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Bradyrhizobium Populations Occur in Deep Soil under the Leguminous Tree *Acacia albida*

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Soil cores were drilled under the leguminous tree *Acacia albida* growing in two different ecoclimatic zones of West Africa: the Sahelian area (100 to 500 mm of annual rainfall) and the Sudano-Guinean area (1,000 to 1,500 mm of annual rainfall). Soil samples were collected at different depths from the surface down to the water table level and analyzed for the presence of rhizobia able to nodulate *A. albida*. In both areas, population densities of rhizobia were substantially greater near the water table than near the surface. In the Sahelian area, rhizobia were present as deep as 34 m at a concentration of 1.3×10^3/g of soil. In the Sudano-Guinean area, population densities at 0.5 to 4.5 m depth were higher than in the Sahelian area and, at several depths, comparable to that of temperate soils supporting legume crops (10^4 rhizobia per g of soil). Surface and deep soil isolates from all four sites were found to be slow-growing rhizobia (*Bradyrhizobium* sp.). The proportion of effective isolates was almost the same within surface and deep soils.

The leguminous tree *Acacia albida* plays a major role in the agro-sylvo-pastoral balance of the Sahelian regions of Africa. In contrast with other Sahelian trees, *A. albida* has the unique phenology of bearing leaves in the dry season and shedding them at the beginning of the wet season. Taking advantage of this inverted phenological cycle, farmers grow crops under the leafless trees during the rainy season and nomads can use the aerial forage for their cattle during the long dry season. It has been repeatedly observed that the soil nitrogen content is several times higher under *A. albida* than outside its cover (9) and that crops benefit from the nutrients brought to the soil surface with the leaf litter. The contribution of symbiotic N_2 fixation by *A. albida* to this input of nitrogen to soil has been questioned since attempts to find root nodules on mature trees were unsuccessful (2). Up to now, only young *A. albida* grown in forest nurseries have been shown to be nodulated and to fix nitrogen actively (2a).

In the Sahelian zone, with an annual rainfall of 100 to 500 mm (10), the ecological distribution of *A. albida* is always dependent on the presence of deep water resources. *A. albida* typically develops a lateral root system near the surface and a main tap root system which can reach the water table at depths reaching 40 m. Surface roots are exposed to the climatic fluctuations, while deep roots grow in more constant temperature and humidity conditions. *A. albida* can also thrive in more humid areas of the Sudano-Guinean zone (1,000 to 1,500 mm of annual rainfall), where this species is mainly found in paddy fields. In such environments, the water table depth varies from 1.5 to 4.5 m during the dry season and *A. albida* only develops surface lateral roots.

Rhizobia isolated from young *A. albida* root nodules have been characterized as slow-growing *Bradyrhizobium* species (3), whereas most Sahelian acacias (e.g., *Acacia senegal*, *Acacia raddiana*, *Acacia nilotica*) nodulate with fast-growing *Rhizobium* species. Consequently, the *A. albida* symbionts will be referred to as bradyrhizobia.

The presence of *A. albida* in two different ecoclimatic regions characterized by a variation of the annual rainfall from 100 to 1,500 mm and by a large difference in the water table depth (35 to 1.5 m, respectively) provides a good model to examine the distribution and effectiveness of bradyrhizobial populations associated with surface and deep roots of this important tree.

The objectives of the present study were (i) to investigate nodule occurrence on the whole root system of *A. albida* in both surface and deep soil horizons in Sahelian and Sudano-Guinean areas, (ii) to examine the distribution of *Bradyrhizobium* populations under *A. albida*, and (iii) to determine the effectiveness of the various *Bradyrhizobium* isolates in order to select the best nitrogen-fixing isolates for use as inocula in forest nurseries.

MATERIALS AND METHODS

Site of sampling. Samples of soil were recovered under a canopy of mature 15- to 20-m high *A. albida* trees. Two sites were chosen in the Sahel and two in the Sudano-Guinean area. In the Sahelian area, two drillings were established, one near Louga (a town 200 km north of Dakar, 300 mm of annual rainfall), the other in a field at Diokoul (a village between Thies and Bambey, central Senegal, 500 mm of annual rainfall). In the Sudano-Guinean area, the two sites were located in the paddy fields of Djinaki (1,100 mm of annual rainfall) and Kabrousse (1,500 mm of annual rainfall), two villages of Casamance (south Senegal).

Method of sampling. Soil samples were collected with lightweight drilling equipment (Dormer's Engineering Equipment, South Murwillumbah, Australia) at different depths. The shell-type auger (rotating drill bit) used for sand (50-mm diameter) can bore down to the water table. Cores were drilled from the surface to the water table and collected in 20-cm increments. Only the internal soil core was collected to avoid contamination with soil from upper horizons. Each 20-cm sample was placed in a separate sterile pouch, immediately stored at 4°C, and brought back to the laboratory for enumeration of rhizobia. Samples were chosen at regular intervals as indicated in Table 1. Furthermore, in each of the ecoclimatic zones, surface soils were sampled and studied

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TABLE 1. Distribution of *Bradyrhizobium* populations in four cores drilled under the canopy of *A. albida* trees from the surface to the water table

Sahelian ecoclimatic zone (100 to 500 mm of annual rainfall)				Sudano-Guinean ecoclimatic zone (1,000 to 1,500 mm of annual rainfall)			
Louga		Diokoul		Djinaki		Kabrousse	
Depth (m)[a]	No. of rhizobia/g of soil[b]	Depth (m)[a]	No. of rhizobia/g of soil[b]	Depth (m)[a]	No. of rhizobia/g of soil[b]	Depth (m)[a]	No. of rhizobia/g of soil[b]
0.1	70	0.1	<1	0.1	13,000	0.1	230
0.5	1,270	0.5	20	0.5	32,000	0.5	42,000
2.5	90	2.5	20	1.0	28,000	1.0	2,300
5.0	160	4.0	50	1.5	28,000	1.5[c]	9,180
7.5	<1	6.0	<1	2.0	43,000		
11.0	<1	8.0	<1	3.0	42,400		
14.0	<1	10.0	<1	4.0	1,500		
17.5	90	11.5	30	4.5[c]	1,500		
21.0	<1	14.0	80				
24.0	40	16.5[c]	30				
27.5	10						
28.5	120						
30.0	20						
32.0	20						
33.5	340						
34.0[c]	1,320						

[a] Mid point of 20-cm-high sample.
[b] As assessed by the most-probable-number method.
[c] Water table level.

for the presence of *Bradyrhizobium* isolates able to nodulate *A. albida*.

Enumeration of bradyrhizobia. *A. albida* seeds were surface sterilized with concentrated sulfuric acid for 1 h. After this treatment, the seeds were washed with sterile water until all traces of acid were removed. The seeds were germinated for 48 h in sterile petri dishes containing water agar (0.8%), and the seedlings were transferred to tubes containing agar slants of Jensen medium (12). The number of bradyrhizobia in soil samples was estimated by the plant infection most-probable-number technique (1). A range of soil dilutions from 10^{-1} to 10^{-6} with four plants per dilution was used. Five days after transplantation in the tubes, the *A. albida* seedlings were inoculated with 1 ml of each soil dilution and placed in a temperature-controlled greenhouse at 30°C. Uninoculated plants did not form nodules, indicating no contamination by exogenous rhizobia. After 4 weeks, the roots were examined for the presence of nodules.

Isolation and cultivation of bradyrhizobia. Nodules formed in tubes were collected from the highest dilution of each soil sample, surface sterilized in 0.1% $HgCl_2$, washed several times with sterile water, squashed on yeast extract-mannitol agar (12), and streaked to obtain isolated colonies. All isolates were incubated at 28°C. Culture purity was verified by repeated streakings of single-colony isolates.

Symbiotic function of *Bradyrhizobium* isolates. Isolates were tested in tubes for their N_2-fixing effectiveness. Four seedlings per isolate were inoculated with 100 μl of fresh broth culture containing approximately 10^8 cells per ml. After 6 weeks, plants were decapitated, the remaining liquid culture medium was poured out of the tubes, and the tubes were immediately sealed with a sleeve-type rubber stopper. Then, acetylene was injected (10%, vol/vol), and after 30 min of incubation, the acetylene reduction activity was measured by determining the concentration of ethylene in the tube by gas chromatography according to standard procedures (4). For some tubes, a time course of acetylene reduction activity was determined to establish whether the activity was linear during the first 90 min of incubation. Isolates were classified into four effectiveness groups according to their acetylene reduction activity (expressed as nanomoles of C_2H_4 produced per hour per plant): highly effective, >600; effective, 350 to 600; partially effective, 100 to 350; ineffective, <100.

RESULTS

Presence of nodules on mature *A. albida* trees. In the higher-rainfall Sudano-Guinean area, we observed an abundant nodulation on surface roots of both young and mature *A. albida* trees growing in paddy fields in Casamance, where the water table is only 1.5 m below the surface. The nodules were attached to fine roots at 10 to 30 cm depth. Nodules were coralloid, and their size varied from 5 to 20 mm long and from 2 to 5 mm wide. When cut, they showed a pink color, an indication of the presence of leghemoglobin and effective nitrogen fixation. In contrast, in the drier Sahelian area, no nodules were found either in surface soils or in soil samples of 0.3 to 0.4 kg recovered during the coring to the water table.

Distribution of *Bradyrhizobium* populations in deep soil under *A. albida* trees in Sahelian and Sudano-Guinean areas. Table 1 shows that bradyrhizobia nodulating *A. albida* are distributed from the surface to the water table level in both climatic regions.

In the Sahelian area of Louga, population densities of bradyrhizobia reached the highest value at 34 m deep (1.32×10^3/g of soil). At all other levels, the population densities were much lower (approximately 1/10th of the density at 34 m) except at 0.5 m (1.27×10^3 bradyrhizobia per g of soil), where lateral roots were present. In Diokoul, *Bradyrhizobium* populations were negligible (<1/g of soil) at the surface and significantly higher near the water table level (30 to 80/g of soil). In both cores, intermediate layers (7.5 to 14 m and 21

TABLE 2. Symbiotic effectiveness of *Bradyrhizobium* isolates from the two Sahelian cores from Louga and Diokoul and from various surface Sahelian soils

Origin and depth (m) of sample	Effectiveness[a]			
	Highly effective	Effective	Partially effective	Ineffective
Louga				
0.5	ORS117	ORS111, -113, -115	ORS110, -112	ORS114, -116
2.5		ORS118, -119, -121	ORS120	
5.0			ORS122	ORS123
17.5		ORS125, -126	ORS124	ORS127
14.0		ORS128		ORS129
27.5	ORS130			
30.0			ORS133, -134	
32.0	ORS136			
33.5			ORS137, -138	
34.0			ORS139, -140	
Diokoul				
0.5		ORS141		
2.5			ORS142	
4.0			ORS143	
11.5			ORS144	
14.0				ORS145, -146
Various surface Sahelian soils	ORS188	ORS186, -189	ORS181, -182, -183, -184, -187, -190, -191, -192	ORS180

[a] Results are given as the mean of four replicates and expressed as nanomoles of C_2H_4 produced per hour per plant: highly effective, >600; effective, 350 to 600; partially effective, 100 to 350; ineffective, <100.

m at Louga, and 4 to 10 m at Diokoul) were practically devoid of bradyrhizobia and root fragments. In the Sudano-Guinean area, where the water table is only 1.5 to 4.5 m deep, population densities of bradyrhizobia were considerably higher and were distributed all along the core. Large population densities comparable to that of temperate soils supporting legume crops are found from 0.5 to 3 m depth in Djinaki (1.3×10^4 to 4.3×10^4 bradyrhizobia per g of soil) and at 0.5 m in Kabrousse (4.2×10^4 bradyrhizobia per g of soil).

Characterization of the *Bradyrhizobium* isolates. We obtained 80 isolates from nodules in culture tubes (Table 2 and 3). All were typically slow-growing on yeast extract-mannitol agar (colony appearance in 5 to 6 days) and thus considered as belonging to the genus *Bradyrhizobium* (8).

In Louga (Table 2), most of the isolates were partially effective or ineffective, while 12 of 28 isolates were classed as effective or highly effective. Of the three highly effective isolates, two (ORS130 and ORS136) originated from deep horizons (27.5 and 32 m). Five isolates were ineffective. In Diokoul, no effective isolates (of six) were obtained from deep horizons. Most surface isolates were found to be partially effective.

TABLE 3. Symbiotic effectiveness of *Bradyrhizobium* isolates from the two Sudano-Guinean cores of Djinaki and Kabrousse and from various surface Sudano-Guinean soils

Origin and depth (m) of sample	Effectiveness[a]			
	Highly effective	Effective	Partially effective	Ineffective
Djinaki				
0.1	ORS148			
0.5	ORS149			
1.0	ORS150	ORS151		
1.5	ORS152	ORS153		
2.0		ORS154		
3.0	ORS155			
4.0		ORS156, -157		
4.5		ORS158, -159		
Kabrousse				
0.1				ORS176
0.5	ORS177			
1.0		ORS178		
1.5	ORS179			
Various surface Sudano-Guinean soils		ORS101, -103, -164, -166, -167, -168, -169, -172, -173, -175	ORS160, -165, -170, -171, -174	ORS161, -162, -163

[a] See Table 2, footnote *a*.

In contrast, at the more humid sites of Djinaki and Kabrousse (Table 3), most of the isolates from the coring were highly effective or effective. For the surface isolates from various sites ($n = 18$), 83% were effective and partially effective and 17% were ineffective.

DISCUSSION

Up to now, it has been generally observed that populations of rhizobia were restricted to the upper soil horizons, mostly because studies were focused on annual crops with relatively shallow root systems. When dealing with deeply rooting trees, rhizosphere microorganisms, including *Rhizobium* and *Bradyrhizobium* species, may occur in the deep soil. In fact, Virginia et al. (14) studied the distribution of rhizobia under *Prosopis glandulosa* in the California Sonoran Desert and reported that population densities as high as 6.1×10^3 cells per g of soil occurred at 4 to 6 m below the ground near the water table. Our results clearly demonstrated that large *Bradyrhizobium* populations were found in soils at the water table level (much deeper than 4 to 6 m) under the canopy of the Sahelian *A. albida* tree; population densities of bradyrhizobia can reach 1.32×10^3/g of soil at a depth of 34 m.

Very few nodules had previously been recovered from *A. albida* in the field. Therefore, soil enrichment by this tree was considered to be due to the ability of the extensive and deep root system of *A. albida* to scavenge soil mineral N concentrated as organic N in the leaves before being released during decomposition of litter at the soil surface. We found actively fixing root nodules on young and mature trees in the wet paddy soils of Casamance, which suggested that mature *A. albida* trees are potentially good nitrogen fixers. Our results suggest that the potential importance of symbiotic N_2 fixation by *A. albida* to soil enrichment has been underestimated.

In the typical Sahelian area, which is the usual habitat of *A. albida*, surface nodulation of this tree is lacking and surface *Bradyrhizobium* populations are low, which suggests that the nitrogen-fixing potential of the tree at the soil surface is negligible. In deep soils, we did not recover nodules during our survey, but we found relatively high *Bradyrhizobium* populations. These densities are consistent with the assumption that nodules might be formed at depth when the conditions are favorable. This assumption will be tested by evaluation of N_2 fixation using the ^{15}N natural abundance method (11, 13) or by excavation of a tree to determine the pattern of nodulation.

Under *P. glandulosa*, two distinct populations of rhizobia were identified by Jenkins et al. (5–7) and Waldon et al. (15). At the soil surface, *Rhizobium* species is dominant, whereas *Bradyrhizobium* species occurs mostly in deep soil. Under *A. albida*, all the isolates that we recovered were typical *Bradyrhizobium* species (8). *Bradyrhizobium* isolates living at the surface or at the water table level are exposed to very different physical and chemical conditions. At the surface of Sahelian soils, the temperature varies from 15 to 50°C and the water content widely fluctuates from the rainy to the dry season, while these conditions should be more constant at depths of 10 to 35 m. Thus, surface and phreatic zones could select for *Bradyrhizobium* species with physiological differences as observed by Waldon et al. (15) for *P. glandulosa* isolates. As shown in Tables 2 and 3, the percentage of effective isolates is about the same within surface and phreatic soils, suggesting that surface populations moved with the roots from the surface into the deep soil. However, isolates with similar effectiveness could have very different physiological tolerances related to free-living survival in the soil at different depths (15).

Deep soil samples (34 m) from Louga showed very low total nitrogen (0.05%) and organic carbon (0.15%) concentrations. This low N and C availability in deep soils could be expected to limit microbial activity, but the presence of the active root system of *A. albida* could greatly improve the deep soil environment for bradyrhizobia in the vicinity of roots. It is also interesting that in the Sahelian area, the largest populations of bradyrhizobia were found at the depth where small *A. albida* root fragments were present in soil samples recovered from the coring. In the Sudano-Guinean area, the presence of a high density of roots from the surface to the water table could also explain the large populations of bradyrhizobia present in the paddy fields of Casamance. We recently found in Kabrousse that population densities of bradyrhizobia were negligible (<1/g of soil) in the soils outside the tree canopy (3a). This suggests that the presence of significant populations of bradyrhizobia depends on root activity and root products.

The variability among the *Bradyrhizobium* isolates is currently being studied in our laboratory, and it is our aim to determine whether there is a distinction between isolates from surface or deep soil, the latter ones being adapted to more constancy in water content and temperature than the former. Furthermore, effective isolates from deep soil will be used in the field to inoculate *A. albida* to test their colonization of its deep root system.

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