USE OF *SESBANIA ROSTRATA* AS GREEN MANURE IN PADDY FIELDS

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OFFICE DE LA RECHERCHE SCIENTIFIQUE ET TECHNIQUE OUTRE-MER

English version July 1983

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by

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INTRODUCTION

Along with water, nitrogen is one of the two major factors governing plant productivity. The nitrogen requirements of plants are considerable: for example, in order to produce 100 kg of grain, rice requires 1.8 to 2 kg of nitrogen regardless of the type of soil and the date for planting (Patnaik and Rao, 1979). A harvest of 4 T of rice per hectare necessitates 80 kg of nitrogen. Nitrogen absorbed by the plant comes from three sources: reserves of soil nitrogen (organic matter nitrogen and inorganic soil nitrogen), fertilizers supplied by the farmer, or biological nitrogen fixation. However, because soil nitrogen reserves are limited, it is necessary to use nitrogen fertilizers. These fertilizers are expensive, as energy requirements for their synthesis are high (approximately 16 hl of natural gas is consumed in the synthesis of 1 kg of anhydrous ammonia). Moreover, they must be purchased with hard currency. Because biological nitrogen fixation presents an appealing alternative to fertilizers, much work has been devoted to harnessing this process.

One solution that has been used for many centuries involves incorporating the shoots of nitrogen-fixing plants into the soil, with plant residues serving as an organic nitrogen fertilizer: hence the term "green manure".

For several centuries, notably in the Far East, many nitrogen-fixing plants have been used: either legumes, such as *Astragalus sinicus*, *Sesbania aculeatea*, *S. paludosa*, *S. cannabina*, *Crotalaria juncea*, *C. striata*, *Vicia cracca*, *Medicago hispida*, *M. officinalis* (Patnaik and Rao, 1979; Vachhani and Murty, 1964; Watanabe and App, 1979), or nonlegumes, such as *Coriaria sinica* (Watanabe, 1980). The plant that has probably been most often used as a green manure is *Astragalus sinicus*, which is cultivated during the winter and incorporated into the soil 20 days before rice is planted. Incorporation of plant residues amounts to 20 to 30 tons of fresh weight per hectare, i.e., approximately 100 kg of nitrogen per hectare (Watanabe, 1980). Progress has recently been made in this field in Senegal with the discovery of *Sesbania rostrata*, a stem-nodulated legume. An advantage of this legume is that, because it nodulates both on roots and stems, it fixes nitrogen more actively than other known legumes.

In this booklet, we begin by presenting the state of the art of the biology of *Sesbania rostrata*. Next, we present the preliminary results of experiments in the use of *Sesbania rostrata* as green manure in paddy fields. Finally, we propose guidelines for field trials aimed at introducing *Sesbania rostrata* in tropical agriculture.

CHAPTER 1. BIOLOGY OF THE NITROGEN-FIXING SYMBIOSIS *SESBANIA ROSTRATA-RHIZOBIUM*

1. Description of *Sesbania rostrata* (Fig. 1 and 2)

The most distinctive characteristic of *Sesbania rostrata* is the presence of predetermined nodulation sites on the stems. These sites resemble small points arranged in vertical lines extending up the stem. When infected by the specific *Rhizobium*, these sites form nitrogen-fixing nodules, known as stem nodules.

2. Climate and soil requirements of *Sesbania rostrata*

Sesbania rostrata is a wild plant that has not yet been selected for plant breeding studies. Climate and soil requirements must be taken into consideration in its cultivation.

2.1. Climate requirements

At the latitude of Senegal (15°N), *Sesbania rostrata* grows well in waterlogged soils during the rainy season (June to September); this plant flowers at the second or third month of its growth cycle. Stem nodulation occurs rapidly (one week after inoculation) and is regular and profuse.

In contrast, at the same latitude during the dry and cold season (December to March), growth is poor, flowering takes places abnormally early, and nodulation is difficult.

This variation in the behavior of *Sesbania rostrata* during the different seasons can be attributed to its sensitivity to photoperiod, temperature, and humidity, this last factor appearing to play a major role in stem infection by *Rhizobium*.

2.2. Soil requirements

Sesbania rostrata grows well only on waterlogged or heavily irrigated soils. It apparently tolerates a certain level of soil salinity, but its salinity threashold has not yet been determined. In rainfed and well-drained soils, *Sesbania rostrata* is particularly susceptible to nematodes, especially *Meloidogyne* sp.

3. Nodulation sites on the stems

As we have already indicated, nodulation sites resembling small points are distributed evenly in three or four vertical lines all along the stem (Fig. 3). Formation of these sites is predetermined and independent of the *Rhizobium* infection.
A developing nodulation site appears as a protuberance on the stem (Fig. 4A). A day or two later, this protuberance swells and becomes an epidermal dome pierced in the center by a root primordium with a length of 0.2 to 0.8mm (Fig. 4B). A circular fissure is formed between the base of root primordium and the epidermal dome (Fig. 5). The root primordium remains inactive on the stem, most likely owing to phytohormonal dormancy, until its base is infected by the specific *Rhizobium* and produces a nitrogen-fixing nodule.

Nodulation sites exhibit two unique characteristics:

1) they are preformed and their exact location is known in advance;

2) they are formed continuously throughout the growth of the stem and remain sensitive to *Rhizobium* infection throughout the life of the plant.

Thus nodule formation can occur at any time during the growth cycle, which is undoubtedly and important ecological advantage of the *Sesbania rostrata*.

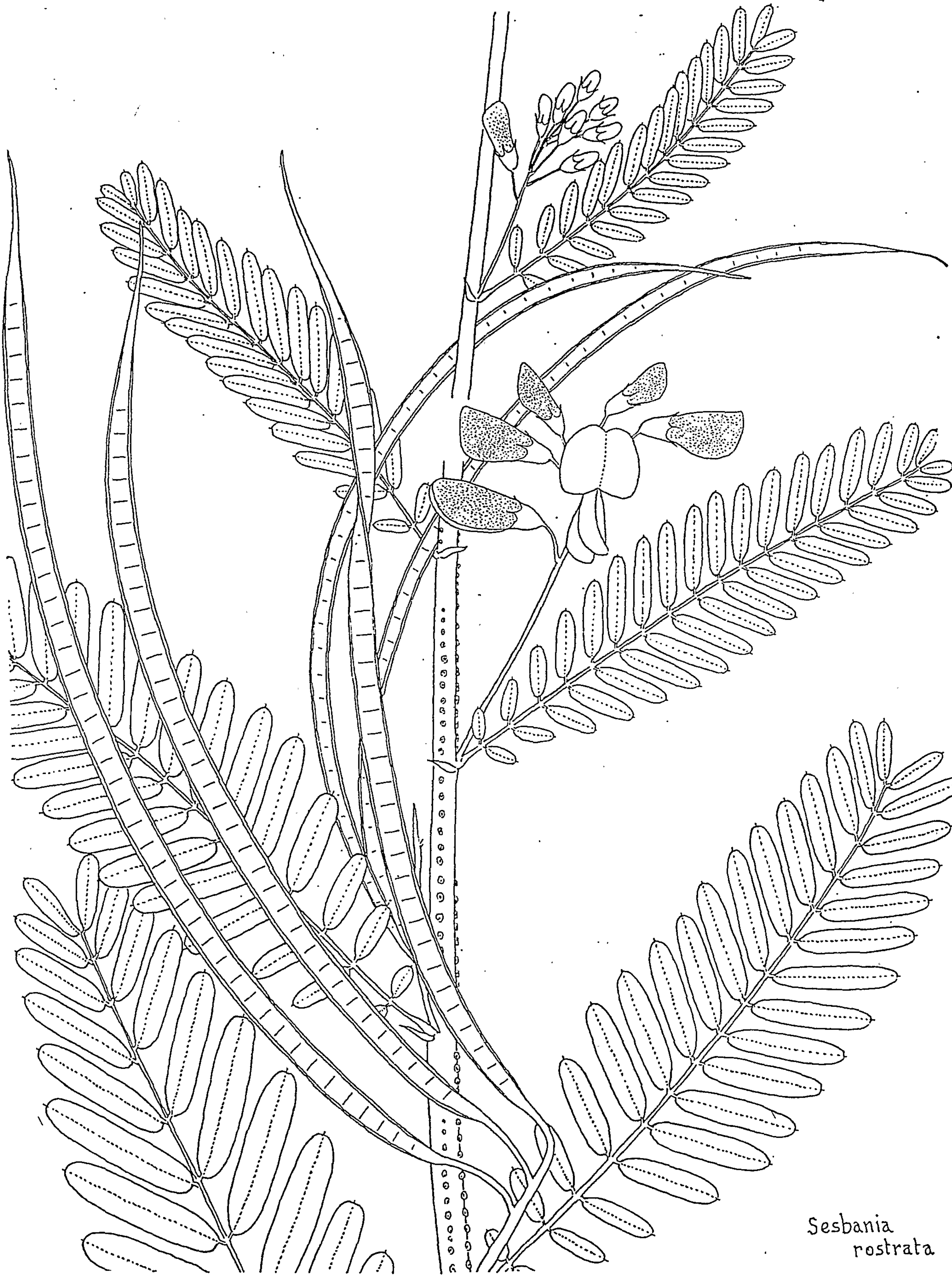

Fig. 1. Sesbania rostrata (Brem). Drawing by J. Berhaut (1976)

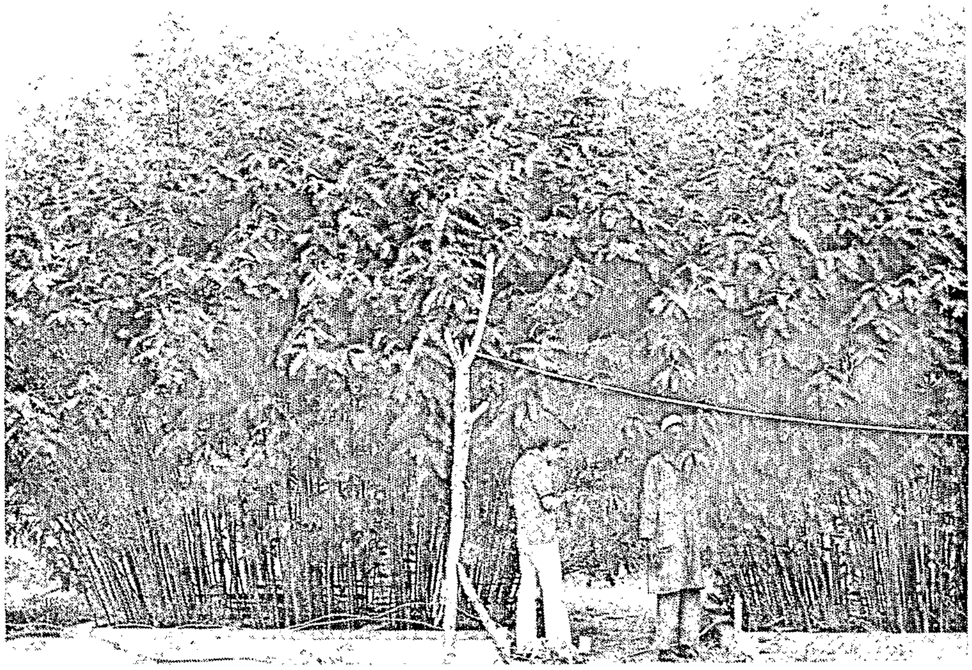

Fig. 2. Within 4 months Sesbania rostrata can grow up to 3-4m, as shown above. Used as green-manure, this plant is chopped into pieces and incorporated into the soil when it is 50 days old and 1.5m high.

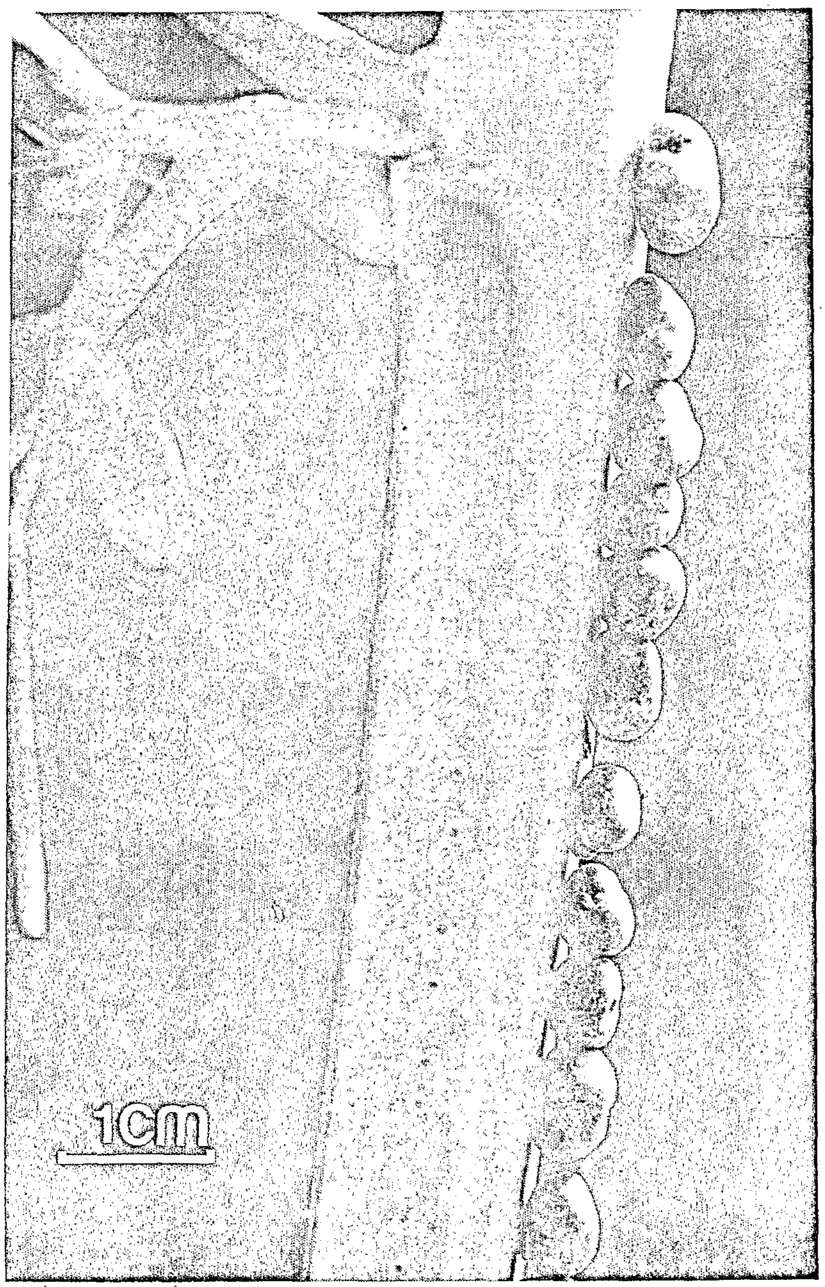

Fig. 3. Stem of Sesbania rostrata exhibiting a row of nodulation sites resembling small points (front) and a row of nodules originating from the infection of nodulation sites by strain ORS 571, the stem Rhizobium strain (right side).

A

B

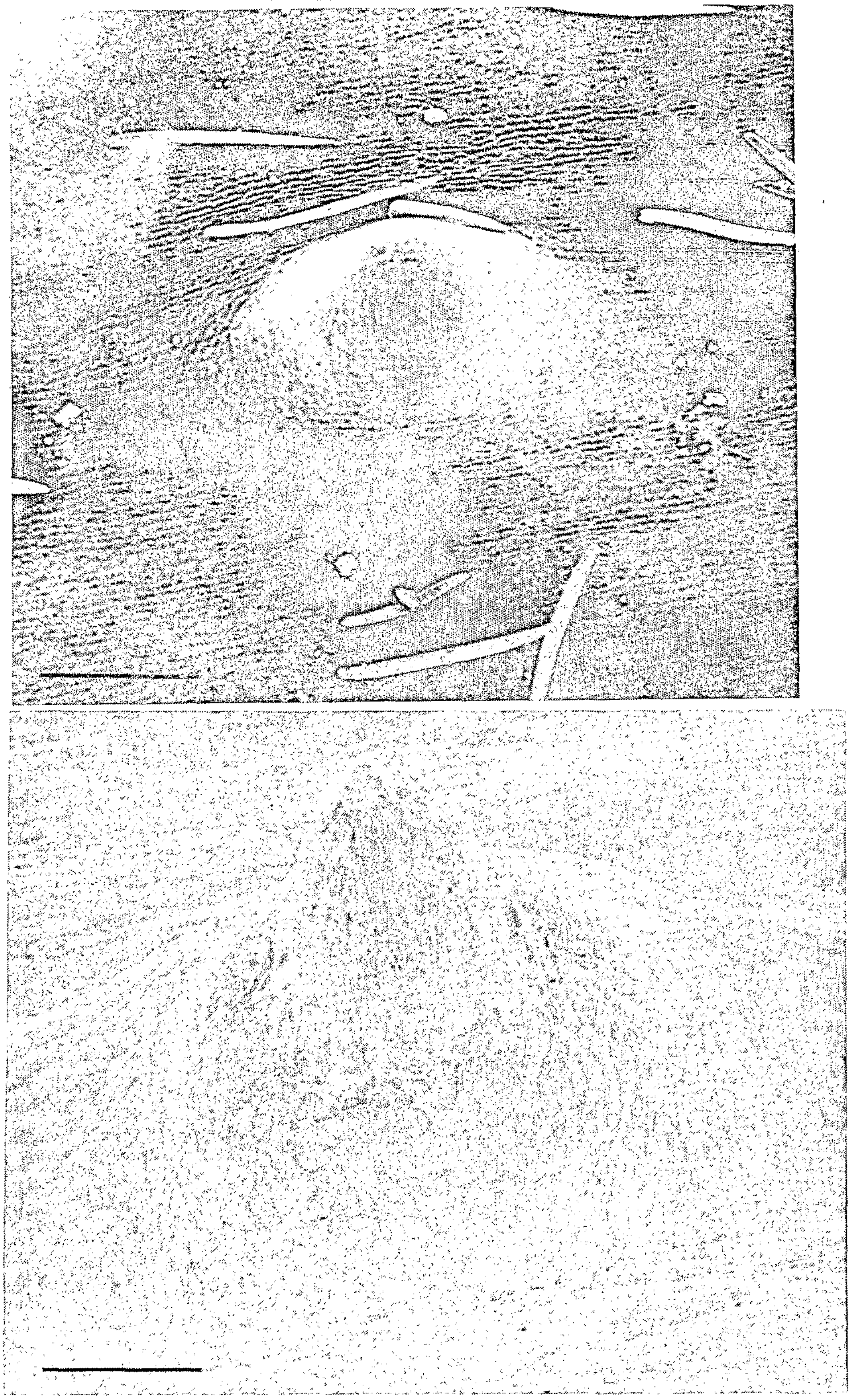

Fig. 4. A. The developing nodulation site resembles a protuberance on the stem.

B. The fully developed uninoculated nodulation site with its epidermal dome pierced by an incipient root (root primordium). Bar represents 25μm (Duhoux and Dreyfus, 1982).

Fig. 5. Longitudinal section of uninoculated nodulation sites showing the circular fissure (CA) formed where the root primordium (EB) emerges through the center of the epidermal dome. Bar represents 50μm (Tsien <u>et al.</u>,1983).

4. Double Nodulation of *Sesbania rostrata*

Sesbania rostrata can bear nodules on roots and stems at the same time.

4.1. Root nodules

When grown in non-inundated soil, the young *Sesbania rostrata* (15 days to 1 month old) displays two types of root nodules. At the level of the crown and at the base of the main(tap)root a cluster of large, elongated nodules appears with an apical meristem. These nodules have two or three lobes and measure from 0.2 to 1.5cm in length. Another type of root nodules appear like beads on a string on lateral roots. These nodules are spherical, small (1 to 2mm in diameter), and numerous. When the plant grows on inundated soil, the large nodules from the crown disappear rapidly and only those nodules located on the surface of the lateral roots remain. These roots float in water and bear many nodules with a green cortex. The total weight of these nodules remains low however (2 to 4 g) compared to that of stem nodules (15 to 40 g).

4.2. Stem nodules

In nature, stem nodules often appear spontaneously over the whole length of the plant, including lateral branches (Fig. 6). Distribution of these stem nodules is often irregular, however. Dust seems to play a significant role in spontaneous nodulation of stems, as plants that grow along dirt roads are generally more nodulated than other plants. Other vectors that may be significant include insects and rain. Not all nodulation sites are spontaneously infected, and the number and weight of stem nodules may vary from one plant to another. Some nodules are spherical, while others are irregular. The red central zone is surrounded by the green cortex. Diameter of the nodules ranges from 0,3 to 0.8cm, and mature plants of 3 to 4m high can bear nodules weighing up to 40g in fresh weight.

5. Genesis of stem nodules

Rhizobia never infect the nodulation sites from inside; they reach the root primordium through the fissure encircling it(Fig.5). Nodule genesis consists of three distinct stages:

1) Development of intercellular pockets of infection and dedifferenciation of the meristematic zone. Initially rhizobia are attached to the surface of the cells located at the base of the root primordium in the circular fissure. Later, they penetrate the intercellular space where they multiply in large numbers, forming pockets of infection (Fig. 7). At the same time, a differenciation of some cortex cells of the root primordium occurs through an activating mechanism which is still not known. The resulting meristematic cells are then infected by the rhizobia.

2) Development of infection threads. Infection threads originate from the intercellular pockets of infection (Fig. 8). These threads divide into several branches that penetrate the meristematic cells.

3) Intracellular infection. Rhizobia are released from the infection threads into the cell cytoplasm 5 or 6 days after infection starts (Fig. 9). When enclosed in the membrane envelope (Fig. 10), the rhizobia begin to fix nitrogen symbiotically. When the rhizobia have reached this stage (known as the bacteroid stage), the nodules exhibit the characteristic red color of leghemoglobin (Fig.11) and they begin fixing nitrogen.

Fig. 6. Spontaneous nodulation of a stem of Sesbania rostrata as observed in nature. Bar represents 1 cm.

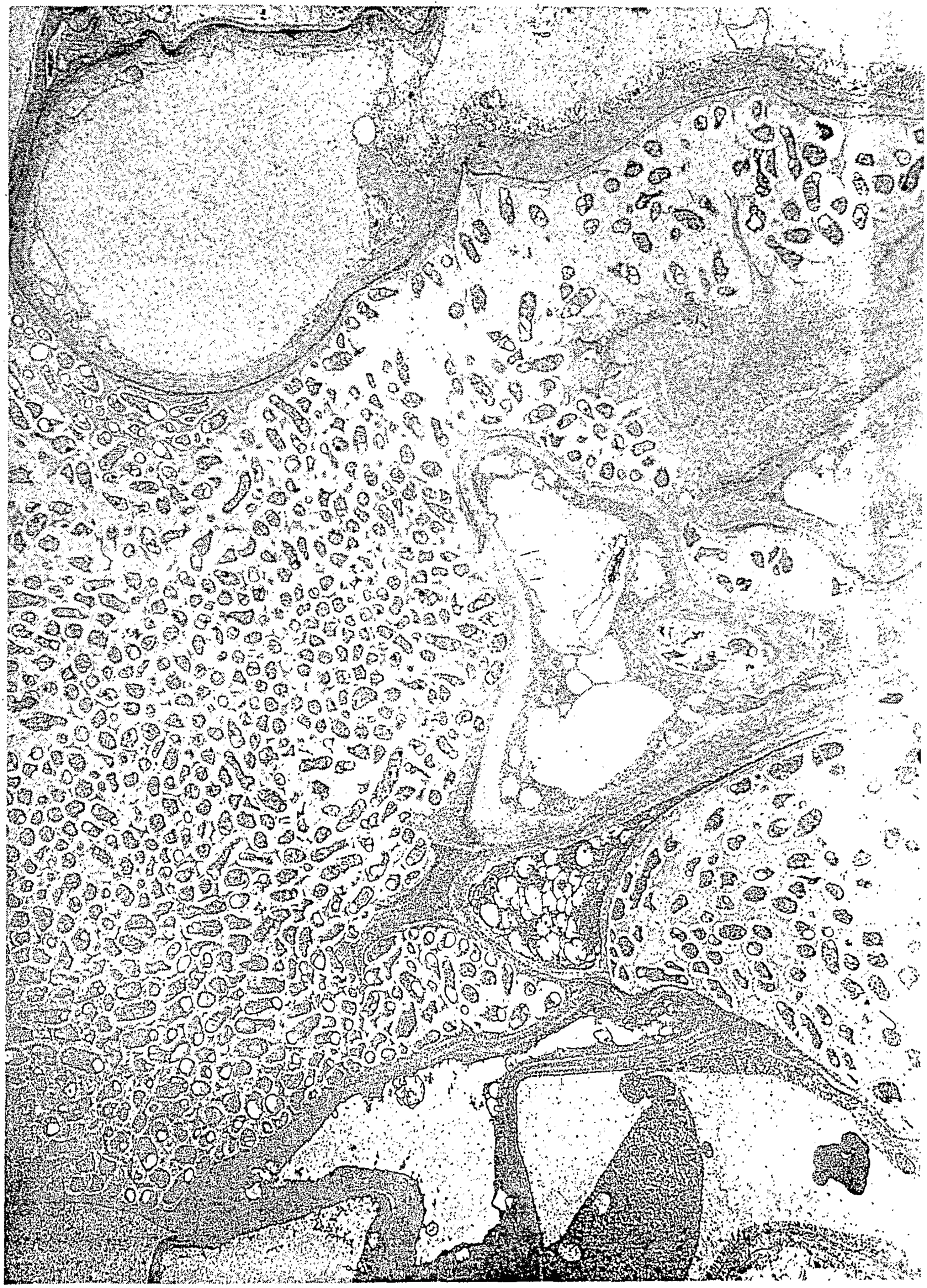

Fig. 7. Multiplication of rhizobia in intercellular spaces 3 days after inoculation sites have been inoculated (Tsien _et al._, 1983).

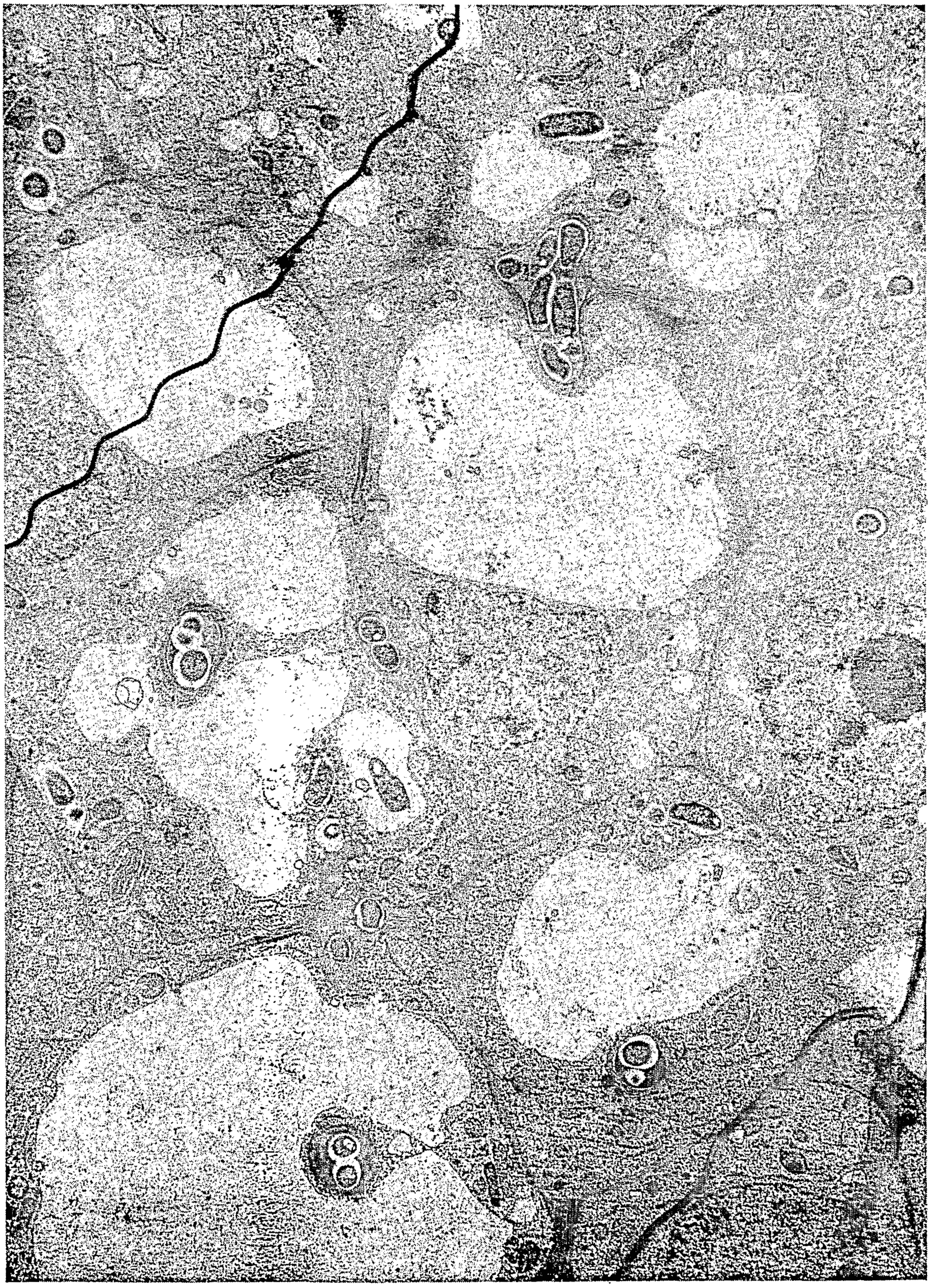

Fig. 8. Infection thread development, X1000 (Tsien et al., 1983)

Fig. 9. Immediately after being released from the infection thread within the host cell rhizobia are enclosed individually within a membrane. X 24.000 (Tsien et al., 1983)

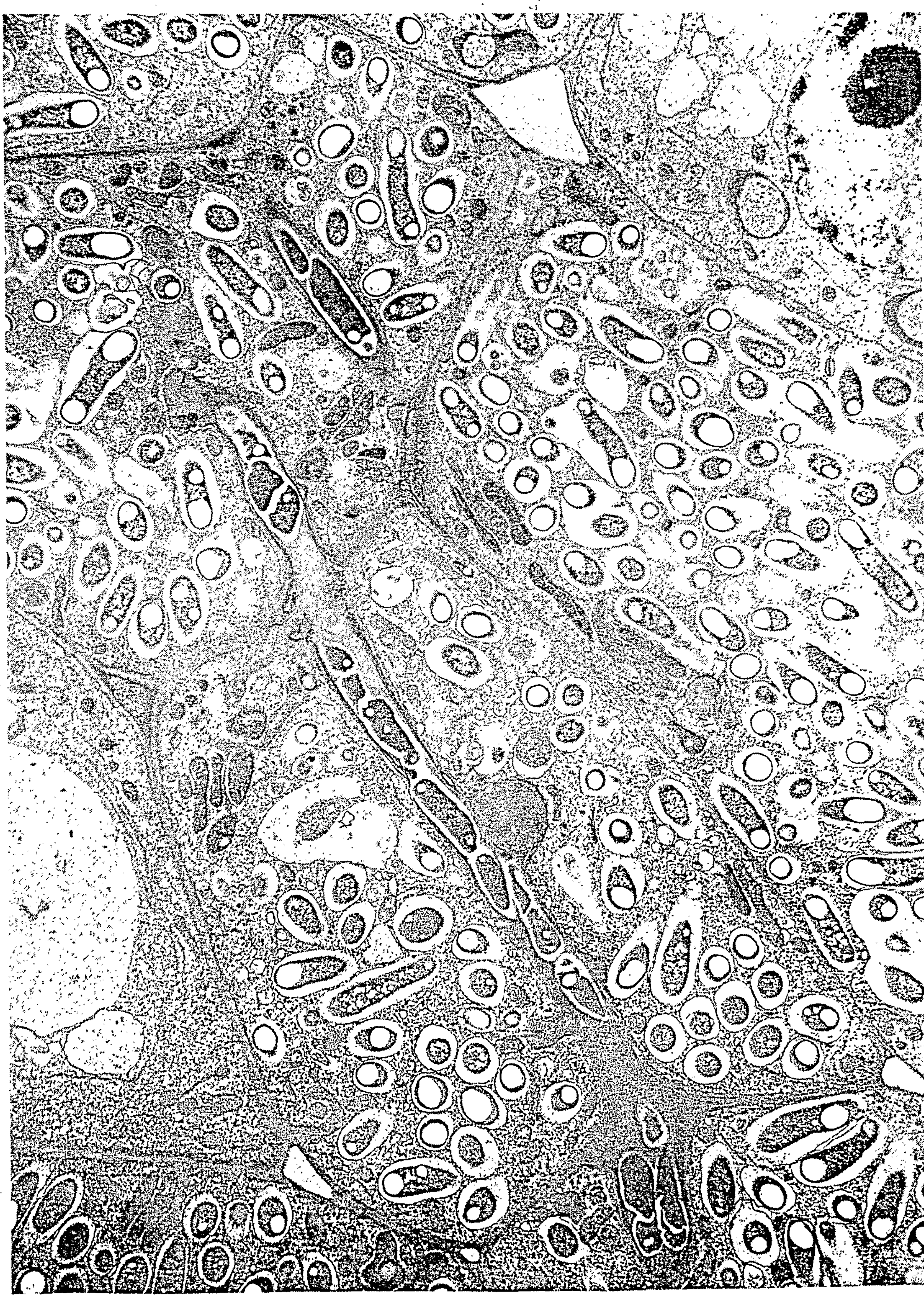

Fig. 10. Six days after inoculation, the host cytoplasm is invaded by the rhizobia X 9.000 (Tsien *et al.*, 1983).

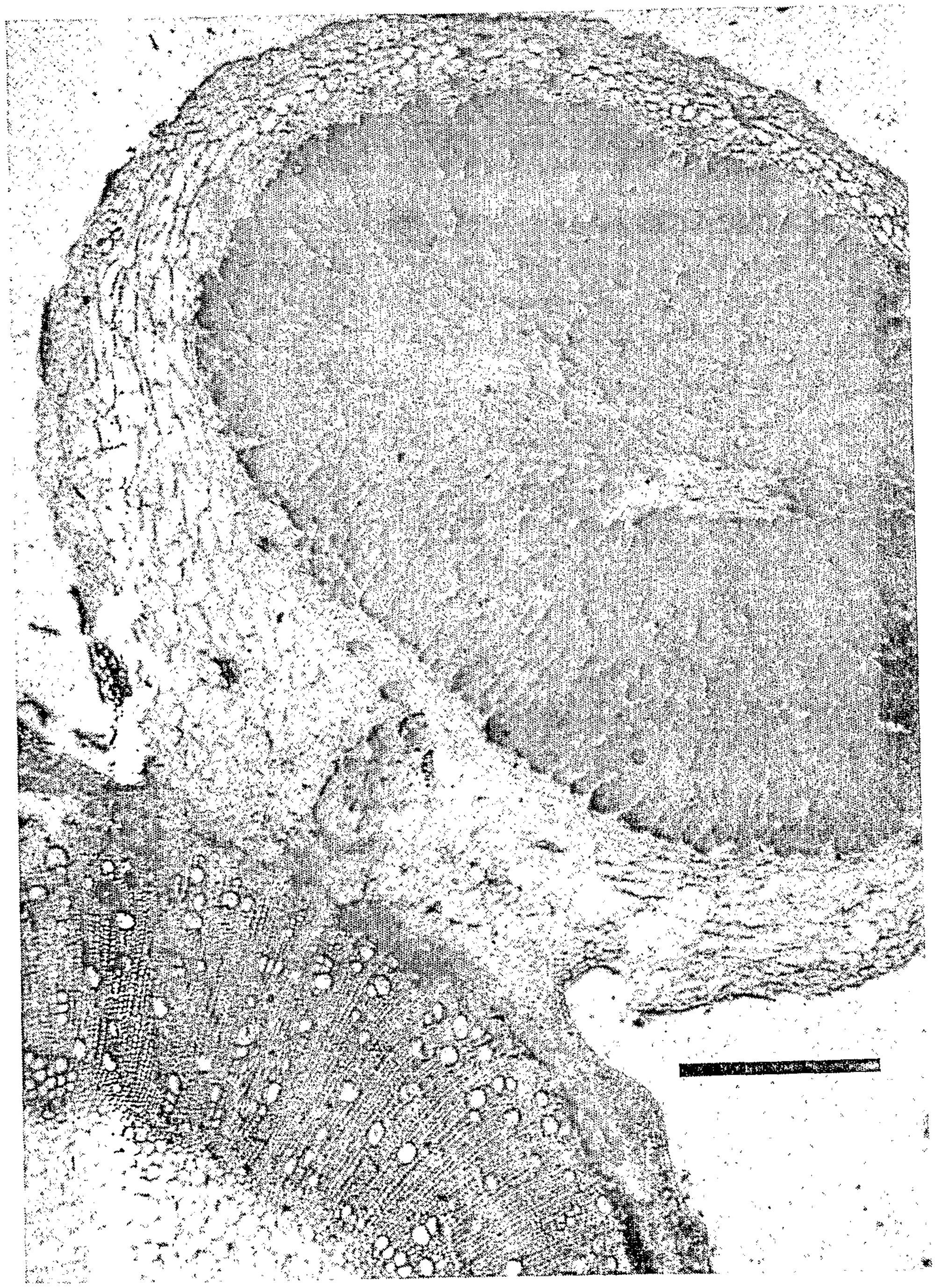

Fig. 11. Section of a mature nodule. Bar represents 0.5mm.

The mode of infection of *Sesbania rostrata* stems is thus unique among the legumes, as it involves both and intercellular invasion by rhizobia, as in peanuts, and the development of infection threads, as in temperate legumes. In contrast with temperate legumes, infection in *Sesbania rostrata* does not occur through root hairs.

6. *Rhizobium* strains of *Sesbania rostrata*

Two types of strains have been isolated: stem strains, capable of nodulating both stems and roots, and root strains, which nodulate only roots. Although the stem strains (type ORS571) are fast-growing, they differ from temperate fast-growing and tropical slow-growing rhizobia. Thus they probably constitute a new species.

A physiological study showed that stem strain ORS571 is able to grow with atmospheric nitrogen as the sole nitrogen source (Dreyfus et al., 1983). This property, unique among all other known rhizobia, has allowed us to obtain nif^- mutants of strain ORS571 by using standard genetic methods. Nitrogen fixation genes of the wild strains have been cloned in a plasmid. Following conjugation and transfert of this plasmid to a nif^- mutant, genetic complementation was observed both ex planta and in planta (Elmerich et al., 1982).

The liquid nutrient medium for the stem strain ORS571 is composed as follows: Na lactate: 10 g; $(NH_4)_2SO_4$: 1 g; K_2HPO_4: 1.67 g; KH_2PO_4: 0.87 g; $MgSO_4$: 0.2 g; NaCl: 0.1 g; $FeCl_3$: 4 mg; yeast extract: 1 g. pH is adjusted to 6.8 and the medium is autoclaved at 120°C for 20 minutes. The same medium is used with agar for cultures in Petri dishes.

7. Inoculation

As we have indicated earlier (p. 5) spontaneous inoculation of stems is always irregular. Thus, inoculation of stems is generally recommended, as is root inoculation (Fig. 12). However, the latter is not necessary when soils already harbor native rhizobia capable of nodulating *Sesbania rostrata*. We recommend strain ORS571, which is both competitive and effective in fixing nitrogen (Fig. 13).

7.1. Seed inoculation

Seeds are inoculated by a culture that is either liquid or adsorbed on a carrier such as peat (Vincent, 1970) or imbedded in a polymer (Jung et al., 1982).

7.2. Inoculation of the stems of *Sesbania rostrata*

Seed inoculation induces complete root nodulation but only partial stem nodulation. Thus, the shoots must be inoculated in order to obtain satisfactory stem nodulation. Either of the following methods can be used:

1) Spraying liquid culture on the shoots. When *Sesbania rostrata* have attained a height of 50-80cm, a culture of the strain ORS571 containing about 10^8 bacteria/ml is sprayed on the shoots using a standard sprayer.
2) Spraying colloidal solution of rhizobia imbedded in a polymer (alginate type). The inoculum made of rhizobia cells imbedded in a polymer and dried as a powder (jung et al., 1982) is mixed with water to obtain a colloidal solution, which is then sprayed on the shoots of *Sesbania rostrata*. An insecticide can be added to the colloidal solution to protect the *Sesbania rostrata* from insects[1].

(1) Patent pending

Fig. 12. Stems of Sesbania rostrata inoculated by spraying with a liquid culture of strain ORS 571. Note the regularity of nodulation.

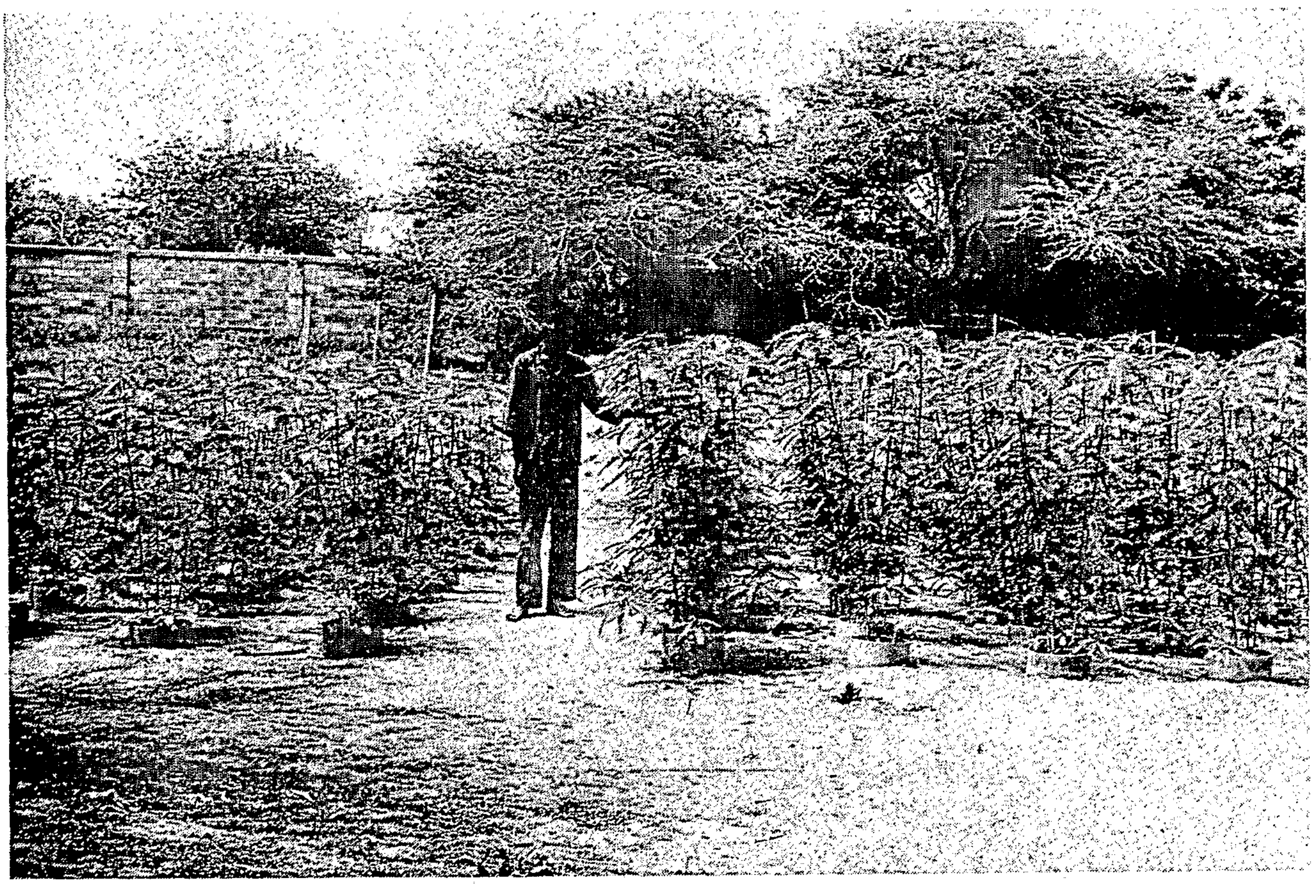

Fig. 13. Effect of stem inoculation (plot on the right) by strain ORS 571 on the growth of <u>Sesbania rostrata</u>.

8. Nitrogen fixation

Nitrogen fixation by stem nodules of *Sesbania rostrata* exhibits two unique properties. First, the *Sesbania rostrata* has a high nitrogen-fixing potential. Nitrogen fixation of *Sesbania rostrata* by the acetylene method is about 600μmole C_2H_4/h/plant, a much higher rate than that of soybean, which ranges from 14 to 120μmole C_2H_4/h/plant. When the difference method and the nitrogen balance method are used, nitrogen fixation by *Sesbania rostrata* has been whown to be about of 200 kg N_2/ha in 50 days, which indicates that this legume is one of the most powerful nitrogen-fixing systems. The second unique property of *Sesbania rostrata* is its ability to nodulate and fix nitrogen even when the rates of combined nitrogen in the soil are high (about 200 kg N/ha). Thus *Sesbania rostrata* is capable of assimilating both soil and atmospheric nitrogen, which constitutes a significant advantage.

CHAPTER 2. PRELIMINARY FINDINGS ON THE USE OF *SESBANIA ROSTRATA* AS GREEN MANURE IN PADDY FIELDS

1. Methodology

Seeds of *Sesbania rostrata* are sown in rows in wet soil. When the plants have attained a height of 30cm, the paddy fields are flooded. Stems are inoculated by spaaying with a culture of the specific *Rhizobium* 20 to 30 days after sowing. When the planss have attained a height of 1.5 m (6 to 7 weeks after sowing), the paddy field is drained and the plants are uprooted and chopped into pieces 10-20cm long which are incorporated into the soil at a depth of 10-15cm. In the first trials ($1m^2$ microplots), rice was planted 2 weeks after incorporation of *Sesbania rostrata* into the soil. Later trials ($25m^2$ plots) whowed that rice could be planted immediately after incorporation of *Sesbania rostrata*, significantly reducing the duration of the *Sesbania* -rice sequence.

2. Effect of *Sesbania rostrata* manure on rice yields

Based on microplot trials conducted at the Bel Air ORSTOM Station in Dakar in the summer of 1980, the use of *Sesbania rostrata* as green manure doubled rice yeelds. These results were later confirmed in trials conducted at the ISRA Station of Djibelor on $25m^2$ plots.

2.1. Microplot trials at the Bel Air ORSTOM Station (Table 1)

2.1.1. *Principal effect*

Yields in dry matter (grains and straw) were more than double those of controls in plots where *Sesbania* *rostrata* green manure was used. The effect of inorganic nitrogen fertilization was significantly less pronounced than that of *Sesbania rostrata* green manure. Moreover, the nitrogen content of grains and straw in the *Sesbania rostrata* plots was significantly higher (by 50%) than that of control plots.

Table 1. Effect of *Sesbania rostrata* green manure on yield and nitrogen content of rice.

Treatments	Dry weight (g/m^2)		Nitrogen content (%)	
	Grain	Straw	Grain	Straw
S. rostrata	584 a	767 a	1.74 a	0.96 a
$(NH_4)_2SO_4$ (1)	381 b	484 b	1.27 b	0.49 b
Control	212 c	276 c	1.14 b	0.58 b

Numbers with the same letters in columns do not differ significantly, P = 0.05.

(1) 60 kg N/ha

2.1.2. *After-effect of Sesbania rostrata green manure.*

Rice was again planted on $1m^2$ plots the following year, without using *Sesbania rostrata* green manure. The residual effect of *Sesbania rostrata* green manure from the preceding year resulted in a 50% yield increase over control plots (328 and 230 g/m^2 respectively).

2.2. <u>Trials on $25m^2$ plots at the ISRA Station at Djibelor</u> (Table 2).

Results obtained at the ISRA Station at Djibelor in the summers of 1981 and 1982 showed that the use of *Sesbania rostrata* green manure doubled the grain yields in comparison with the control plots. The application of organic matter (compost + farm yard manure) to the soil had a less marked effect.

Table 2. Effects of *S. rostrata* green manure and application of organic matter on grain yields of rice

Treatments	Grain yield (kg/25m^2)	
	1981	1982
S. rostrata	7.30	11.25
Organic matter	3.44	7.30
Control	2.99	5.75

Note: Each figure represents the average of six replications

3. Cause of observed effects

3.1. Nitrogen input (Table 3)

The nitrogen balance calculated from microplot trial data showed that the use of *Sesbania rostrata* green manure induced a large gain of nitrogen (59 ± 27 g N/m^2), resulting from nitrogen fixation by *Sesbania rostrata* stem nodules (Rinaudo et al., 1982). This gain in nitrogen explains the increase in grain yields.

Table 3. Effect of *Sesbania rostrata* green manure on the shoots and soil nitrogen content

Treatments	Total nitrogen (g/m^2)		
	Shoots	Soil	Shoots + Soil
S. rostrata	18 ± 4 a	177 ± 14 a	195 ± 14 a
$(NH_4)_2SO_4$ (1)	7 ± 1 b	139 ± 5 b	146 ± 6 b
Control	4 ± 1 c	132 ± 12	136 ± 13 b

Numbers with the same letters in columns do not differ significantly, P = 0.05

(1) 60 kg N/ha.

3.2. Input of organic matter

The incorporation of *Sesbania rostrata* into the soil results in an input of 2 kg of organic matter (dry weight) per m^2 or 20 T/ha. For soils low in organic matter, such an input significantly improves soil structure.

3.3. Effect on certain pathogenic nematodes of rice

Another set of experiments conducted on $1m^2$ microplots that had been infested with *Hirschmanniella oryzae*[1] suggested that *Sesbania rostrata* probably acts as a plant trap for nematodes (Germani et al., 1983). *Sesbania rostrata* also acts to limit the pathogenic nematode population. The rice cultivated after *Sesbania rostrata* incorporation grows in an environment where the infestation rate is low enough to protect the crop against harmful precocious attacks by nematodes. This protective effect is another beneficial characteristic of *Sesbania rostrata* green manure in paddy fields.

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[1] The same effect has been observed with *Hirschmanniella spinicaudata* (Germani, personal communication, 1983).

CHAPTER 3. GUIDELINES FOR *SESBANIA ROSTRATA* TRIALS

These trials should be conducted in three successive stages. The first stage consists in determining whether it is possible to introduce *Sesbania rostrata* in a given geographic region. If the results of these trials are positive, one can proceed to the second stage, which involves assessing the effect of *Sesbania rostrata* green manure on rice yields under irrigated conditions. If the results of this second stage are encouraging, one can advance to the final stage, which consists in perfecting the application of *Sesbania rostrata* green manure both in paddy fields (one or more harvest per year) and drained soils.

Stage 1: Assessment of *Sesbania rostrata* behavior in a given geographic region.

1. Objectives

1.1. To determine whether *Sesbania rostrata* thrive under the climatic and soil conditions of the region.

1.2. If so, to determine the optimal time or times for planting *Sesbania rostrata* in order to maximise biomass, nodulation, and seed production.

1.3. To examine the problems posed by various pests.

2. Trial Design

For each of the soil types to be studied (e.g. soils with different levels of salt or organic matter content), a plot of 2 x 6m divided into four subplots of 2 x 1.5m should be delimited. *Sesbania rostrata* should be planted on each subplot at 3-month intervals (Fig. 14).

Each subplots contains three rows of *Sesbania rostrata* (designated A, B and C). Row A should be harvested 50 days and row C 100 days after seeding in order to assess biomass production. *Sesbania rostrata* in row B should be maintained throughout the growth cycle so that the seeds can be harvested. Because these trials are primarily exploratory, replications are not essential.

2.1. Preparation of soil and application of PK fertilizer

The soil should be plowed and PK fertilizer applied at the standard rates used in the region. For example, the following rates are used in Senegal: 30 kg P/ha; 8 kg K/ha.

2.2. Treatment of seeds

Before planting, the seeds should be treated as indicated on page 18. They should then be inoculated according to the technique outlined on page 8 (this inoculation induces nodulation on the root system and at the bottom of the stem).

2.3. Seeding

Seeding density is shown in Fig. 14 (one seed every 10cm on rows 50cm apart). *Sesbania rostrata* should be seeded in wet but not flooded soil.

2.4. Irrigation and stem inoculation

When *Sesbania rostrata* has attained a height of more than 30cm, soil should be flooded. When the plants have reached a height of 50-80cm, shoots should be inoculated by spraying a culture of *Rhizobium* (cf page 8).

3. Data collection (1)

3.1. Characteristics of experimental sites

3.2. Seeding

3.3. Measure height of *Sesbania rostrata* from soil to top (last growing point) every 2 weeks.

3.4. Determine total biomass (fresh weight and, if possible, dry weight at 80°C) when shoots are cut: 50 days after seeding for row A, 100 days after seeding for row C, and at the end of the growth cycle for row B.

Table 4 presents a timetable for these measurements (12 sets in all). If possible, determine the total N percentage of leaves and stems of *Sesbania rostrata* for row A. Leaves and stems should be weighed separa rately, and carefully sampled preceding nitrogen analysis.

Table 4. Timetable for data collection in the subplots

	Dates of sowing in each subplot			
	D	D+3 months	D+6 months	D+9 months
Row A	D+50 days	D+3 months + 50 days	D+6 months + 50 days	D+9 months + 50 days
Row C	D+100 days	D+3 months +100 days	D+6 months +100 days	D+9 months +100 days
Row B	D+T	D+3 months +T	D+6 months +T	D+9 months +T

D = Starting day of trial; T = total duration of *Sesbania rostrata* growth cycle.

3.5. Nodulation. Note the date of inoculation and appearance of nodules. Determine number and weight (fresh and dry) of stem nodules at harvest for rows A and C.

(1) See data sheet on page 23.

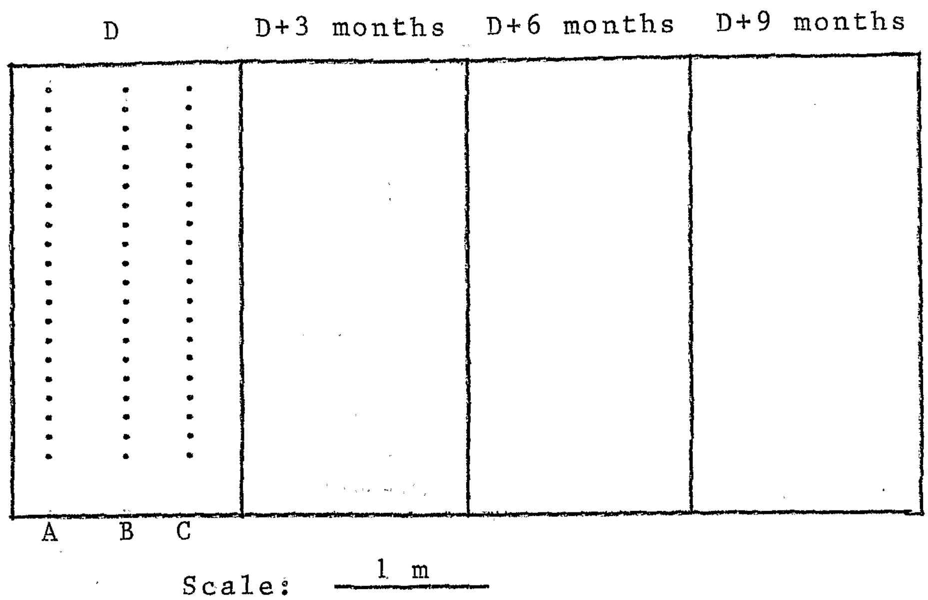

Fig. 14. Assessment of *Sesbania rostrata* behavior. For each of the soil types to be studied, a plot of 2 x 6 m divided into 4 subplots of 2 x 1.5m should be delimited. *Sesbania rostrata* should be planted on each subplot at 3-month intervals. In the first subplot, seeding should take place on "D-day"; in the second, on D+3 months; in the third, D+6 months; and in the fourth, D+9 months. Each subplot should contain 3 rows of *Sesbania rostrata* (designated A, B, and C). Seeds should be spaced at 10cm apart in each row, with a total of 20 seeds per row. The plants in row A should be cut 50 days and plants in row C 100 days after sowing. Plants in row B should be maintained throughout the growth cycle so that the seeds can be harvested.

3.6. Flowering and fructification. Note the date of flowering and fructification an determine the seed yield.

3.7. Pests. Note the dates and conditions of pest attacks. Apply necessary treatments. Pests that attack *Sesbania rostrata* are not well known. However, we have observed that young plants can be attacked by a black aphid, flowers can be eaten up by coleopteran, and seeds can be destroyed by hymenopteran. The destructive effect of these pests is greatly reduced in the summer, when environmental conditions are more favorable to the growth of *Sesbania rostrata*.

4. Seed harvest

Sesbania rostrata produces seeds in abundance as soon as the days get shorter. Seeds should be harvested as they mature. Treat the seeds with insecticide.

Stage 2: Assessment of the effect of *Sesbania rostrata* green manure on rice yields under irrigated conditions.

1. Objectives.

1.1. Assess the increase of rice yields (grains) (grains) resulting from use of *Sesbania rostrata* as green manure.

1.2. Compare this response to that observed with three different levels of added N fertilizer (e.g. 50, 100, and 150 kg N/ha).

1.3. Assess the after-effects of *Sesbania rostrata* green manure.

2. Trial design

The effects of the following three treatments should be compared:

Treatment A: *Sesbania rostrata* green manure

Treatment B: nitrogen fertilizer at 3 levels

Treatment C: control.

The trial should be conducted using randomized blocks with five to six replications. The minimum surface of each plot should be $20m^2$. Figure 15 presents an example of a trial design with six replications ($28m^2$ plots). This experimental design could be incorporated into a larger design aimed, for instance, at comparing different types of fertilization.

2.1. Fertilizer application

The PK fertilizer should be applied at the standard rates used in the region, as indicated page 15. For plots where treatment B is used, urea nitrogen fertilizer should be used, as other forms of fertilizer introduce other elements, such as S or P, which would distort the results. Application should be staggered.

2.2. *Sesbania rostrata* seeds

2.2.1. *Stocking up seeds*

A sufficient amount of seeds must be stocked in stage 1, prior to the start of field trials. The experiments require 100 seeds (2 g) per m^2, or 20 kg per ha.

2.2.2. *Seed treatment*

As is true for many tropical legumes, *Sesbania rostrata* seeds germinate rapidly and homogeneously only when they are treated chemically or scarified in a mortar. Chemical treatment consists of submerging seeds into concentrated sulphuric acid for 30 minutes and then rinsing them quickly in a large amount of water. Seeds can also be scarified by mixing them with an equivalent quantity of siliceous coarse sand and then pouring this mixture into a mortar and delicately rotating the pestle in a scraping motion until the grains become pock-marked.

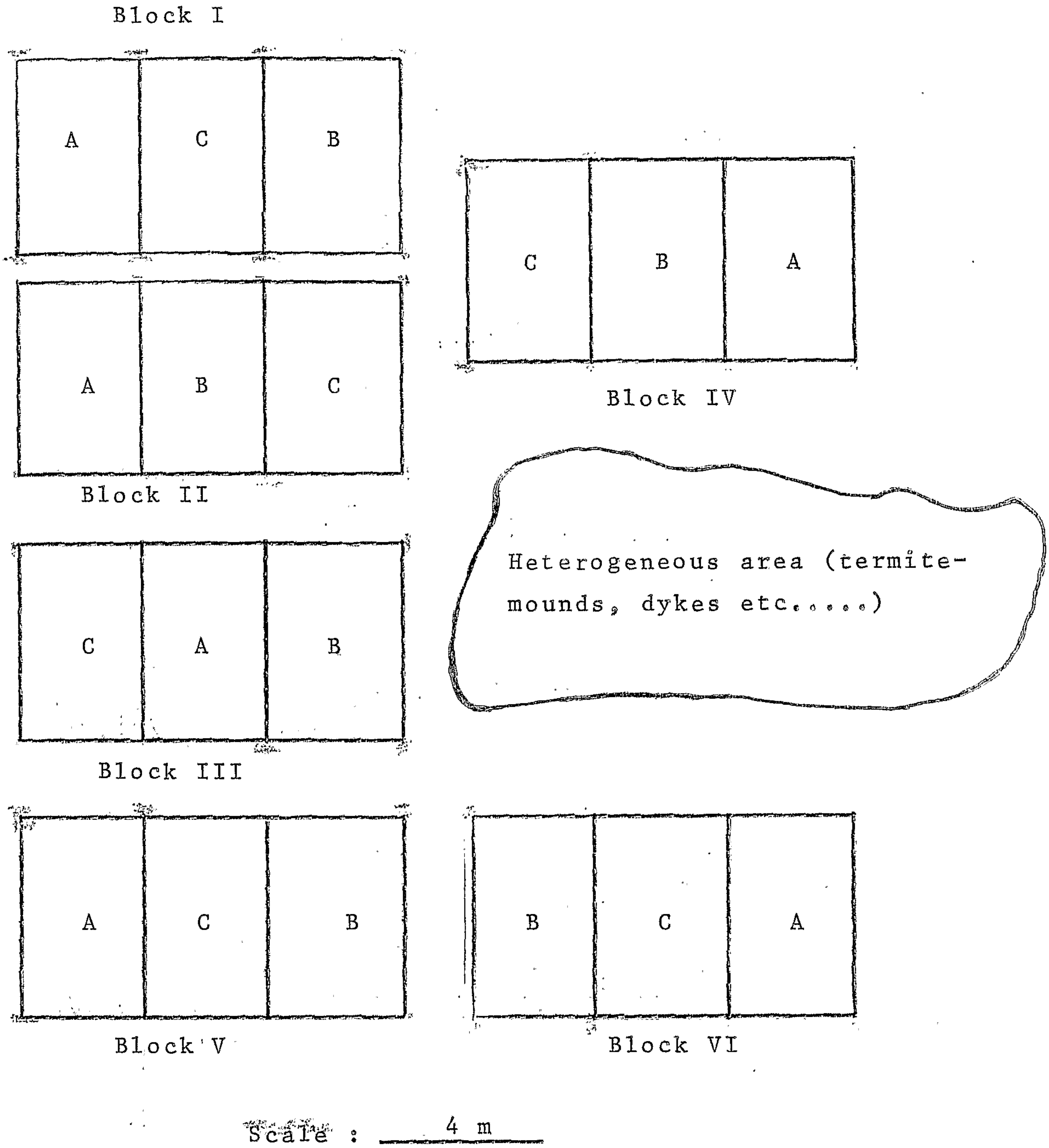

Fig. 15. Example of an experimental design with four replications, each plot having a surface of 28 m^2. In this example, the blocks have not been juxtaposed so as to avoid an heterogeneous zone. The three treatments are designated A, B, and C.

It is advisable to inoculate the seeds before seeding by pouring a *Rhizobium* culture over the seeds (cf page 8). Note: Laboratory trials have shown that *Sesbania rostrata* could be easily multiplied using cuttings; however, field trials have not yet demonstrated the applicability of this method to agriculture.

2.2.3. *Seeding*

S. rostrata should be seeded at the rate prescribed on page 15, in wet but not flooded soils.

2.3. *Sesbania rostrata* culture

The following precautions should be taken in cultivating *Sesbania rostrata*:

1) Do not flood paddy fields until the plants have attained a height of 30cm.
2) Stems should be inoculated by one of the two methods described on page 8 when the plants have reached a height of at least 50cm.

A description of culture medium for the specific *Rhizobium* of *Sesbania rostrata* appears on page 7.

2.4. Incorporation of *Sesbania rostrata* into the soil

When *Sesbania rostrata* is 50 days old and has reached a height of 1.5m, the paddy field should be drained. Plants should be uprooted or severed at soil level when the soil has been sufficiently drained (about 5-7 days later). Plants should be chopped into segments 10-20cm long and incorporated into the soil at a depth of 10-15 cm.

While incorporation of *Sesbania rostrata* into the soil has been carried out by hand up to now, it would be advantageous to mechanize this operation.

2.5. Rice planting

Rice can be planted immediately after *Sesbania rostrata* is incorporated into the soil.

3. Data collection[1]

3.1. Characteristics of the experimental site

3.2. Seeding

3.3. Fertilizer application

3.4. Nodulation

Determine the number and weight of nodules that have appeared on 10 plants randomly sampled before they are incorporated into the soil.

3.5. Biomass

Determine total biomass (fresh and, if possible, dry weight at 80°C) when shoots are cut. If possible, determine the total N percentage of leaves and stems of *Sesbania rostrata*.

Stage 3: Perfecting and extending the application of *Sesbania rostrata* green manure

In this final stage, it is desirable not only to perfect the method of applying *Sesbania rostrata* manure in paddy fields, but to extend its use to rainfed crops. Following are some general suggestions for trials that could be conducted.

1. Irrigated rice culture

Sesbania rostrata can be inserted into the rice crop by growing *Sesbania rostrata* and rice either sequentially (page 17), or simultaneously. In the latter case, *Sesbania rostrata* should be grown between rows of rice and incorporated into the soil when the legume is from 30 to 50 days old.

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(1) See the data sheet on page 24.

Up until now, trials have been conducted only for cultural systems involving only one crop a year. It may be useful to study the possibility of inserting *Sesbania rostrata* in a cultural system involving two or three rice crops per year.

2. Rainfed rice culture and other rainfed crops

Because rainfed rice culture has expanded significantly over the last two decades, it may be worthwhile to develop methdds of using *Sesbania rostrata* either as green manure (incorporation *in situ*) or as organic nitrogen fertilizer for rainfed crops (incorporation into soils distant from plots where *Sesbania rostrata* is grown). To produce organic nitrogen fertilizer from *Sesbania rostrata*, one should grow, harvest, transport, and incorporate *Sesbania rostrata* into the soil of the rainfed crop as fresh or composted organic fertilizer. For this purpose, *Sesbania rostrata* can be grown in soils unsuitable for other crops (e.g., temporary ponds, saline soils, etc...), provided that these soils are sufficiently wet.

ACKNOWLEDGMENTS

The published and unpublished studies carried out in the ORSTOM laboratory at Dakar were supported by the French Ministry of Industry and Research, grant 81.G.1451

We thank M. Boureau for the photograph and Deborah Gold for the translation from French into English.

ANNEXE 1. HOW TO OBTAIN *SESBANIA ROSTRATA* SEEDS AND THE SPECIFIC STRAIN OF *RHIZOBIUM*

1. Seeds

Seeds can be obtained from the laboratory of soil microbiology of ORSTOM, B.P. 1386, Dakar, Senegal. Because our stock is limited, individual orders must not exceed 500 seeds. This quantity should be sufficient to set up a field trial as described on pages 14.

2. Specific strain of *Rhizobium* (ORS571)

This strain can also be obtained from the above laboratory.

ANNEX 2. DATA SHEET N°1: ASSESSMENT OF *SESBANIA ROSTRATA* BEHAVIOR IN A GIVEN GEOGRAPHIC REGION

- Experiment site
- Latitude, longitude
- Soil type
- Climatic characteristics
- Date of seeding *Sesbania rostrata*
- Percentage of germinated seeds
- Date of stem inoculation
- Height of stems at harvest
- Fresh and dry weight of stems
- Fresh and dry weight of leaves
- Percentage of nitrogen in stems
- Percentage of nitrogen
- Date of appearance of stem nodules after inoculation
- Number and weight (fresh and dry) of stem nodules at harvest
- Dates of flowering (beginning and end)
- Dates of seed maturation (beginning and end)
- Weight of harvested seeds
- Pests
- Remarks

Note: The completed form should be returned to
Dr. B. Dreyfus, Laboratoire de Microbiologie des Sols
ORSTOM, B.P 1386, DAKAR, Sénégal.

ANNEX 3. DATA SHEET N°2: ASSESSING THE EFFECT OF *SESBANIA ROSTRATA* GREEN MANURE ON RICE YIELDS UNDER IRRIGATED CONDITIONS

- Experiment site
- Latitude, longitude
- Soil type
- Climatic characteristics
- Quantity of fertilizer applied (P, K, urea N in kg/ha)
- Date of seeding *Sesbania rostrata*
- Percentage of germinated seeds
- Date of stem inoculation
- Date of appearance of stem nodules
- Number and weight of stem nodules before incorporation of *Sesbania rostrata* shoots into the soil
- Height (cm) and weight (fresh and dry) in g/m^2 of *Sesbania rostrata* shoots before incorporation into the soil
- Date of rice planting
- Date of rice harvest
- Rice yield kg grains/ha and percentage of nitrogen in grains in the various treatments
- Remarks

Note: The completed form should be returned to Dr. B. Dreyfus, Laboratoire de Microbiologie des Sols ORSTOM, B.P 1386, DAKAR, Sénégal.

REFERENCES

BERHAUT, J. (1976). Flore illustrée du Sénégal, Tome V, p 515, Clairafrique, Dakar.

DREYFUS, B. (1982). La symbiose entre *Rhizobium* et *Sesbania rostrata*, légumineuse à nodules caulinaires. Thèse de doctorat d'état, Université Paris VII.

DREYFUS, B. et DOMMERGUES, Y.R. (1980). Non-inhibition de la fixation d'azote atmosphérique par l'azote combiné chez une légumineuse à nodules caulinaires, *Sesbania rostrata*. C.R. Acad. Sci. Paris, D, 291, 767-770.

DREYFUS, B. et DOMMERGUES, Y.R. (1981). Nitrogen-fixing nodules induces by *Rhizobium* on the stem of the tropical legume *Sesbania rostrata*. FEMS Microbiol. Letters. 10, 313-317.

DREYFUS, B., ELMERICH, C., and DOMMERGUES, Y.R. (1983). Free-living *Rhizobium* strain to grow under N_2 as the sole nitrogen source. Appl. Environ. Microbiol., 45, 711-713.

DUHOUX, E. et DREYFUS, B. (1982). Nature des sites d'infection par le *Rhizobium* de la tige de la légumineuse, *Sesbania rostrata*. C.R. Acad. Sc. Paris, t. 294, sér. III, 407-411.

ELMERICH, C., DREYFUS, B.L., REYSSET, G. and AUBERT, J.P. (1982). Genetic analysis of nitrogen fixation in a fast-growing *Rhizobium*. The Embo J., 1 , 499-503.

GERMANI, G., REVERSAT, G. et LUC, M. Effect of *Sesbania rostrata* on Hirschmanniella oryzae in flooded rice. J. Nematol. (Sous presse).

JUNG, G. MUGNIER, J., DIEM, H.G., and DOMMERGUES, Y.R. (1982). Polymer-entrapped *Rhizobium* as an inoculant for legumes. Plant Soil, 65, 219-231.

PATNAIK, S et RAO, M.V. (1979). Sources of nitrogen for rice production. In: Nitrogen and Rice, International Rice Research Institute, Los Banos, Philippines, 25-43.

RINAUDO, G., DREYFUS, B, and DOMMERGUES, Y.R. (1982). *Sesbania rostrata* as a green-manure for rice in West Africa. In: Biological Nitrogen Fixation Technology for Tropical Agriculture. (P.H. GRAHAM and S.C. HARRIS, eds.) Centro International de Agricultura Tropical, Cali, Colombia, 441-445.

RINAUDO, G., DREYFUS, B., and DOMMERGUES, Y.R. (1982). Influence of *Sesbania rostrata* green-manure on the nitrogen content of rice crop and soil. Soil Biol. Biochem. 15, 111-113.

TSIEN, B., DREYFUS, B. and SCHMIDT, E.L. (1983). Initial stages in the morphogenesis of the nitrogen-fixing stem nodules of *Sesbania rostrata* J. Bacteriol. (in press).

VACHHANI, M.V. and MURTY, K.S. (1964). Green manuring for rice. Indian Council of Agric. Res. Rep. Ser. No 17, 50 p.

VINCENT, J.M. (1970). A manual for the practical study of root-nodule bacteria. IBP Handbook No 15, Blackwell Scientific Publication, Oxford and Endinburgh, 164 p.

WATANABE, I. (1980). Report on the INSFFER field observation tour to China, 3-14 September 1980 (mimeo).

WATANABE, I. and APP, A. (1979). Research needs for management of nitrogen fixation in flooded rice crop system. In: Nitrogen and Rice, International Rice Research Institute, Los Banos, Philippines, 485-490.

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