Acacia raddiana, Acacia izilotica) at depths reaching 40 m. Surface roots are characterized as slow-growing (Bradyrhizobium) and to fix nitrogen actively (2a).

In the Sahelian zone, A. albida, Rhizobia isolated from young root nodules on mature trees were unsuccessful (2). 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 N₂ 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 radiiana, 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.
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 containing 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 10⁻⁷ with four plants per dilution was tested in tubes for their N₂-fixing effectiveness. Four isolates 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% HgCl₂, washed several times with sterile water, squeezed 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 N₂-fixing effectiveness. Four seedlings per isolate were inoculated with 100 µl of fresh broth culture containing approximately 10⁸ 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 C₂H₂ 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 × 10³/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 × 10² 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 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** | **Djimani** |
| **Depth (m)** | **No. of rhizobia/g of soil** | **Depth (m)** | **No. of rhizobia/g of soil** | **Depth (m)** | **No. of rhizobia/g of soil** |
| 0.1 | 70 | 0.1 | 13,000 | 0.1 | 230 |
| 0.5 | 1,270 | 0.5 | 20 | 0.5 | 42,000 |
| 2.5 | 90 | 2.5 | 30 | 2.5 | 43,000 |
| 5.0 | 160 | 5.0 | <1 | 5.0 | 32,000 |
| 7.5 | <1 | 7.5 | <1 | 7.5 | 1,500 |
| 11.0 | <1 | 11.0 | <1 | 11.0 | 4,500 |
| 14.0 | <1 | 14.0 | <1 | 14.0 | 4,500 |
| 21.0 | <1 | 21.0 | 30 | 21.0 | 1,500 |
| 24.0 | 40 | 24.0 | 80 | 24.0 | 1,500 |
| 27.5 | 10 | 27.5 | 30 | 27.5 | 1,500 |
| 28.5 | 120 | 28.5 | 30 | 28.5 | 1,500 |
| 30.0 | 30 | 30.0 | 30 | 30.0 | 1,500 |
| 32.0 | 20 | 32.0 | 30 | 32.0 | 1,500 |
| 33.5 | 340 | 33.5 | 30 | 33.5 | 1,320 |
| 34.0 | 1,320 | 34.0 | 30 | 34.0 | 1,320 |

*Mid point of 20-cm-high sample.*

*As assessed by the most-probable-number method.*

*Water table level.*

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*Note*:

- The table lists the distribution of *Bradyrhizobium* populations in four cores drilled under the canopy of *A. albida* trees from the surface to the water table level in both climatic regions.

- The data are presented for both the Sahelian and Sudano-Guinean ecoclimatic zones.

- The population densities are given for different levels of depth, ranging from 0.1 m to 34 m from the surface, with values expressed as the number of rhizobia per gram of soil.

- The table includes data for Louga and Djimani, with additional columns for other areas, such as Diokoul and Kabrousse, showing similar patterns.

- The study highlights the variation in population densities across different levels of depth and the significance of these levels in relation to the water table.
TABLE 2. Symbiotic effectiveness of _Bradyrhizobium_ isolates from the two Sahelian cores from Louga and Diokoul and from various surface Sahelian soils

<table>
<thead>
<tr>
<th>Origin and depth (m)</th>
<th>Highly effective</th>
<th>Effective</th>
<th>Partially effective</th>
<th>Ineffective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louga</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>ORS117</td>
<td>ORS111, -113, -115</td>
<td>ORS110, -112</td>
<td>ORS114, -116</td>
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<tr>
<td>2.5</td>
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<td>ORS120</td>
<td>ORS122</td>
<td>ORS123</td>
</tr>
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<td>5.0</td>
<td>ORS125, -126</td>
<td>ORS124</td>
<td>ORS127</td>
<td>ORS129</td>
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<td></td>
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<td>27.5</td>
<td>ORS130</td>
<td></td>
<td></td>
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<td>30.0</td>
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</tr>
<tr>
<td>34.0</td>
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<td></td>
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</tr>
<tr>
<td>Diokoul</td>
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</tr>
<tr>
<td>0.5</td>
<td>ORS141</td>
<td></td>
<td></td>
<td>ORS145, -146</td>
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</tr>
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</tr>
<tr>
<td>11.5</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>14.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Various surface Sahelian soils</td>
<td>ORS188</td>
<td>ORS186, -189</td>
<td>ORS181, -182, -183, -184, -187, -190, -191, -192</td>
<td>ORS180</td>
</tr>
</tbody>
</table>

* Results are given as the mean of four replicates and expressed as nanomoles of C₂H₄ 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 x 10⁴ to 4.3 x 10⁴ bradyrhizobia per g of soil) and at 0.5 m in Kabrousse (4.2 x 10⁴ 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

<table>
<thead>
<tr>
<th>Origin and depth (m)</th>
<th>Highly effective</th>
<th>Effective</th>
<th>Partially effective</th>
<th>Ineffective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Djinaki</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0.1</td>
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<td></td>
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<td></td>
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<tr>
<td>0.5</td>
<td>ORS149</td>
<td></td>
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<tr>
<td>1.0</td>
<td>ORS150</td>
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<td></td>
</tr>
<tr>
<td>3.0</td>
<td>ORS155</td>
<td>ORS156, -157</td>
<td>ORS158, -159</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kabrousse</td>
<td></td>
<td></td>
<td></td>
<td>ORS176</td>
</tr>
<tr>
<td>0.1</td>
<td>ORS177</td>
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<td></td>
<td></td>
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<tr>
<td>0.5</td>
<td>ORS178</td>
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<tr>
<td>1.0</td>
<td>ORS179</td>
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<td>1.5</td>
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<td></td>
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</tr>
</tbody>
</table>

* See Table 2, footnote a.
In contrast, at the more humid sites of Djinaki and 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.

**ACKNOWLEDGMENTS**

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