Root-Nodule Bacteria of Tropical Legumes

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The legume-*Rhizobium* symbiosis has been mostly studied with agricultural legumes of the temperate regions. The *Leguminosae*, one of the largest families in the plant kingdom, however, comprises over 550 genera and 13,000 species, and most of them occur in tropical or subtropical regions. Of these, about 1,200 species (10% of all the leguminous species) have been examined for nodulation and not all of them nodulate[1].

The legumes are grouped into the so-called cross-inoculation groups on the basis of their nodule production-kinship with nodule bacteria[5]. Many tropical or subtropical legumes are comprised in the cowpea group which is the largest one of the cross-inoculation groups. It has often been pointed out that unsolved problems remain especially in this cowpea group from the conception of cross-inoculation.

The nodule-bearing legumes undoubtedly play a greater part in nitrogen economy in agriculture as well as in nature. The recognition of the legume-*Rhizobium* symbiosis, therefore, will be particularly important in the tropics or subtropics.

In the present article, descriptions are made on general characters of nodule bacteria from some tropical or subtropical legumes and their symbiotic phenomena with host plants.

Isolates from various legume comprised in the cowpea group: *Albizzia moluccana, Calopogonium mucunoides Centrosema pubescens, Crotalaria anagyroides, C. usaramoensis Desmodium gyroides Mucuna capitata, Tephrosia maxima, T. noctiflora. T. Vogelii,* and *Vigna sinensis*

1) *General characters in artificial media*

The cowpea group comprises many genera and species of *Leguminosae*, whereas *Rhizobium* of this group has hitherto been mostly considered one of slow growers, just like the soybean bacteria, *Rhizobium japonicum*.

Detailed survey, however, revealed the presence of various types of *Rhizobium* in addition to the slow grower type. The difference in characters among each type is shown in Table 1 and Fig. 1[6].

Of these types, type B corresponds to the one which has been considered as the representative of cowpea bacteria. Type A is easily distinguished from type B by agar slope culture, but both types are very similar to each other in other characters.

Although most isolates are unable to grow on potato, the method of its preparation is important. In case of bouillon, too, notice must be placed on the composition. Most of isolates are unable to grow in the following bouillon A, but are able to grow when the bouillon B is used.

A: Meat extract (Liebig) 5g
Peptone 10g

Table 1. General characters of each type of isolates from some tropical legumes

	Yeast water mannitol agar slope	Potato	Bouillon	Litmus reaction	Milk zone	Nitrate reduction	Utilization of raffinose or lactose*		Isolated from
A	Fairly rapid growth, filiform, thin, watery, transparant to translucent	⊕	—	—	—	+	—	—	*Albizzia, Calopogonium, Centrosema, Crotalaria anagyroides, Desmodium, Mucuna, Tephrosia, Vigna*
B	Slow growth, raised, glistening, opaque, white	—	—	—	—	+	—	—	*Calopogonium, Centrosema, Crotalaria, Mucuna Tephrosia maxima, T. noctiflora, Vigna*
A B	Fairly rapid growth, slightly raised, moist, glistening, translucent, white	—	—	—	+	+	—	—	*Crotalaria usaramoensis*
C	Very slow growth, scanty, filiform, glistening, opaque, white, viscous	—	—	—	—	+	—	—	*Centrosema*
A C	Fairly rapid growth, scanty, glistening, opaque, white, slightly, viscous	—	—	—	⊕	+	—	—	*Albizzia*
D	Fairly rapid growth, raised, glistening, translucent, white	⊕	—	—	+	?	+	+	*Calopogonium, Tephrosia maxima, T. Vogelii*
E	Fairly rapid growth, filiform, glistening, opaque, white	+	+	—	—	+	—	+	*Vigna*
F	Fairly rapid growth, raised, glistening, opaque, white	—	—	—→⊕	+	+	+	+	*Phaseolus angularis*
G	Similar to F, but less moist	—	—	—→⊕	+	+	+	+	*Vigna*

Potato & Bouillon: —: No growth ⊕: Slight growth +: Growth, Litmus milk: Reaction —: Alkaline ⊕: Slightly acid
Zone: —: Not formed, +: Formed, ⊕<+ * Depend upon the growth in asparagine raffinose or lactose medium

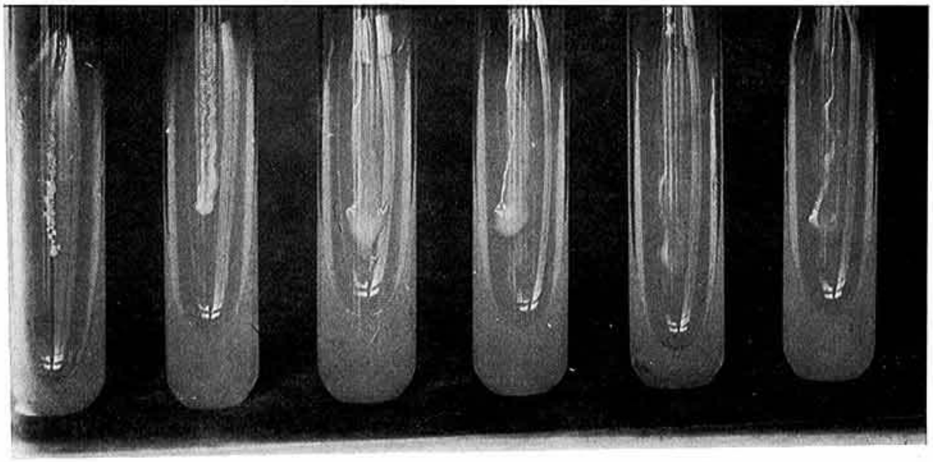
NaCl 5g
Distilled water .... 1,000 ml
B: Meat extract (Liebig) 3g
Peptone 5g
Distilled water .... 1,000 ml

Except type D, nitrate is reduced, but the course of reduction differed among types. Nitrite accumulation is especially large in case of type E and some of type A, whereas nitrite is hardly detected in case of types B, AB, F and G. Types B, AB, C, F and G reduce nitrate to gaseous nitrogen. Such characters can be also verified in the growth zone in nitrate agar shake culture.

The use of asparagine lactose or raffinose medium enables us to know the presence of a very distinct difference among isolates. Types F and G are acid sensitive (critical pH 5.1), whereas type E is acid tolerant (critical pH 3.4>). Type E seems peculiar in showing growth in bouillon as well as on potato. In addition, maximum temperature of most types is 32 to 35°C., whereas that of type E is extremely high (42°C).

2) Symbiotic relations with hosts

The extent of symbiotic nitrogen fixation of each type with various host legumes is shown in Table 2 and Figs. 2 to 7[8]. According to these results, it is known that the range



AC C B B D D A

1-a Isolate from *Mimosa* and each type of cowpea bacteria

1-b Type A of cowpea bacteria (under counter-light)

Fig. 1. Growth on soil extract mannitol agar

Type AC
Type C
Type B
Type B
Type D
Type D
} of cowpea bacteria

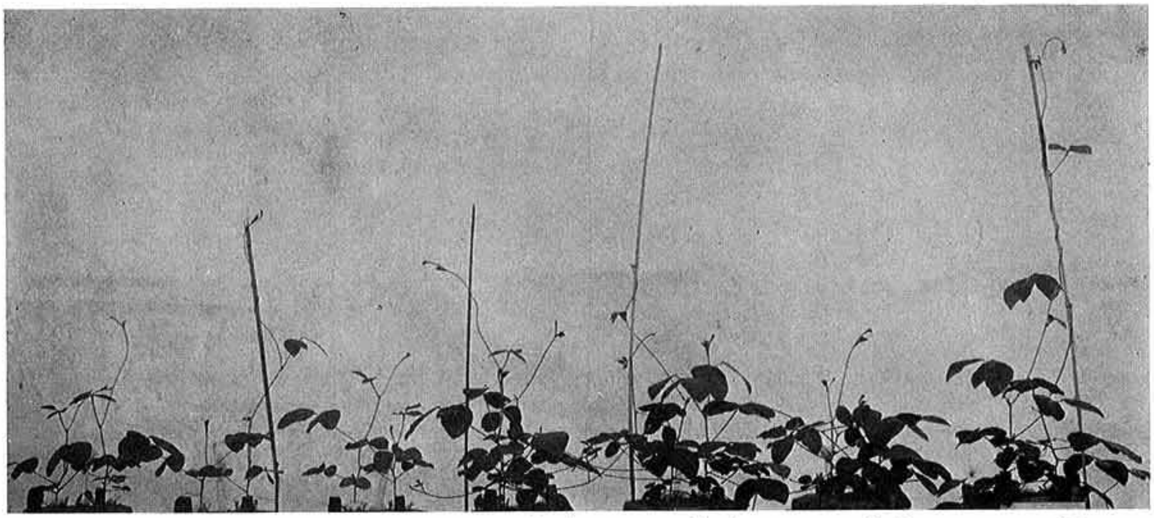
Remarks: Descriptions in Tables 1 and 3 are made on growth on yeast extract mannitol agar, but the general appearance is similar on both media

Table 2. Symbiotic relationship between each type of isolates and kind of host plant

Host	Type of isolates									
	A-1	A-2*	B	AB	C	AC	D	E	F	G
Albizzia	E	E	IE	—	ME	E	IE	IE	—	—
Arachis hypogaea	IE	—	ME	—	E	E	—	—	—	—
Calopogonium	E	ME-IE	ME-IE	E	IE	IE	IE	—	—	IE
Centrosema	IE	IE	E	IE	E	IE	IE	—	—	IE
Crotalaria anagyroides	E	IE	ME	—	ME	IE	IE	IE	—	—
C. usaramoensis	ME	IE	ME	E	ME-IE	—	ME-IE	—	—	IE
Desmodium	E	ME	ME	ME	IE	E	IE	—	—	IE
Tephrosia maxima	E-ME	ME	E-ME	ME	ME-IE	ME-IE	ME-IE	—	—	ME
T. noctiflora	E	ME-IE	ME-IE	E	ME	ME	IE	—	—	ME-IE
Vigna	E	E	E-ME	E	E-ME	E	IE	—	—	E

E: Effective, ME: Moderately effective, IE: Ineffective * See the text

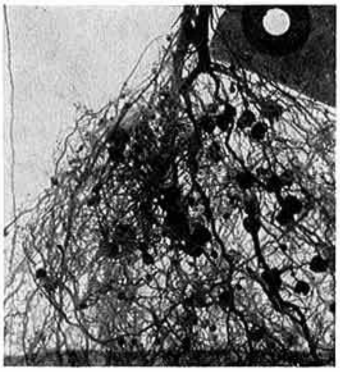
×D ×G ×C ×AC ×B_2 ×B_1 Control (not inoculated)



×$(A-2)_3$ ×$(A-2)_2$ ×$(A-2)_1$ ×$(A-1)_3$ ×$(A-1)_2$ ×$(A-1)_1$ ×AB

Fig. 2. Response of *Calopogonium mucunoides* to each type of cowpea bacteria

2-a Growth of host plant



2-b Nodules of *Calopogonium mucunoides* (× Type AB)

of type of isolates with which each host symbioses effectively varies.

The difference of nodulation pattern will distinctly be found among each host—type of isolate combination. In addition, the recognition of host specificity and strain variation will be possible.

From these phenomena, it may be easily understood that the selection of effective strain to each individual host has been made for artificial inoculation.

In this respect, attention must be focused on the point that the host legumes used are

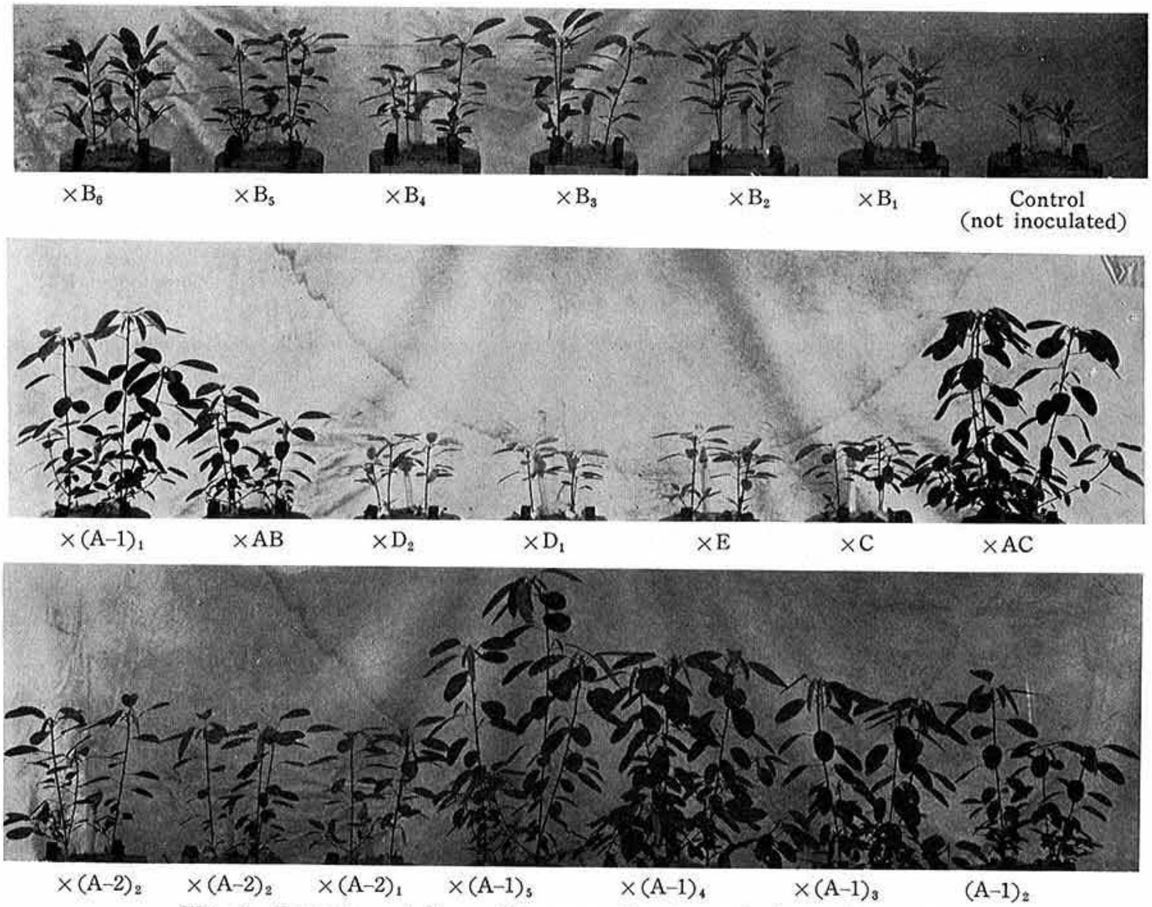


Fig. 3. Response of *Desmodium gyroides* to each type of cowpea bacteria

divided into the following two groups depending upon their relation to type A.

Group 1. *Arachis hypogaea*, *Centrosema pubescens*, *Crotalaria usaramoensis*—type A is ineffective

Group 2. *Vinga sinensis*, *Albizzia moluccana*, *Calopogonium mucunoides*, *Crotalaria anagyroides*, *Desmodium gyroides*, *Tephrosia maxima*, *T. noctiflora*—type A is effective

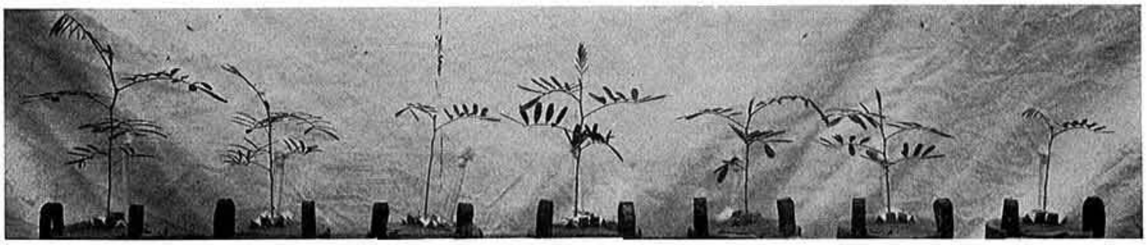
In Table 2, it is found also that all of the isolates grouped into type A do not always behave in a similar manner. Some of type A are effective to *Crotalaria anagyroides*, *Calopogonium*, *Desmodium* and *Tephrosia noctiflora*, but others are ineffective.

As a result, the isolates type A characterized by the characters in artificial media apart from host must be subdivided into A-1 and A-2 from the relationship with hosts.

This phenomenon appears to suggest that the selection of effective strain is hardly made without examining its behavior to host. On this point, however, it seems very interesting that there exists a distinct difference between A-1 and A-2, i.e.

	Nitrite accumulation in nitrate medium	Nodule production on soybean root
Type A-1	None or less	Negative
Type A-2	Remarkable	Positive

Although it remains to be studied whether the behavior to nitrate is a key character to subdivide type A, these results strongly



$\times$AC $\times B_5$ $\times B_4$ $\times B_3$ $\times B_2$ $\times B_1$ Control (not inoculated)

4-a-1

$\times (A\text{-}1)_1$ $\times (A\text{-}2)_1$ $\times$AB $\times$D $\times$G $\times$C

4-a-2

$\times (A\text{-}1)_5$ $\times (A\text{-}2)_3$ $\times (A\text{-}2)_2$ $\times (A\text{-}1)_4$ $\times (A\text{-}1)_3$ $\times (A\text{-}1)_2$

4-a-3

Fig. 4 Response of *Tephrosia noctiflora* to each type of cowpea bacteria

4-a Growth of host plant

suggest the importance of detailed study of rhizobial strain from two aspects, in artificial media apart from host and in the relationship with host.

As Allen and Allen stated on the basis of their cross-inoculation studies of tropical legumes with *Vigna sinensis*, the cowpea group, now so very large, will have to be subdivided into smaller or sub-groups[2),3)]. The difference in the response of each host to the strain type A seems to support their opinion.

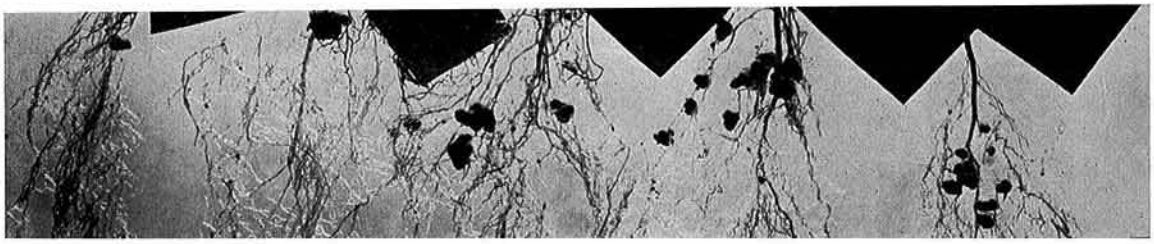
Isolates from *mimosa invisa*, *Leucaena glauca* and *Scsbania aegyptiaca*

1) General characters in artificial media

General characters of isolates from these legumes are shown in Table 3[6)]. Characters of isolates from *Mimosa* and *Leucaena* are almost the same, whereas two types were obtained from *Sesbania*. Isolates from *Mimosa* and *Leucaena* may be especially characterized

×B_4 ×B_3 ×B_2 ×B_1 Control (not inoculated)

4-b-1

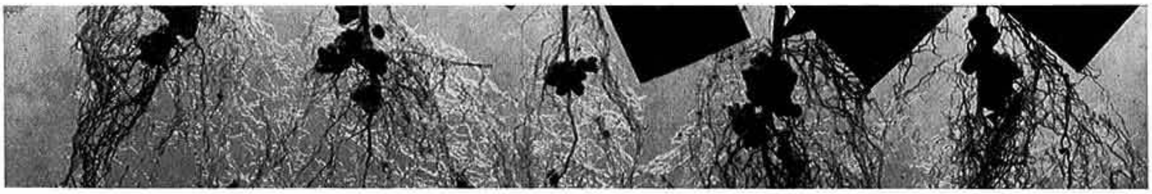


×D ×G ×C AC ×B_5

4-b-2

×$(A\text{-}1)_2$ ×$(A\text{-}1)_1$ ×$(A\text{-}2)_1$ ×AB

4-b-3



×$(A\text{-}1)_5$ ×$(A\text{-}2)_3$ ×$(A\text{-}2)_2$ ×$(A\text{-}1)_4$ ×$(A\text{-}1)_3$

4-b-4

4-b Nodulation pattern

by the reaction change in litmus milk and a remarkable accumulation of nitrite in nitrate medium.

Isolates from *Sesbania* are easily divided into two types by use of potato, bouillon and nitrate or lactose medium. Type A from *Sesbania* produces gaseous nitrogen in nitrate media.

Behavior to host

1) Cross-inoculation

A part of cross-inoculation studies is shown in Table 4. Although there exist irregularities in the results, each of *Mimosa* and *Sesbania* was tentatively proposed as an independent cross-inoculation group, respectively[7]. *Leucaena* was included in the *Mimosa*

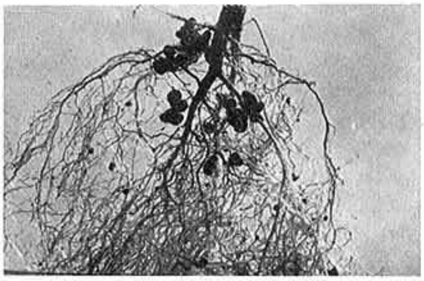


Fig. 5. Nodules of *Albizzia moluccana* (× Type AC)

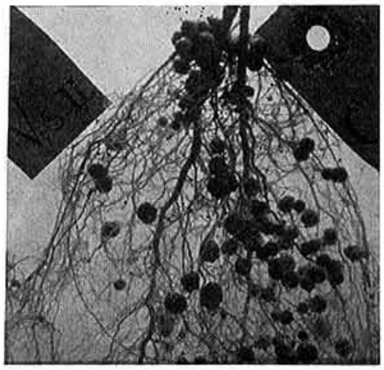


Fig. 6. Nodules of *Centrosema pubescens* (× Type C)

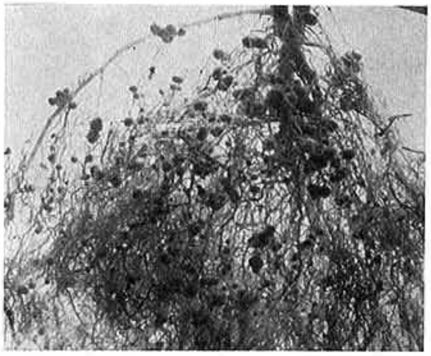


Fig. 7. Nodules of *Tephrosia maxima*

group. But the relationship between *Mimosa* and *Dalea* group remains to be examined.

Attention must be placed on the behavior of type E (cowpea, see Table 1). This strain produces nodule on *Mimosa* and *Leucaena*. According to the definition of the so-called cross-inoculation group, *Mimosa* may be considered as one of members of the cowpea group.

In addition, there is a paper in which *Mimosa strigillosa* was included in the cowpea

Table 3. General characters of isolates from *Mimosa, Leucaena* and *Sesbania*

Isolated from	Yeast water mannitol agar slope	Potato	Bouillon	Litmus milk reaction	Litmus milk zone	Nitrate reduction	Utilization of reffinose or lactose	
Mimosa invisa	Fairly rapid growth, raised, glistening, opqaue, white	−	−	−→⊕	+	+	+	+
Leucaena glauca	〃	−	−	−→⊕	+	+	+	+
Sesbania aegyptiaca (A)	Rapid growth, flat, moist, glistening, translucent, white	+	+	−	+	+	+	+
〃 (B)	Rapid growth, raised, glistening, opaque, white, sticky	−	−	−	+	+	+	−

Table 4. Cross-inoculation test

Isolate from	Host				
	Mimosa	*Leucaena*	*Sesbania*	*Vigna*	*Astragalus sinicus*
Mimosa	+	+	±	+	−
Leucaena	+	+	±	+	−
Sesbania (A)	−	+	+	+	−
Sesbania (B)	−	⊕	+	+	−
Dalea	+	⊕	±	+	±
Vigna (type E)	+	⊕	−	+	±
Astragalus sinicus	−	−	−	−	+

Nodulations: −: negative, +∼±: positive, +>⊕>±

group[4].

But among the rhizobial strains of the cowpea group, the one which can infect *Mimosa* is almost confined to type E, and most of the cowpea bacteria showed no positive relation with *Mimosa*.

Legumes belonging to the same genus are not always placed in the same group. The most striking example is found in case of *Phaseolus*. Therefore, in some cases some members of *Mimosa* may find their position in a different group.

2) Nitrogen fixation

As shown in Table 5 and Figs 8 to 10, isolates from *Mimosa* and *Leucaena* were equally

Table 5. Effect of four rhizobial strains to *Mimosa* and *Leucaena*

Isolate from	I		II	
	Mimosa	*Leucaena*	*Mimosa*	*Leucaena*
Mimosa	E	E	E	—
Leucaena	E	E	—	E
Delea	I E	E	I E	I E
Vigna(type E)	—	—	E	I E

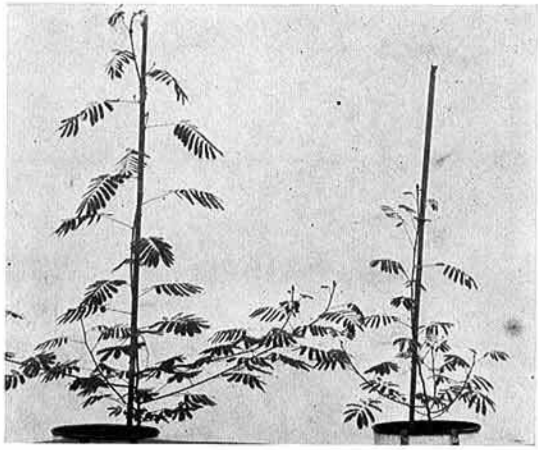
I: In midsummer, II: In early autumn,
E: Effective, I E: Ineffective

effective to both hosts. *Dalea* bacteria were effective to *Leucaena*, but ineffective to *Mimosa* in midsummer.

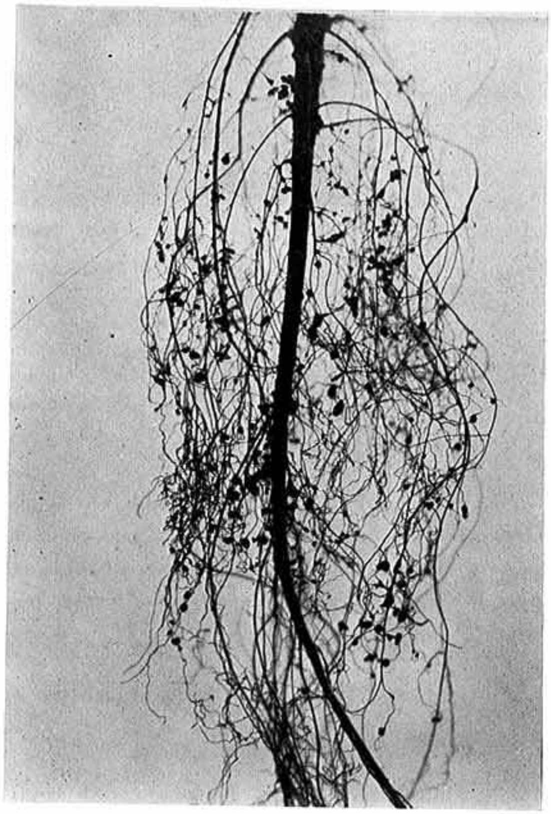
It is noticeable, however, that the effectiveness of *Dalea* bacteria to *Leucaena* was greatly changed in another season[8], though this season was unfavorable for the growth of *Leucaena*. A great difference is observed in the nodulation pattern between Figs. 8 and 10 or between Figs. 9 and 10.

Attention must be drawn on strain type E which was originally obtained from *Vigna*. As already shown in Table 2, it was ineffective to two members of the cowpea group, whereas it was effective to *Mimosa* as equally as the isolate from its original host.

From these results, it seems more rational to transfer this type E to the *Mimosa* group. From the view of nodule production, it is to be noticed that *Phaseolus vulgaris* and *Vigna sinensis* are rather low in their specificity to rhizobial strains.

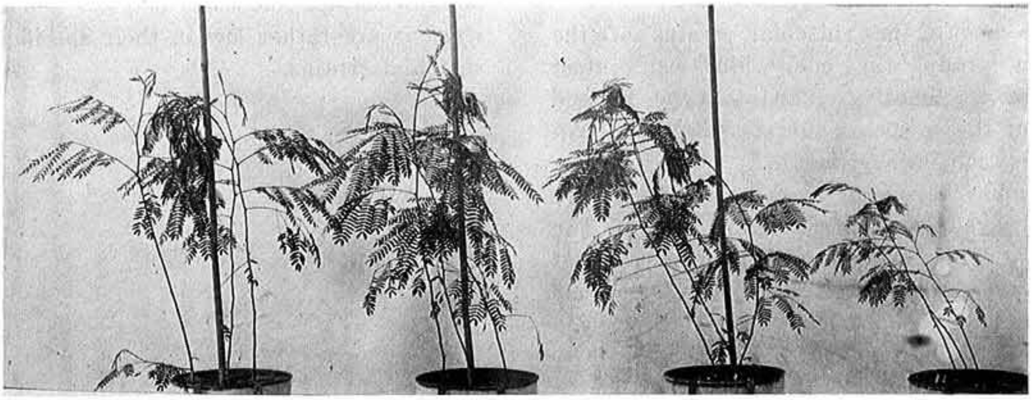


8-a Growth:
Left: × Isolate from *Mimosa*
Right: Control (not inoculated)

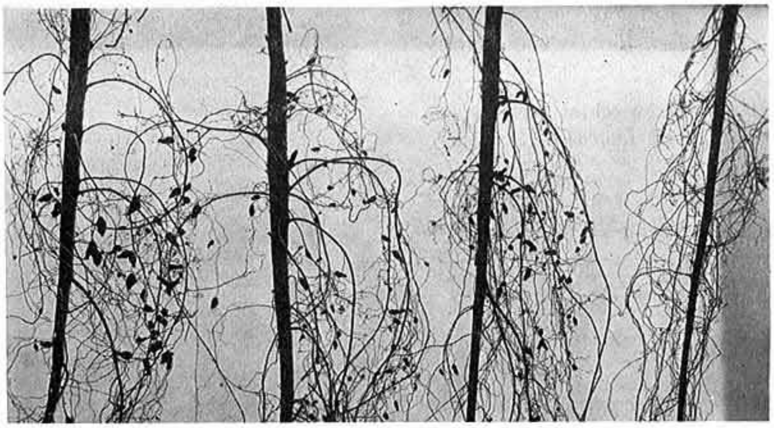


8-b Nodulation

Fig. 8. Effect of inoculation to *Mimosa invisa*



9-a Growth



9-b Nodulation pattern

Fig. 9. Response of *Leucaena glauca* to isolates from *Dalea* and *Leucaena*
From left to right:
Isolate from *Dalea*
Isolate from *Leucaena*
Isolate from *Leucaena*
Control

Two types of isolates from *Sesbania* were both effective to their original host, though type A was somewhat more effective than type B. The nodulation pattern differed between types A and B. (Fig. 11).

A perspective of rhizobia of tropical legumes

The data on tropical legumes and their nodule bacteria have been gradually increased. But much more efforts must be made along this line. This is because the kind of legume is so numerous in the tropics or subtropics as compared with temperate regions.

As stated above, many legumes in the tropics are comprised in the cowpea group and this group is exceptionally large. Therefore, the presence of various types of rhizobia will be expected.

The rhizobial strains described in this paper were mostly isolated by the author. Concerning cowpea bacteria, types A and B seem most popular in Japanese soils.

Leucaena *Mimosa*

10-a

10-b (*Mimosa*)

10-b (*Leucaena*)

Fig. 10. Response of *Mimosa* and *Leucaena* to various isolates (in autumn)

10-a Growth
10-b Nodulation pattern

From left to right:

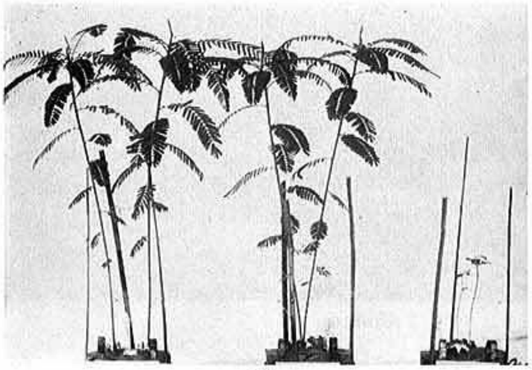
Leucaena
× Isolate from *Vigna* (Type E)*
× Isolate from *Leucaena*
× Isolate from *Dalea*
Control

Mimosa
× Isolate from *Vigna* (Type E)*
× Isolate from *Mimosa*
× Isolate from *Dalea*
Control

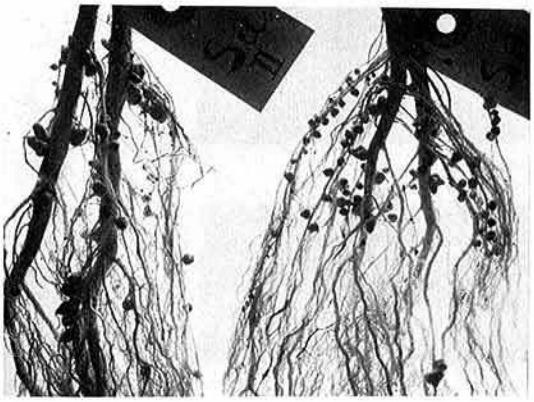
* See cowpea bacteria

Since type A was also isolated from soils of Indonesia and Hai-Nan Tao Island, its distribution may be fairly wide. And it will be worthy of note that isolates grouped into type A-1 or A-2, irrespective of its origin, showed a similar behavior to hosts.

When isolation of rhizobia is tried from soils containing various types of *Rhizobium*, it is difficult to determine which type is obtained. In this connection, it is noticeable that type C of cowpea bacteria was isolated only from *Centrosema*, but not from other hosts, by use of soils where various types of them co-exist. This suggests the importance of kind of host when rhizobial distribution in soil is surveyed or when rhizobial collection is



11-a



11-b

Fig. 11. Response of *Sesbania aegyptiaca* to two types of isolates
11-a Growth of host plant
From left to right:
× Type A
× Type B
Control (not inoculated)
11-b Nodulation pattern
Left: Type A
Right: Type B

undertaken.

According to the author's experience, such strain as type C of cowpea bacteria appears not to be found in Japanese soils. The origin of this type C will be certainly Indonesian soil.

It may be added that the peculiar type E of cowpea bacteria was obtained from Indonesian soils by use of *Vigna*, and that both type G (cowpea) and type A of *Sesbania* organisms were isolated from Taiwan soils.

Strain variation of rhizobia will be induced by various factors under natural conditions. Of these, soils which are formed in intimate relation to climate and vegetation will undoubtedly be one of the important factors.

For the survey of rhizobia, besides the use of nodules from originally growing legumes, it will be necessary to sow various leguminous seeds in the soils from various regions and to isolate rhizobia from nodulated legumes. By such procedure, various types of rhizobial strain will be obtained.

A survey of rhizobial distribution in the tropical or subtropical soils will be one of the most interesting problems. Although the cowpea bacteria were subdivided into about nine types, another type or types may be added in the future.

In this connection, the publication of "World Catalogue of *Rhizobium* Collection" which is being worked by Allen and Hamatova as one of the contributions from PP/IBP Nitrogen Fixation Section is eagerly waited for.

In the tropics or subtropics, leguminous plants are cultivated as food, green-manure, cover, shade, and barricade-crop, etc. A large contribution will be undoubtedly introduced to tropical or subtropical agriculture by an active utilization of legume-*Rhizobium* symbiosis.

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