

Occurrence of nodulation in unexplored leguminous trees native to the West African tropical rainforest and inoculation response of native species useful in reforestation

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Summary

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- Despite the abundance and diversity of timber tree legumes in the West African rainforest, their ability to form nitrogen-fixing nodules in symbiosis with rhizobia, and their response to rhizobial inoculation, remain poorly documented.
- In the first part of this study the occurrence of nodulation was determined in 156 leguminous species growing in six natural forest areas in Guinea, mostly mature trees. In the second part, an *in situ* experiment of rhizobial inoculation was performed on eight selected tree species belonging to three genera: *Albizia*, *Erythrophleum* and *Millettia*.
- Of the 97 plant species and 14 genera that had never been examined before this study, 31 species and four genera were reported to be nodulated. After 4 months of growing in a nursery and a further 11 months after transplantation of plants to the field, we observed a highly significant ($P < 0.001$) and positive effect of inoculation with *Bradyrhizobium* sp. strains on the growth of the eight tree species tested.
- The importance of determining the nodulation ability of unexplored local trees and subsequently using this information for inoculation in reforestation programmes was demonstrated.

Key words: *Albizia*, *Bradyrhizobium*, *Erythrophleum*, high-value timber, *Millettia*, natural forest, nitrogen-fixing trees.

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Introduction

The Leguminosae is the most represented botanical family in terms of specific diversity among trees of the West African natural rainforest, as recorded in the Côte d'Ivoire where 26% of all commercial timber species are legumes (Dupuy *et al.*, 1997). A large majority (75%) of the timber legume species present in the Guinean rainforest, as observed in our study, are represented throughout all humid zones of West and Central Africa (Lock, 1989). Due to their nitrogen-fixing symbiosis

with rhizobia, many native leguminous trees could play an important role in the restoration of N-depleted soils, and might be used as priority pioneer species for the rehabilitation of degraded and overexploited rainforests. However, despite their abundance, diversity and economic importance as high-value timber species in West Africa, very few have been observed for their ability to nodulate and fix atmospheric N symbiotically with rhizobia (Allen & Allen, 1981; de Faria *et al.*, 1989; Wester & Högberg, 1989). Such unexplored symbiotic associations putatively include a high genetic diversity

of associated microorganisms, as shown by recent findings describing new species of rhizobia or new genera of legume-nodulating bacteria (Chen *et al.*, 2001; Moulin *et al.*, 2001; Sy *et al.*, 2001). It is considered that only 20% of the known species of Leguminosae worldwide have been examined for nodulation (Sprent, 1995). Nevertheless, studies on nodulation and N-fixing status of tree species are more advanced in other tropical humid regions, especially in the Amazonian basin (Norris, 1969; Bradley *et al.*, 1980; de Faria *et al.*, 1989; Moreira *et al.*, 1992; de Faria & de Lima, 1998; Guehl *et al.*, 1998; Moreira *et al.*, 1998; Roggy & Prévost, 1999). The leguminous woody species found in the latter region are also predominant, representing 40% of the total phytomass (Puig *et al.*, 1990) as well as the most diversified botanical family (Sabatier & Prévost, 1990).

In this context, and because of the lack of information on the N-fixing status of most legume trees native to the West African rainforest, a large and systematic survey of the occurrence of nodulation was carried out on all these species encountered in six different natural forest areas in the south of Guinea. In the second part of our study, the N₂-fixing potential of some of the species surveyed, which were chosen for their economic interest, good silvicultural potential and profuse seed production, was evaluated after isolation of rhizobial isolates from the nodules collected in the different sites. Thus, in order to test the effect of rhizobial inoculation on plant growth, a two-step *in situ* experiment set up in the nursery and in the field was performed on eight species belonging to three different genera: *Albizia adianthifolia*, *Albizia altissima*, *Albizia ferruginea*, *Albizia zygia*, *Erythrophleum guineensis*, *Erythrophleum ivorensis*, *Millettia rhodantha* and *Millettia zechiana*.

Materials and Methods

General survey of nodulation status of leguminous trees, shrubs and vines in natural conditions

The survey of nodulation was performed in six different stations of natural forest in the south-eastern part of Guinea: Béro, Diécké, Monts Nimba, Ziama, Pic de Fon and N'zérékoré. All these stations are located in the climatic zone of the humid dense forest from 7°30' to 9°30' latitude, with annual rainfall varying from 1800 to 2200 mm. The total area of these protected forests varies from 13 000 to 120 000 ha, and the altitude from 400 to 1300 m.

Before the field survey an exhaustive list of legume species was prepared from those described in a relevant flora (Adam, 1971). The general taxonomy used in this study follows Polhill & Raven (1981), although it was recently modified at the tribe level by Bruneau *et al.* (2001). The field observations were carried out without any particular sampling protocol, and roots of the targeted species were systematically observed for nodulation when encountered. Herbarium specimens

represented by whole plants or different plant samples were collected and are now kept in the herbarium of the Institut de Recherche Agronomique de Guinée in Seredou.

Effect of rhizobial inoculation on the growth of *Albizia*, *Erythrophleum* and *Millettia* spp. in nursery and field conditions

Isolation of bacterial strains Nodules were collected from mature *A. adianthifolia*, *A. ferruginea*, *A. zygia*, *E. guineensis*, *M. rhodantha* and *M. zechiana* growing in the natural forests of the Ziama reservation (details in Table 1). After 1 month of storage in tubes containing silica gel, the nodules were rehydrated by immersion for 30 min in sterile water, surface sterilized in 30% H₂O₂ for 10 min and rinsed 10 times in sterile water before being crushed. The crushed nodules were transferred into Petri dishes containing a yeast extract mannitol medium (Vincent, 1970) for bacterial isolation. After incubation at 28°C for 2 wk and several purification subcultures on the same culture medium, seven rhizobial isolates were finally obtained: STM 916 from *A. adianthifolia*; STM 922 from *A. ferruginea*; STM 923 from *A. zygia*; STM 934 from *E. guineensis*; STM 931 from *M. rhodantha*; and STM 851 and STM 926 from *M. zechiana*. These different bacterial isolates formed N-fixing nodules after inoculation to *Macroptilium atropurpureum* in *in vitro* monoxenic conditions of culture (Trinick *et al.*, 1991) and were confirmed as *Bradyrhizobium* sp. strains in previous experiments (Munive, 2002).

Plant material Seeds of *A. adianthifolia*, *A. altissima*, *A. ferruginea*, *A. zygia*, *E. guineensis* [= *suaveolens* (Guill. & Perr.) Brenan], *E. ivorensis*, *M. rhodantha* and *M. zechiana* were collected from adult trees grown naturally in Ziama reservation area. Each of the three genera represented here belong to a different subfamily of the Leguminosae: *Albizia* to Mimosoideae; *Millettia* to Papilionoideae; *Erythrophleum* to Caesalpinoideae. The germination pretreatment consisted of immersing the seeds in warm tapwater followed by soaking for 6 h.

Inoculation procedure and plant growth conditions in nursery

Inoculation of plants was performed in the experimental nursery of Centre de Recherche Agronomique de Seredou located within the Ziama natural forest area. At the end of the dry season in early January 2001, seeds were directly sown into 12 × 17 cm (diameter × height) polybags filled up with nonsterile forest topsoil from Ziama. The germination of *Albizia*, *Millettia* and *Erythrophleum* species occurred 1, 2 and 3 wk after sowing, respectively. All plants were inoculated 1 month after sowing. The bacterial inoculants consisted in 7-d-old pure rhizobial cultures grown on yeast mannitol medium (Vincent, 1970), and each plant received 1 ml culture containing 10⁹ cells. Except for *M. zechiana* and *A. altissima*, which were inoculated with two and three different strains of *Bradyrhizobium* sp., respectively, each plant species was inoculated

Table 1 Observation of nodulation in leguminous species of the primary rainforest in Guinea (West Africa)

Subfamily, tribe, species	Plant habit; height (m)	Location	Nodulation	Newly observed species†§	Newly observed genus†
CAESALPINIOIDEAE					
Caesalpinieae					
<i>Bussea occidentalis</i> Hutch.	T; 25	Ziama	–	Yes	Yes
<i>Chidlowia sanguinea</i> Hoyle	T; 25	Ziama	+	Yes	Yes
<i>Delonix regia</i> (Hook.) Raf.	T; 12	Nimba	–	No*	No
<i>Erythrophleum ivorense</i> A. Chev.	T; 40	Ziama	+	No**	No
<i>Erythrophleum suaveolens</i> (Guill. & Perr.) Brenan	T; 40	Béro	+	No**	"
<i>Mezoneuron benthamianum</i> Baillon	S; 5	Diécké	–	Yes	No
Cassieae					
<i>Cassia aubrevillei</i> Pellegr.	T; 15	Nimba	–	Yes	No
<i>Cassia sieberiana</i> DC.	T; 15	Béro	–	Yes	"
<i>Chamaecrista kirkii</i> (Oliver) Standley	H; 1.5	Nimba	–	No**	No
<i>Chamaecrista mimosoides</i> (L.) Greene	H; 1.5	Nimba	+	No**	"
<i>Dialium aubrevillei</i> Pellegr.	T; 30	Ziama	–	Yes	No
<i>Dialium dinklagei</i> Harms	T; 20	Diécké	–	Yes	"
<i>Dialium guineense</i> Willd.	T; 15	Béro	–	No*	"
<i>Dialium pobeguinii</i> Pellegr.	T; 15	Ziama	–	Yes	"
<i>Distemonanthus benthamianus</i> Baillon	T; 35	Ziama	–	Yes	Yes
<i>Duparquetia orchidacea</i> Baillon	S; 8	Nimba	–	Yes	Yes
<i>Senna alata</i> (L.) Roxb.	S; 5	Diécké	–	No*	No
<i>Senna occidentalis</i> (L.) Link	H; 1.5	Nimba	–	No*	"
<i>Senna podocarpa</i> (Guill. & Perr.) Lock	S; 5	Nimba	–	Yes	"
<i>Senna siamea</i> (Lam.) Irwin & Barneby	T; 20	Béro	–	No*	"
Cercideae					
<i>Bauhinia thonninguui</i> Schum.	T; 9	Pic de Fon	–	Yes	No
<i>Griffonia simplicifolia</i> (DC.) Baillon	S; 5	N'zérékore	–	Yes	Yes
Detarieae					
<i>Azelia africana</i> Pers.	T; 30	Béro	–	No*	No
<i>Azelia bella</i> var. <i>gracilior</i> Keay	T; 35	Ziama	–	Yes	"
<i>Copaifera salikounda</i> Heckel	T; 30	Ziama	–	Yes	No
<i>Daniellia ogea</i> (Harms) Holland	T; 30	Béro	–	Yes	No
<i>Daniellia thurifera</i> Bennett	T; 35	Ziama	–	Yes	"
<i>Detarium heudelotianum</i> Baillon	T; 20	Nimba	–	Yes	No
<i>Detarium macrocarpum</i> Harms	T; 25	Diécké	–	No*	"
<i>Detarium senegalense</i> J. F. Gmel.	T; 25	Béro	–	Yes	"
<i>Guibourtia copallifera</i> Bennett	T; 25	Ziama	–	Yes	No
<i>Guibourtia ehie</i> (Chev.) J. Léonard	T; 30	Ziama	–	Yes	"
<i>Guibourtia leonensis</i> J. Léonard	T; 30	Diécké	–	Yes	"
<i>Tessmannia baikiaeoides</i> Hutch. & Dalz.	T; 10	Nimba	–	Yes	Yes
Amherstieae					
<i>Anthonotha crassifolia</i> J. Léonard	T; 20	Béro	–	Yes	No
<i>Anthonotha fragrans</i> (Baker f) Exell & Hillc.	T; 30	Ziama	–	No*	"
<i>Anthonotha macrophylla</i> P. Beauv.	T; 12	Diécké	–	Yes	"
<i>Cryptosepalum tetraphyllum</i> (Hook. f) Benth.	T; 25	Ziama	–	Yes	No
<i>Gilbertiodendron bilineatum</i> (Hutch. & Dalz.)	T; 15	Ziama	–	Yes	No
<i>Gilbertiodendron limba</i> (Scott Elliot) J. Léonard	T; 15	Ziama	–	Yes	"
<i>Paramacrolobium coeruleum</i> (Taub.) J. Léonard	T; 30	Ziama	–	No**	No
<i>Pelligriniodendron diphyllum</i> (Harms) J. Léonard	T; 20	Ziama	–	Yes	Yes
MIMOSOIDEAE					
Parkieae					
<i>Parkia bicolor</i> A. Chev.	T; 30	Nimba	–	Yes	No
<i>Parkia biglobosa</i> (Jacq.) Don	T; 15	Béro	–	No**	"
<i>Pentaclethra macrophylla</i> Benth.	T; 25	Ziama	+	No*	No
Mimoseae					
<i>Adenopodia scelerata</i> (A. Ch.) Brenan	V; 30	Nimba	–	Yes	Yes
<i>Aubrevillea kerstingii</i> (Harms) Pellegr.	T; 30	Ziama	–	Yes	Yes
<i>Aubrevillea platycarpa</i> Pellegr.	T; 30	Ziama	+	Yes	"
<i>Calpocalyx aubrevillei</i> Pellegr.	T; 25	Ziama	+	Yes	No
<i>Calpocalyx brevibracteatus</i> Harms	T; 25	Ziama	–	Yes	"
<i>Dichrostachys cinerea</i> (L.) Wight & Arn	S; 8	Béro	+	No**	No
<i>Entada africana</i> Guill. & Perr.	T; 12	Béro	+	Yes	No

Table 1 Continued

Subfamily, tribe, species	Plant habit; height (m)	Location	Nodulation	Newly observed species‡§	Newly observed genus‡
<i>Entada gigas</i> (L.) Fawc. & Rend.	V; 30	Diécké	+	Yes	"
<i>Entada mannii</i> (Oliv.) Tisser.	S; 15	Ziama	+	Yes	"
<i>Mimosa invisus</i> Collad.	S; 0.5	Diécké	+	No**	No
<i>Newtonia aubrevillei</i> (Pellegr.) Keay ssp. <i>aubrevillei</i>	T; 30	Ziama	–	Yes	No
<i>Newtonia duparquetiana</i> (Baillon) Keay	T; 25	Nimba	–	Yes	"
<i>Piptadeniastrum africanum</i> (Hook. f.) Brenan	T; 40	Ziama	+	No**	No
<i>Tetrapleura tetraptera</i> (Schum. & Thonn.) Taub.	T; 20	Ziama	+	Yes	Yes
<i>Xylocarpus evansii</i> Hutch.	T; 25	Diécké	+	Yes	No
Ingeae					
<i>Albizia adianthifolia</i> (Schum.) W. F. Wright	T; 20	Diécké	+	No**	No
<i>Albizia altissima</i> Hook. f.	T; 20	Ziama	+	Yes	"
<i>Albizia dinklagei</i> (Harms) Harms	T; 20	Ziama	+	Yes	"
<i>Albizia ferruginea</i> (Guill. & Perr.) Benth.	T; 20	Béro	+	No**	"
<i>Albizia glaberrima</i> (Schum. & Thonn.) Benth.	T; 15	Nimba	+	No**	"
<i>Albizia zygia</i> (DC.) J. f. Macb.	T; 20	Ziama	+	No**	"
PAPILIONOIDEAE					
Swartzieae					
<i>Bobgunnia fistuloides</i> (Harms) J.H. Kirkbr. & Wiersema	T; 15	Diécké	–	Yes	No
Sophoreae					
<i>Amphimas pterocarpoides</i> Harms	T; 25	Ziama	+	Yes	Yes
<i>Angylocalyx oligophyllus</i> (Baker) Baker f.	S; 4	Nimba	–	Yes	Yes
<i>Baphia nitida</i> Lodd.	T; 10	Nimba	+	No**	No
<i>Baphia cappariifolia</i> ssp. <i>polygalacea</i> Brummit	T; 8	Diécké	+	Yes	"
Dalbergieae					
<i>Dalbergia afzeliana</i> G. Don	V; 35	Nimba	–	Yes	No
<i>Dalbergia albiflora</i> Hutch. & Dalz. ssp. <i>albiflora</i>	V; 15	Ziama	–	Yes	"
<i>Dalbergia bignoniae</i> Berhaut	V; 25	Béro	+	Yes	"
<i>Dalbergia dalzielii</i> Hutch. & Dalz.	V; 6	Diécké	–	Yes	"
<i>Dalbergia hostilis</i> Benth.	V; 15	Ziama	+	Yes	"
<i>Dalbergia oblongifolia</i> G. Don	S; V; 10	Diécké	–	Yes	"
<i>Dalbergia rufa</i> G. Don	S; V; 15	Diécké	–	Yes	"
<i>Dalbergia saxatilis</i> Hook. f.	S; V; 20	Nimba	–	Yes	"
<i>Pterocarpus erinaceus</i> Poir.	T; 15	Béro	+	No**	No
<i>Pterocarpus mildbraedii</i> Harms ssp. <i>mildbraedii</i>	T; 20	Ziama	+	Yes	"
<i>Pterocarpus santalinoides</i> DC.	T; 15	Ziama	–	No**	"
Abreae					
<i>Abrus canescens</i> Baker	H; 2.5	Nimba	+	No**	No
<i>Abrus pulchellus</i> Thwaites	H; 2	Ziama	+	No**	"
<i>Abrus pulchellus</i> ssp. <i>tenuiflorus</i> (Benth.)	H; 2	Nimba	+	No**	"
Millettieae					
<i>Dalbergiella welwitschii</i> (Baker) Baker f.	S; V; 20	Nimba	–	Yes	No
<i>Leptoderris brachyptera</i> (Benth.) Dunn	S; V; 20	Ziama	+	Yes	No
<i>Leptoderris fasciculata</i> (Benth.) Dunn	S; V; 20	Nimba	+	Yes	"
<i>Lonchocarpus cyanescens</i> (Schum. & Thonn.) Benth	S; V; 30	Nimba	–	Yes	No
<i>Millettia barberi</i> (Benth.) Dunn	S; V; 20	Nimba	–	Yes	No
<i>Millettia dinklagei</i> Harms	S; V; 15	Nimba	+	Yes	"
<i>Millettia griffoniana</i> Baillon	T; 15	Nimba	–	Yes	"
<i>Millettia lane-poolei</i> Dunn	T; 7	Béro	–	Yes	"
<i>Millettia lucens</i> (Scott Elliot) Dunn	S; V; 15	Béro	–	Yes	"
<i>Millettia rhodantha</i> Baillon	T; 12	Ziama	+	Yes	"
<i>Millettia warneckeae</i> Harms	S; V; 7	Ziama	–	Yes	"
<i>Millettia zechiana</i> Harms	S; 10	Ziama	+	Yes	"
<i>Platysepalum hirsutum</i> (Dunn) Hepper	S; V; 20	Nimba	–	Yes	Yes
<i>Tephrosia flexuosa</i> G. Don	S; 1.5	Nimba	–	Yes	No
<i>Tephrosia nana</i> Schweinf.	H; 2	Nimba	–	Yes	"
Indigofereae					
<i>Indigofera atriceps</i> Hook. f. ssp. <i>atriceps</i>	H; 3	Nimba	+	No**	No
<i>Indigofera dendroides</i> Jacq.	H; 1.3	Béro	–	No**	"
<i>Indigofera heudelotii</i> Benth. ex Baker var. <i>heudelotii</i>	H; 2.5	Béro	–	Yes	"
<i>Indigofera macrophylla</i> Schum. & Thonn.	S; V; 10	Ziama	–	Yes	"

Table 1 Continued

Subfamily, tribe, species	Plant habit; height (m)	Location	Nodulation	Newly observed species†§	Newly observed genus‡
<i>Indigofera paniculata</i> Pers. ssp. <i>paniculata</i>	H; 1.5	Ziama	–	No**	"
<i>Indigofera simplicifolia</i> Lam.	H; 1.5	Béro	–	No**	"
Desmodieae					
<i>Desmodium adscendens</i> (Sw.) DC.	H; 75	Béro	–	No**	No
<i>Desmodium incanum</i> (Sw.) DC.	H; 1	Nimba	–	No**	"
<i>Desmodium ramosissimum</i> G. Don	H; 1	Nimba	–	Yes	"
<i>Desmodium salicifolium</i> (Poir.) DC.	H; 1.5	Ziama	+	No**	"
<i>Desmodium velutinum</i> (Willd.) DC.	S; 4	Nimba	–	No**	"
<i>Droogmansia scaettaiana</i> A. Chev. & Sillans	H; 2	Nimba	+	Yes	No
Phaseoleae					
<i>Calopogonium mucunoides</i> Desv.	H; V; 4	Nimba	+	No**	No
<i>Canavalia ensiformis</i> (L.) DC.	H; V; 4	Nimba	–	No**	No
<i>Dioclea reflexa</i> Hook. f.	S; V; 15	Nimba	–	No**	No
<i>Dolichos dinklagei</i> Harms	S; 1.5	Nimba	–	Yes	No
<i>Dolichos nimbaensis</i> Schnell	H; 1.5	Nimba	+	Yes	"
<i>Dolichos tonkouiensis</i> Portères	S; 1.5	Nimba	+	Yes	"
<i>Eriosema glomeratum</i> Hook. f.	H; 1.5	Nimba	+	No**	No
<i>Eriosema parviflorum</i> ssp. <i>collinum</i> Hepper	H; 0.25	Nimba	+	Yes	"
<i>Eriosema parviflorum</i> E. Mey. ssp. <i>parviflorum</i>	H; 1.5	Nimba	–	No**	"
<i>Erythrina milbraedii</i> Harms	T; 30	Ziama	+	Yes	No
<i>Erythrina senegalensis</i> DC.	S; 8	Diécké	+	No**	"
<i>Glycine wightii</i> Verdc. ssp. <i>wightii</i>	H; V; 5	Nimba	–	No**	No
<i>Mucuna flagellipes</i> Hook. f.	S; V; 5	Béro	–	Yes	No
<i>Mucuna poggei</i> Taub.	S; V; 20	Béro	+	No**	"
<i>Mucuna pruriens</i> (L.) DC. var. <i>pruriens</i>	V; 5	Béro	–	No**	"
<i>Mucuna pruriens</i> var. <i>utilis</i> (Wall.ex Wight) Bak. ex Burck	V; 10 m	Nimba	–	No**	"
<i>Mucuna sloanei</i> Fawc. & Rend.	H; V; 8	Nimba	–	No**	"
<i>Physostigma venenosum</i> Balf.	S; V; 6	Béro	–	Yes	Yes
<i>Rhynchosia brunnea</i> Baker f.	H; V; 5	Diécké	+	Yes	No
<i>Rhynchosia mannii</i> Baker	H; V; 8	Diécké	–	Yes	"
<i>Rhynchosia minima</i> (L.) DC.	H, V; 2	Béro	–	No**	"
<i>Rhynchosia pycnostachya</i> (DC.) Meikle	H, V, 8	Nimba	–	Yes	"
<i>Teramnus micans</i> (Baker) Baker f.	H; V; 4	Nimba	–	Yes	No
<i>Vigna gracilis</i> (Guill. & Perr.) Hook. f.	H; V; 4	Ziama	–	No**	No
<i>Vigna multiflora</i> Hook. f.	H; V; 6	Nimba	–	Yes	"
<i>Vigna nigrizia</i> Hook. f.	H; V; 3	Nimba	–	Yes	"
<i>Vigna racemosa</i> (G.Don) Hutch. & Dalz.	H; V; 6	Nimba	+	No**	"
<i>Vigna reticulata</i> Hook. f.	H; V; 6	Nimba	+	No**	"
<i>Vigna unguiculata</i> (L.) Walp.	H; V; 4	Nimba	+	No**	"
<i>Vigna venulosa</i> Baker	H; V; 2	Nimba	+	Yes	"
<i>Vigna vexillata</i> (L.) A. Rich.	H; V; 4	Nimba	+	No**	"
Aeschynomeneae					
<i>Aeschynomene pulchella</i> Baker	H; 0.5	Ziama	–	Yes	No
<i>Aeschynomene sensitiva</i> Sw.	H; 2.5	Ziama	+	No**	"
<i>Cyclocarpa stellaris</i> Baker	H; 0.5	Nimba	–	No**	No
<i>Kotschya lutea</i> (Portères) Hepper	S; 2	Nimba	+	Yes	No
<i>Kotschya ochreatea</i> (Taub.) Dewit & Duvign. var. <i>ochreatea</i>	S; 3 m	Nimba	+	Yes	"
<i>Ormocarpum megalophyllum</i> Harms	S; 1.5	Nimba	–	Yes	No
<i>Zornia glochidiata</i> DC.	H; 0.6	Nimba	+	No**	No
<i>Zornia latifolia</i> Smith	H; 1	Nimba	+	No**	"
Crotalariaeae					
<i>Crotalaria cylindrocarpa</i> DC.	H; 2	Ziama	+	Yes	No
<i>Crotalaria doniana</i> Baker	H; 2	Béro	–	Yes	"
<i>Crotalaria lachnosema</i> Stapf	H; 1.75	Béro	+	Yes	"
<i>Crotalaria lathyroides</i> Guill. & Perr.	S; 2	Béro	+	No**	"
<i>Crotalaria spectabilis</i> Roth	H; 1.25	Béro	–	No**	"

†T, tree; S, shrub; V, vine; H, herbaceous species.

‡After Ezedinma *et al.* (1979); Allen & Allen (1981); Högberg & Pearce (1986); de Faria *et al.* (1989); Wester & Högberg (1989); Moreira *et al.* (1992); Högberg & Alexander (1995); Doignon-Bourcier *et al.* (1999); Sprent (2001).

§When it is not a newly observed species: *, nodulation reported as negative; **, species already observed as nodulated.

with a homologous bacterial strain, or a strain isolated from a species of the same genus when no homologous strain was available. Thirty plants were used per plant species \times *Bradyrhizobium* strain association, as well as for the control treatments represented by uninoculated plants. Treatments were spaced apart to prevent cross-contamination. After inoculation with rhizobia, plants were grown in the shade for 4 months while shoot height and diameter at collar level were measured on all plants at 1 month intervals.

Experimental design of field trial The seedlings of the eight species used for the inoculation experiment described above and grown for 4 months in the nursery were transferred directly to the field close to the nursery in Sereidou in early June, this date corresponding to the beginning of the long wet season. The open planting area was prepared by hand-cleaning, and no fertilization treatment was applied to the plants. For each tree species, lines of 30 plants were planted per inoculation treatment. Each species tested, except *A. altissima* and *M. zechiana*, was represented by one line of 30 trees inoculated with a single *Bradyrhizobium* strain and one line of 30 uninoculated control trees planted just beside. For *A. altissima* and *M. zechiana*, which were inoculated with two and three *Bradyrhizobium* strains, respectively, one line of 30 uninoculated control trees and two or three adjacent lines of 30 inoculated trees each were planted. Tree spacing was 2 m between trees of the same line, and 4 m between each line. All tree species and inoculation treatments were planted within the same trial, making a total area of 0.46 ha (30 trees per line \times 19 lines).

Collection of growth data and statistical analysis One month after inoculation and every month during the 4 month growing period in the nursery, shoot height and stem diameter of plants were measured on 30 replicates per inoculation treatment (uninoculated control plants and plants inoculated with the different *Bradyrhizobium* strains) in the eight tree species tested. The height of trees and their stem diameter at ground level were recorded 11 months after field transplantation.

The different inoculation treatments were compared through a one-way ANOVA using the Statistical Analysis System computer program (SAS Institute, 1985). The means obtained from the different inoculation treatments applied to *A. altissima* and *M. zechiana* were ranked according to the Newman and Keuls multiple range test (Dagnélie, 1969).

Results and Discussion

Occurrence of nodulation in natural forest conditions

The occurrence of nodulation was investigated in 156 species belonging to different subfamilies and tribes of the Leguminosae, growing in diverse locations of the humid dense forest in Guinea. The different species observed and their characteristics are listed in Table 1. About 40% of the species analysed were represented

by tall mature trees (mean height of observed trees = 22.3 m), while 12% were shrubs, 16% vines, and 32% herbaceous species. Among the Papilionoideae subfamily, 40 out of 90 species (45%) belonging to 10 different tribes were found to be spontaneously nodulated in natural conditions of Guinean forest. Members of this subfamily were represented by the same proportion of ligneous and herbaceous species in our survey (50 and 49%, respectively). No difference in nodulation occurrence was found between both plant habits, with about half the species examined being nodulated in each plant type. The only species observed belonging to the tribe Swartzieae was not nodulated, while the nine other tribes contained nodulated species. A higher proportion of species of the Mimosoideae subfamily, mostly represented by tree species (75%), was nodulated: it occurred in 17 out of 24 species (71%) distributed across the four tribes observed. By contrast, with four positive observations among 42 species analysed (81% of tree species), a low proportion (12%) of species belonging to the Caesalpinioideae was nodulated, and restricted to the Caesalpinieae and Cassieae tribes. Nodulation was not observed in the three other Caesalpinioideae tribes: Cercideae, Detarieae and Amherstieae.

Our observations are consistent with those reported in the literature: we found a high proportion of nodulated species among the Papilionoideae and Mimosoideae and a minority within the Caesalpinioideae. Overall, nodulated species represent 90, 97 and 23%, respectively, of the total number of species observed in these three subfamilies (Allen & Allen, 1981; de Faria *et al.*, 1989; Sprent, 1995). The proportions of nodulated species obtained in our survey were lower than those reported in the literature, especially in the Papilionoideae subfamily, as spontaneous nodulation is often inhibited by different edaphic or climatic factors in natural conditions. So far, nodulation in the Leguminosae family has been examined in only \approx 60% of genera and 20% of species, those remaining unexplored being mostly tropical (Sprent, 1995). Although the trees surveyed in the present study are tall, well known and quite frequent in their natural habitat, a large majority (72%) had never been observed for nodulation before this study. Of the 97 new species and 14 new genera that had never been examined before, 31 species and four new genera are reported here to be nodulated (Table 1). Thus this study shows that an exhaustive list of nodulated leguminous species is far from being finalized, and can easily be expanded by new observations carried out in unexplored biotopes. Within the Caesalpinioideae subfamily, *Chidlowia sanguinea*, the only species of this genus in the Caesalpinieae tribe, is reported here to nodulate for the first time. According to Sprent (2001), only eight genera within this subfamily have been described and confirmed as nodulated, seven of them belonging to the Caesalpinieae tribe and the other one, *Chamaecrista*, to the Cassieae. Some nodulation data from other genera of Caesalpinieae were obtained more recently, but need to be confirmed. The nodulation status of the genus

Senna, formerly included with *Chamaecrista* in the genus *Cassia*, is more controversial and is subject to conflicting reports (Allen & Allen, 1981). The exclusive occurrence of nodulation in the Caesalpinieae and Cassieae tribes among the Caesalpinioideae subfamily was shown to be related to their taxonomic position, which is close to that of the Mimosoideae and Papilionoideae subfamilies. In addition, the absence of nodulation in the other Caesalpinioideae tribes is explained by their inclusion in well differentiated and distant clades, as found by phylogenetic studies based on the analysis of the chloroplast gene *rbcL* (Doyle *et al.*, 1997). Among the Mimosoideae, *Aubrevillea platycarpa* and *Tetrapleura tetraptera* are also the first tree species of new genera that had never been described as being nodulated. The other important tree species of the Mimosoideae subfamily reported to be nodulated for the first time belong to the following genera: *Pentaclethra*, *Calpocalyx*, *Entada*, *Xylocarpus* and *Albizia* (see the corresponding species in Table 1). Lastly, among the Papilionoideae, nodulation had never been reported in the genus *Amphimas*, nor at the specific level in 21 species belonging to 14 other genera.

Effect of rhizobial inoculation on tree growth in nursery and field experiments

As reported in Table 2, the effect of inoculation on plant growth was significantly positive for all species grown in nursery conditions. Positive effects on shoot height and stem diameter were observed as little as 1 month after inoculation

and at each time of measurement until the fourth month of nursery growing, just before transferring plants to the field (only shoot heights recorded 4 months after inoculation are shown in Table 2). After 4 months' nursery growing we observed a significant and positive effect of inoculation with *Bradyrhizobium* on both stem height and diameter at collar level ($P < 0.001$ for both parameters) in *M. rhodantha* (+62.5 and +53.5%, respectively, compared with uninoculated control plants); *E. ivorensis* (+37.3 and +39.7%); *E. guineensis* (+20.3 and +27.7%); *A. adianthifolia* (+69.7 and +94.0%); *A. ferruginea* (+35.3 and +37.1%); and *A. zygia* (+58.8 and +29.3%). We also observed a significant and positive effect of inoculation, as well as a variability in the efficiency of the different strains tested, in *A. altissima* (+19.7% in height and +50.6% in diameter with the most efficient strain, STM922) and *M. zechiana* (+113.0% in height and +43.8% in diameter with the most efficient strain, STM851).

As indicated in Table 2, 11 months after transplantation of inoculated plants to the field, the positive effects of inoculation observed in the nursery remained significant in all species. The differences in height and diameter measured between inoculated plants and uninoculated control plants were similar to those obtained after 4 months' nursery growing, and the height differences were enhanced in the following species: *A. zygia* (+92%); *A. ferruginea* (+97%); *E. guineensis* (+87%); *M. rhodantha* (+82%). Conversely, we noted an important decline of the inoculation effect in *A. adianthifolia*, although it remained significantly positive (+33% in height, +16% in diameter).

Table 2 Effect of rhizobial inoculation on the growth of eight leguminous tree species in the nursery 4 months after inoculation and 11 months after transfer to the field

Tree species	Rhizobium strain treatment	Plant height 4 months after inoculation (cm)	Plant height 11 months after field transfer (cm)	Stem diameter 11 months after field transfer (mm)
<i>Albizia adianthifolia</i>	STM 916	14.05 ^a ± 0.25	30.1 ^a ± 1.5	9.8 ^a ± 1.1
	Uninoculated	8.28 ^b ± 0.26	22.6 ^b ± 2.3	8.4 ^b ± 1.2
<i>Albizia altissima</i>	STM 916	11.82 ^a ± 0.63	26.7 ^a ± 2.2	9.6 ^a ± 1.4
	STM 922	12.04 ^a ± 0.31	26.6 ^a ± 2.0	9.5 ^a ± 1.7
	STM 923	11.89 ^a ± 0.38	26.8 ^a ± 2.7	8.0 ^b ± 1.2
	Uninoculated	10.06 ^b ± 0.38	24.4 ^b ± 2.3	6.7 ^c ± 1.2
<i>Albizia ferruginea</i>	STM 922	20.50 ^a ± 0.45	59.4 ^a ± 8.2	12.4 ^a ± 2.2
	Uninoculated	15.15 ^b ± 0.42	30.0 ^b ± 3.2	9.7 ^b ± 1.5
<i>Albizia zygia</i>	STM 923	14.88 ^a ± 0.39	50.0 ^a ± 3.3	9.0 ^a ± 1.4
	Uninoculated	9.37 ^b ± 0.23	26.0 ^b ± 3.7	7.8 ^b ± 0.7
<i>Erythrophleum guineensis</i>	STM 934	22.37 ^a ± 0.76	57.3 ^a ± 8.9	11.7 ^a ± 1.5
	Uninoculated	18.60 ^b ± 0.53	30.8 ^b ± 2.6	10.0 ^b ± 1.1
<i>Erythrophleum ivorensis</i>	STM 934	17.23 ^a ± 0.46	38.2 ^a ± 3.1	10.2 ^a ± 1.4
	Uninoculated	12.55 ^b ± 0.38	29.1 ^b ± 2.5	8.4 ^b ± 0.9
<i>Millettia rhodantha</i>	STM 931	26.10 ^a ± 1.02	59.5 ^a ± 9.3	11.4 ^a ± 1.0
	Uninoculated	16.00 ^b ± 0.65	32.7 ^b ± 5.2	10.1 ^b ± 1.1
<i>Millettia zechiana</i>	STM 851	27.12 ^a ± 1.07	62.5 ^a ± 11.0	11.8 ^a ± 1.2
	STM 926	18.47 ^b ± 0.68	39.7 ^b ± 8.6	11.0 ^b ± 1.5
	Uninoculated	12.73 ^c ± 0.45	31.6 ^c ± 2.6	9.6 ^c ± 1.1

Means ± SE were calculated from 30 replicates per treatment. One-way ANOVA showed significant effects of inoculation on height and diameter at $P < 0.01$ in each tree species both in the nursery and 11 months after transfer to the field. Means followed by different letters are significantly different according to the Newman and Keuls test at $P = 0.05$.

The inoculation experiments presented here were performed using rhizobial strains isolated from the trees observed and described in Table 1. Other studies focusing on the effect of rhizobial inoculation on tree legume species showed very large differences between the growth of inoculated plants and that of uninoculated control plants at the end of the nursery growing period. This was the case with *Acacia mangium*, a fast-growing tree species mainly planted for pulp production in South-East Asia, which exhibited increments of ≈ 40 –100% in stem height between inoculated and uninoculated plants according to different nursery conditions (Galiana *et al.*, 1998). Similar increments were observed in other plantation species such as *Albizia procera*, *Albizia lebbek* and *Leucaena leucocephala* grown in nursery conditions (Aryal *et al.*, 1999). However, in contrast with the present study, such large differences were obtained with selected rhizobial strains obtained after several steps of screening performed among an initial large population of isolated strains. In other cases the inoculation effect on plant growth is nil or very low at the end of the nursery stage, as it was found in several dry-zone tree species including *Acacia tortilis*, *Acacia nilotica*, *Acacia senegal*, *Faidherbia albida* and *Prosopis juliflora*, even after a preliminary step of rhizobial strain selection (Brunck *et al.*, 1990). The absence of growth response of a given plant species to inoculation is sometimes attributed to a low level of specificity of their associated rhizobia, as it is often observed in host species nodulating with *Bradyrhizobium* (Turk & Keyser, 1992). In this case, other local *Bradyrhizobium* strains are able to take over the inoculated strains and contaminate all the plants in nonsterile nursery conditions. However, many plant species associated with *Bradyrhizobium* sp. strains known to have a wide host spectrum, such as *A. mangium* (Galiana *et al.*, 2002), can have a marked growth response to inoculation. We observe similar marked inoculation effects in our experiments performed on *Albizia*, *Erythrophleum* and *Milletia* species where associated strains were genetically identified as *Bradyrhizobium* sp. strains, as attested by sequencing of the 16S–23S ribosomal DNA intertranscript (Munive, 2002).

Most inoculation studies involving tree legume species have been performed on fast-growing species or species traditionally used in pure plantations or agroecosystems. On the other hand, very few data are available on rhizobial inoculation of long-rotation legume trees such as those found in natural ecosystems. Our results, obtained in *in situ* conditions, show the need to identify the nodulation status of unexplored local trees before inoculating them with rhizobia in the context of plantation projects or reforestation programmes in degraded humid tropical forests.

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