Shivcharn and Ampornpan (1992), otherwise, reported that N fertilization induces the level of root colonization, or that colonization may be suppressed or inhibited. The nodule number decreased in the soils of both locations when N was applied. Inorganic N sources are widely reported to decrease the nodulation of legumes and to reduce N_2 fixation (Ayanaba and Dart, 1977; Franco, 1977). The degree of inhibition achieved depends on many factors including the concentration and form of N, the time of application and also the host plant and bacterial strain used. According to Kumar Rao and Dart (1981), with *Cajanus cajan*, both nodulation and nitrogenase activity are depressed by soil N concentrations higher than 25 mg $N.kg^{-1}$ as NO^{3-}.

The results showed that there was no significant effect of residual P on the studied parameters, except the effect of P on % AMF in the Zouzouvou soils. The mechanism causing reduced mycorrhizal colonization upon P fertilization are not fully understood, but seem to be due to an enhanced P concentration in the plant tissue (Jooner, 2000). Phosphorus is normally the most limiting growth nutrient of leguminous crops in tropical and subtropical regions. This particularly applies to soils of high iron or aluminium oxide content where P is strongly bound and largely unavailable for crop uptake. The weak response of *Cajanus cajan* to P application in an Alfisol suggests that *Cajanus cajan* was able to efficiently utilize iron-bound P (Fe-P). It was found that *Cajanus cajan* can acquire P from Alfisols by excreting an organic acid and its derivates which can specifically chelate Fe ligands (Ae *et al.*, 1990).

Arbuscular mycorrhizal fungi (AMF) contribute to soil structure and nutrient cycling. However, agricultural practices like tillage, crop sequence, plant breeding, use of fertilizers, and pesticides may alter the AMF population, species and root colonization (Ana *et al.*, 2001).

The level of P fertilization at which AMF development and activity are best enhanced depends on the levels of available P to both plant and AMF in the soil, the host plant, the AMF species and the soil environment (Lee and Wani, 1991). These authors reported that previously applied rock phosphate has a residual value. In addition, it does not reduce the level of AMF infection as soluble fertilizers do.

The addition of rock phosphate significantly increased the arbuscular mycorrhizal fungi (AMF) infection of *Mucuna* (from 24 to 33 %) and Lablab roots (from 15 to 28 %) to a similar extent at all fields tested (Vanlauwe *et al.*, 2000). These authors suggested that this increased AMF infection was likely caused by specific processes in the rhizosphere of the legumes as AMF infection of the maize roots (8 %) was not affected by rock phosphate addition.

The results show that P fertilizers reduced the AMF less importantly than did N. There have been many other studies showing that high concentrations of P fertilizers can reduce the intensity of AMF colonization (Thompson, 1991). Acute deficiency of P can prevent nodulation by legumes. Phosphorus and sulphur are required for nodule metabolism and tend to be concentrated in the nodules when the plant is deficient in these nutrients (Giller, 2001). It has been suggested that, as nodulated plants often have a less well-developed root system than non-nodulated plants, the ability of nodulated plants to capture nutrients, particularly P, is decreased (Giller, 2001).

At both locations, cotton is produced with governmental support and allocation of credit to the farmers to purchase inputs. Intensified cotton cropping is often accompanied by an increased reliance on chemicals for pest and disease control. It was reported that some herbicides decrease rhizobial growth, nodulation, nitrogen fixation or yield of legumes (Slattery *et al.*, 2001). The supply of adequate levels of essential nutrients is fundamental for the efficient functioning of all legume symbioses. In many regions legume crops and pastures are grown on nutrient deficient soils, and sustaining agricultural productivity on these soils requires the efficient management of the limited nutrient reserves and the appropriate use of fertilizers (O'Hara *et al.*, 2001).

Conclusion

In the studied area it was observed that three major constraints (N, P and soil borne diseases) could limit the growth and establishment of *Cajanus cajan*. N application did not have a large effect on the shoot dry weight. However, N deficiency reduced the percentage of AMF. This was more acute in soils from farmers' fields at Eglimé than at Zouzouvou. N application reduced the nodule number and the nodule fresh weight.

The shoot dry weight and the percentage of AMF were affected by P deficiency. This deficiency was more prevalent in the Zouzouvou soils than in the Eglimé soils. The results show that *Cajanus cajan* roots could be subjected to soil borne diseases and insects. This could affect not only the plant-soil system nutrition, but also the soil biota and could hamper *Cajanus cajan* growth.

Balanced plant nutrition in a rotational cropping system, associated with symbiotic properties, suitable varieties and an integrated pest control management could be a sustainable means to improve *Cajanus cajan* growth and production in the derived savanna of the Benin Republic.

Reference

Ae N., Arihara J., Okada K., Yoshira T. and Johansen C., 1990. Phosphate uptake by *Cajanus cajan* and its role in cropping systems of the Indian Subcontinent. Science 248, 477-480.

Ana B., Merendez J.M., Scervino A. and Godes M., 2001. Arbuscular mycorrhizal populations associated with natural and cultivated vegetation on a site of Buenos Aires province Argentina. Biology Fertility Soil 33, 373-381.

Ayanaba A.P. and Dart, J., 1977. Biological Nitrogen fixation in farming systems of the Tropics. Proceeding of a Symposium held at the International Institute of Tropical Agriculture. Ibadan, Nigeria, October 1975, John Wiley & Sons 'Chischester'.

Buwalda J.G. and Goh K.M., 1982. Host-Fungus competition for carbon as a cause of Growth Depression in Vesicular Arbuscular Mycorrhizal RYEGRASS. Soil Biology & Biochemistry 14, 103-106.

FAO, 1991. World Soil Resources: An Explanatory Note on the FAO World Resources Map at 1:25000000 Scales. Food and Agriculture Organization of the United Nations, Rome, Italy, 58 p.

FAO/ISRIC/ISSS, 1998. World ReferenceBase for Soil Resources, World Soil Resources Report Nr 84. Food and Agriculture Organisation of the United Nations, Rome, Italy.

Faure P. and Volkoff B., 1998. Some factors affecting regional differentiation of the Soils in the Republic of Benin (West Africa). Catena 32, 281–306.

Franco C.A., 1977. Contribution of the legume Rhizobium symbiosis to the ecosystem and food production. In: Vincent, J.M., Whitney, A.S. and Bose, J., Eds. Exploiting the Legume Rhizobium Symbiosis in Tropical Agriculture. University of Hawaii, Hawaii, USA, pp. 237–252.

Gibson A.H., 1977. The influence of the environment and managerial practices on the legume-Rhizobium symbiosis. In: Hard R.W.F. and Gibson A.H. (Eds). A Treatise on Dinitrogen Fixation-Section IV: Agronomy and Ecology. John Wiley and sons, New York, USA, pp. 393–450.

Giller K.E., 2001. Nitrogen fixation in Tropical cropping system, 2nd Edition. CAB International Wallingford, UK.

Giller K.E. and Cadisch G., 1995. Future benefits from biological nitrogen fixation: An ecological approach to agriculture. Plant and Soil 174, 255-277.

Giovanneti M. and Mosse B., 1980. An evaluation of methods for measuring vesicular-arbuscular infection in roots. New Phytolologist 84, 489–500.

Honlonkou A.N., Manyong V.M. and Nguessan T., 1999. Farmers' perception and the dynamics of adoption of a resource management technology: The case of Mucuna fallow in southern Benin, West Africa. International Forestry Review 1 (4).

IITA (International Institute of Tropical Agriculture)., 1982. Automated and semi-automated methods for soil and plant analysis Manuel series n°7, p 33, IITA. Ibadan, Nigeria.

Jooner E.J., 2000. The effect of long-term fertilization with organic or inorganic fertilizers on mycorrhiza-mediated phosphorus uptake in Subterranean clover. Biology and Fertility of Soils 32, 435-440.

Kang B.T. and Shannon D.A., 2001. Agroforestry with Focus on Alley Cropping. In: Tian, G., Ishida, F. and Keatinge, D., Eds. Sustaining soil fertility in West Africa. Soil Science Society of America and America Society of Agronomy, Special Publication Nº 58, pp. 197-224.

Kowalchuk G.A., Os G.H.V., Aartrijk J.V. and Veen, J.A.V., 2003. Microbial community responses to disease management soil treatments used in flower bulb cultivation. Biology and Fertility of Soils 37, 55-63.

Kumar Rao, J.V.D.K. and Dart P.J., 1981. Effect of different plant growth media on nodulation, growth and nutrient uptake of pigeon pea. In: Proceedings of the International Workshop on Pigeon peas, volume 2, 15-19 December 1980, ICRISAT Center, Patancheru, A.P., India: ICRISAT, pp. 403-408.

Ledgard S.F., 2001. Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. Plant and Soil 228, 43–59.

Lee, K.K. and Wani S.P., 1991. Possibilities for Manipulating Mycorrhizal Association, In: Johansen, C., Lee, K.K. and Sahrawat, K.L., Eds. Phosphorus Nutrition of Grain Legumes in the Semi-Arid Tropics. International Crops Research for the Semi-Arid Tropics, Patancheru, India. pp. 107-116.

Manyong V.M. and Degand J., 1997. Measurement of the sustainability of African smallholder farming systems: Case study of a systems approach. IITA Research 14/15: 1–6.

Nene Y.L., Susan D.H. and Sheila V.K., 1990. The Cajanus cajan. CAB International. International Crops Research Institute for the semi-Arid Tropics (ICRISAT), Patancheru, India.

O'Hara G.W., 2001. Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation: A review. Australian Journal of Experimental Agriculture 41, 417–433.

Phillips J.M. and Hayman D.S., 1970. Improved procedure for cleaning roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycology Society 55, 158-161.

Sanginga N., Wirkom L.E., Okogun A., Akobundu I.O., Carsky R.J. and Tian G., 1996. Nodulation and estimation of symbiotic nitrogen fixation by herbaceous and shrub legumes in Guinea savanna in Nigeria. Biology and Fertility of Soils 23, 441-448.

SAS (Statistical Analysis System), 1992. The General Linear Model Procedure In: SAS Technical Report. SAS/STAT Software changes and Enhancements. SAS Institute Inc, Cary NC, USA.

Schreiner R.P. and Bethlenfalvay G.J., 1997. Mycorrhizae, biocides and biocontrol. 3. Effect of three different fungicides on developmental stages of three AM fungi. Biology and Fertility of Soils 24, 18-26.

Shivcharn S.D. and Ampornpan L., 1992. The influence of inorganic nutrient fertilization on the growth, nutrient composition and vesicular-arbuscular mycorrhizal colonization of pretransplant rice (Oryza sativa L.. plants. Biology and Fertility of Soils 13, 85–91.

Slattery J.F., Coventry D.R. and Slattery W.J., 2001. Rhizobial ecology as affected by the soil environment. Australian Journal of Experimental Agriculture 41, 289–298.

Thompson J.P., 1991. Improving the Mycorrhizal Condition of the Soil through Cultural Practices and Effects on Growth and Phosphorus Uptake by plants. In: Johansen, C., Lee, K.K., Sahrawat, K.L., Eds. Phosphorus Nutrition of Grain Legumes in the Semi-Arid Tropics. International Crops Research Institute for the Semi-Arid Tropics.

Vanlauwe B., Nwoke O.C., Diels J., Sanginga N., Carsky R.J., Deckers J. and Merckx R., 2000. Utilization of rock phosphate by crops on representative toposequence in the northen Guinea savannah zone of Nigeria: response by Mucuna pruriens, Lablab purpureus and maize. Soil Biology and Biochemistry 32, 2063-2077.

Versteeg M.N. and Koudokpon V., 1993. Participative farmer testing of four low external input technologies to address soil fertility decline in Mono Province (Benin. Agricultural Systems 42, 265-276.

Volkoff B., 1976. Carte pédologique de Reconnaissance de la République Populaire du Bénin à 1/20000 Feuille d'Abomey (2. Notice explicative N°66(2). ORSTOM, 40 pp.

Volkoff B. et William P., 1976. Carte Pédologique de Reconnaissance de la République Populaire du Bénin à 1/20000 Feuille de Porto-Novo (1). Notice explicative N° (66) 1. ORSTOM, 39 p.