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seed samples in this study had received the benefit of a partial hand scalping of the threshing material so averages for typical crude lots will be lower The mechanically processed seed was all passed twice through the cleaner whereas commercially it is highly probable that only one pass will be made and slightly lower values will result

The relative demand for crude and graded seed will be influenced by the planting system used and the costs of seed transport Crude seed will undoubtedly be used where seed is produced on the same farm and where mechanical planters are unavailable or too costly Graded seed will facilitate more precise and mechanical planting and will reduce storage and transport costs Where seed is combine-harvested mechanical scalping could be desirable before drying or deawning

## Regional Seed Production Potentials

As part of an overall assessment of regional seed production potentials a field experimental phase was initiated in six regions during 1979 Actual regional locations and collaborators are shown in Table 29 The initial emphasis was to initiate the project at locations in higher latitude regions with some seasonality of rainfall distribution and low frost incidence and to collaborate with institutions already active in germ plasm evaluation

The field experiment aims to define phenology seed yields and the incidence of weeds pests and diseases while additional climatic edaphic agronomic and economic data are collected from other sources for each region

The genetic materials included were nominated by both the collaborator and CIAT Each material was sown in pure stands in 80m² plots with three replications

Adverse seasonal conditions had negative effects on the number of entries established and within site variability at Santa Cruz Sete Lagoas and Valledupar Weeds were a problem for the legumes at Sete Lagoas reflecting site history The first year seed harvests of grasses and legumes were made at Chapare Brasilia Felixlandia CIAT Quilichao and Santa Cruz while only grasses were harvested at Sete Lagoas

# SOIL MICROBIOLOGY

The objective of the Soil Microbiology Section is to maximize the benefits of biological nitrogen fixation to adapted forages in the acid infertile soils of tropical Latin America with priority given to the legume/*Rhizobium* symbiosis The research strategy is (1) to maintain and enlarge the CIAT *Rhizobium* germplasm resource (2) to evaluate the symbiotic nitrogen fixation potential of *Rhizobium* strains with adapted legumes and (3) to test the symbiotic potential of selected strains in field situations initially at CIAT Quilichao Carimagua and Brasilia and then in regional trials throughout the Tropical Pastures Program s target area

## Rhizobium Collection

The addition of 55 *Rhizobium* strains in 1979 brought the bank number to 2098 Information about the entire collection is recorded in a retrieval system detailed in the CIAT *Rhizobium* Catalogue

Nodule collection especially from *Zornia* spp was carried out in the Llanos Orientales of Colombia (Meta and Vichada departments) Complete information for each site was collected and soil samples were taken for analysis

## Strain Selection

The five stages of *Rhizobium* strain selection have remained unchanged since 1977 (CIAT Annual Report 1977)

### Stage I (Aseptic tube culture)

Although Stage I testing was delayed due to unavailability of a growth chamber alternate systems were explored

In a hemotoxilin study with the Soil Nutrition Section nodulation occurred in a non aerated system indicating the potential for replacement of the current ly used agar plates with nutrient solution Several nutrient solutions were tested and growth pouches were selected for a comparative study with the routine enclosed plant agar tube method The former system employs plastic bags filled with nutrient solution and a paper wick One apparent advantage is that six times as many treatments can be placed in the same space in the light room Plant appearance days to nodulation and location and abundance of nodules of several legumes was observed in both systems Norris and Date as well as Hoagland s nutrient solutions (modified to simulate acid soil stresses with or without 3 ppm Al) were used in the growth pouches while Jensen s agar was used in tubes Preliminary results show more rapid and enhanced plant growth in growth pouches One hundred and nine strains were proven infective on a member of the host genus from which they were isolated

**Stage II (Sand culture with nutrient solution pH 7)**

The lack of a suitable inert matrix to substitute the previously used river sand limited the number of tests completed in 1979

*Zornia latifolia* 728 and *Stylosanthes capitata* 1315 were not able to establish sufficient root growth in the N free quartz sand to permit nodulation initiation Starter nitrogen at the rate of 5 ppm N will be used

**Stage III (Pot culture in site soil)**

Evaluation of nine *Desmodium* isolates on *D ovalifolium* 350 grown in soil from CIAT Quilichao was repeated due to anomalous results compared to those obtained in Stages II and IV The first Stage III test indicated that strains varied significantly in their ability to affect dry matter production *Rhizobium* sp CIAT 283 ranking first among inoculating strains (Table 30) However it was not better than native *Rhizobium* as indicated by its statistical equivalence with the non inoculated control without applied N In the second Stage III test *Rhizobium* sp CIAT 283 produced more dry matter than the control with applied N although

Table 30 Comparison of rankings obtained for Rhizobium strains in Stage II and two identical Stage III studies with Desmodium ovalifolium CIAT 350

| Rhizobium strain CIAT No | Mean dry matter yield | | | | | |
|---|---|---|---|---|---|---|
| | Stage III November 1978 (g/2 shoots) | | Stage III July 1979 (g/2 shoots) | | Stage II July 1978 (g/2 pl ) | |
| +N control | 1[1] | 4.98a[3] | 2[1] | 12.52a | 2[1] | 0.411a |
| N control | 2 | 4.07ab | 6 | 11.46a | 37[2] | 0.054g |
| 283 | 3 | 3.95ab | 1 | 14.14a | 11 | 0.202b d fg |
| 359 | 4 | 3.75b | 8 | 10.91a | 3 | 0.402a |
| 388 | 5 | 3.59b | 11 | 10.39a | 5 | 0.335ab |
| 297 | 6 | 3.42b | 10 | 10.66a | 9 | 0.254b d [illegible] |
| 533 | 7 | 3.32bc | 7 | 11.42a | 8 | 0.264ab d |
| 512 | 8 | 3.29bc | 4 | 12.09a | 4 | 0.347 b |
| 529 | 9 | 3.02bc | 5 | 11.68a | | 0.29 ab [illegible] |
| 507 | 10 | 2.86bc | 3 | 12.09a | 1 | 0.419a |
| 299 | 11 | 2.33c | 9 | 10.68a | 6 | 0.334ab |

1 Overall rank by means
2 In the Stage II experiment some of the best out of 39 strains and the nitrogen control are shown
3 Mean values within column followed by the same letter are not significantly different at a 0.05 level

not significantly. In the Stage II test CIAT 283 was the least effective.

The lack of correlation between Stage III tests may have been due to seasonal effects on rhizobial performance. This needs to be determined with duplicate experiments established in the field and harvested at time intervals to measure wet and dry season effects on foliage production and inoculation response.

To test the response of *Z. latifolia* 728 and *S. capitata* 1315 to 15 *Rhizobium* strains, a contamination free system enabling more plants to be placed in the same space ($50/m^2$ vs $9/m^2$) was adopted.

Statistical analysis of *Zornia* shoot dry matter production in CIAT Quilichao and Carimagua soils indicated no significant difference between strains nor between strains and the plus and minus N controls. The high N content of the CIAT Quilichao soil may mask inoculation responses. In Carimagua soil, results may indicate the presence of efficient native *Rhizobium* strains. Antisera will be prepared to determine the identity of the nodulating strains.

Although dry matter yields of *S. capitata* CIAT 1315 in Carimagua soil were not analyzed statistically, a visual evaluation of nodulation indicated that strains CIAT 301 and 871 were superior. Mean dry matter production of *S. capitata* 1315 in the CIAT Quilichao soil (Table 31) shows *Rhizobium* strains CIAT 301 and 1363 to be superior.

**Stage IV (Field trials)**

In Stage II and Stage III tests (CIAT Annual Report 1978), strain dependent statistical differences were observed for *D. ovalifolium* 350 inoculation response. In Stage IV experiments, although results were statistically significant for the first cutting, this was not so for further cuttings in 1979. The inoculant technology experiment at CIAT Quilichao utilizing three strains and three pelletization technologies again did not show significant treatment effects after the first cutting, nor for treatment yields accumulated over all harvests.

The purpose of a 31 strain, single replicate experiment with *Leucaena leucocefala* CIAT 871 was to determine if any strain could nodulate this legume in an acid soil. However, by accumulating dry matter over two harvests as replications, a Duncan multiple range test was run on the inoculation response. At Carimagua, no statistically different yield responses to strains were observed (Table 32). The five highest ranking strains for the first harvest were different from those of the second.

Table 31. *Stylosanthes capitata* CIAT 1315 mean dry matter production under greenhouse conditions in soil from CIAT Quilichao (Stage III).

| Rhizobium strain CIAT No. | Rank[1] | Mean dry matter yield (g/2 shoots) |
|---|---|---|
| 71 | 12 | 1.76ab[2] |
| 79 | 10 | 1.[illegible]8 b |
| 301 | 1 | 2.10a |
| 763 | 5 | 1.[illegible] b |
| 765 | 4 | 1.9[illegible] ab |
| 870 | 11 | 1.78 b |
| 871 | 7 | [illegible].88ab |
| 882 | 3 | 2.00ab |
| 887 | 16 | 1.[illegible]3ab |
| 1238 | 8 | 1.83 b |
| 1358 | 6 | 1.91ab |
| 1359 | 13 | 1.[illegible] b |
| 1363 | 2 | 2.10a |
| 1460 | 14 | 1.71ab |
| 1468 | 17 | 1.[illegible]9b |
| +N control | 15 | 1.5[illegible]ab |
| N control | 9 | 1.82ab |

1 By mean dry matter production.
2 Mean values followed by the same letter are not significantly different at a 0.05 level.

After the second harvest, roots of five plants from each treatment were rated for nodulation. The treatment nodulation scores were not significantly correlated with dry matter yield. In treatments with greater than average dry matter production, only half had better than average nodulation. Native *Rhizobium* or contamination from inoculated plants produced nodules on plants in non inoculated treatments. Some strains found to be initially good nodulators such as CIAT 1967 and 1920 (Carimagua Annual Report 1978) were poorly infective this year, while other strains improved. At CIAT Quilichao, statistical yield differences were recorded for accumulated harvests.

The establishment of a study with *S. capitata* 1315 and *Z. latifolia* 728 for strain selection was undertaken at CIAT Quilichao and Carimagua.

**Verification of Stage IV results.** Recorded inoculation responses should be verified by determining that

Table 32 Dry matter production accumulated over two harvests by Leucaena sp CIAT 871 inoculated with Rh zobium strains in Carimagua

| Rh obium strain CIAT No | Mean dry matter yield (kg/ha) | Nodulat on[2] score (se ond harvest) | Rhi ob um st a n CIAT No | Mean d y matter yield (kg/ha) | Nodulat on s o e (se ond ha v t) |
|---|---|---|---|---|---|
| 1920 | 610 | 3 | 42 | 70 | 6 |
| 1967 | 540 | 5 | 685 | 362 | 5 |
| 1919 | 533 | 10 | 1921 | 361 | 7 |
| 1931 | 524 | 11 | 1963 | 352 | 8 |
| 1939 | 480 | 10 | 1961 | 345 | 12 |
| 1964 | 471 | 9 | 847 | 338 | 5 |
| 1966 | 468 | 7 | 1923 | 337 | 7 |
| 1965 | 460 | 10 | 1959 | 301 | 10 |
| 1934 | 449 | 10 | 1732 | 284 | 5 |
| +N control | 433 | 2 | 1937 | 259 | 8 |
| 1944 | 429 | 4 | 1747 | 251 | 7 |
| 1740 | 418 | 5 | 1742 | 250 | 8 |
| 1735 | 415 | 6 | 1947 | 237 | 8 |
| 346 | 399 | 4 | N control | 234 | 1 |
| 843 | 394 | 6 | 1962 | 137 | 2 |
| 194? | 375 | 10 | 1927 | 121 | 10 |
| 1917 | 371 | 13 | 9 | 85 | 1 |

1 Nd significant differences were found between strains
2 Sum of five plants scored by visual inspection (3 = abundant 2 = moderate 1 = scarce 0 none average 6 9)

the nodules formed on the plant correspond to the inoculating strain This is especially necessary under the septic conditions in the field where competitive native *Rhizobium* may be nodulating the plant In order to accomplish this several additions to the laboratory s antisera collection have been made

## Inoculation Response Potential

Due to the high N content in the CIAT Quilichao soil the potential for dry matter production responses to inoculation is questionable A control of ground corn stalk (2 t/ha) was incorporated in the *S capitata* 1315 experiment established at CIAT Quilichao If there is a large difference between this treatment and the minus N control strain selection at CIAT Quilichao should be re evaluated as high soil N may be limiting inoculation response

## Inoculant Recommendations Extrapolability Study

A host range study is in progress to determine the infectivity and efficiency of several *Rhizobium* strains on various promising lines of *Stylosanthes*

As in Stage I infectivity of strains under defined acid soil conditions was studied in growth pouches Information on the range of infectivity will be used in planning Stage II and III experiments to study the efficiency of a strain under defined aseptic conditions and in septic site soil This information will help clarify the limitations to strain recommendation

This type of information is apparently necessary as related *Desmodium* species reacted very differently to *Rhizobium* at the Carimagua field station *D ovalifolium* 350 nodulated readily in the non inoculated plots None of the three strain x three inoculant technology treatments significantly increased dry matter production over the plus and minus N controls Native *Rhizobium* appear to be equally effective as the best strains selected from among 70 *Desmodium* isolates at preliminary screening levels On the other hand *D heterocarpon* 365 (taxonomically the same species as *D ovalifolium D heterocarpon* var *heterocarpon)* appeared chlorotic and nodulated poorly An effective inoculant would prove to be of great advantage for this accession Stage I II and III experiments to select suitable inoculant strains are in progress

## Comparison of Acid and Rich Media for Rhizobium Culturing

Phenotypic differences between strains isolated from a single nodule have been observed Isolates grown on routine (rich) laboratory medium may demand special growth factors and may not be suited for acid soils Furthermore strain storage on such media has been considered a cause of loss of competence (survival and infectivity) under field conditions On the other hand strains were less efficient when maintained on acid medium A study is in progress to determine the best isolation medium and to determine the correlation between type of isolation maintenance medium and strain efficiency with time under defined acid conditions

Table 33 Growth and stabil ty of *Rhizob um* train in various ac d screeni ig med a and h ige n the pH of the latter

| Culture medium[1] | Rh ob um CIAT No [2] | C ll d ns ty (cells/ml) In t al | C ll d ns ty (cells/ml) F nal | pH In t al | pH F nal |
|---|---|---|---|---|---|
| 1 | 270 A | 2 2 $10^{4}$ | 8 28 $10^{3}$ | 4 7 | 4 75 |
| | B | | 1 2 $10^{8}$ | | 4 50 |
| | 861 A | 1 8 $10^{4}$ | 1 10 | | 4 7 |
| | B | | 1 10 | | 4 7 |
| | 907 A | 3 5 $10^{3}$ | 1 49 $10^{3}$ | | 4 7 |
| | B | | 1 75 $10^{3}$ | | 4 7 |
| 2 | 270 A | 2 2 $10^{4}$ | 4 7 $10^{8}$ | 4 7 | 6 9 |
| | B | | 9 1 $10^{7}$ | | 7 3 |
| | 861 A | 1 8 $10^{4}$ | 0 $10^{1}$ | | 4 8 |
| | B | | 0 $10^{1}$ | | 6 2 |
| | 907 A | 3 5 $10^{3}$ | 1 2 $10^{8}$ | | 4 5 |
| | B | | 6 9 $10^{8}$ | | 4 5 |
| 3 | 270 A | 2 $10^{4}$ | 1 2 $10^{7}$ | | 6 0 |
| | B | | | | |
| | 861 A | 1 8 $10^{4}$ | $> 10^{1}$ | | 1 |
| | B | | $> 10^{1}$ | | 5 2 |
| | 907 A | 3 5 $10^{3}$ | 1 $10^{6}$ | | 5 5 |
| | B | | 8 $10^{6}$ | | 6 2 |
| 4 | 270 A | 2 2 $10^{4}$ | 4 4 $10^{7}$ | 4 7 | 4 9 |
| | B | | 3 7 $10^{6}$ | | 6 5 |
| | 861 A | 1 8 $10^{4}$ | 1 0 $10^{9}$ | | 4 8 |
| | B | | | | |
| | 907 A | 3 5 $10^{3}$ | $> 10^{1}$ | | 4 3 |
| | B | | 2 $10^{7}$ | | 4 9 |
| 5 | 270 A | 2 2 $10^{4}$ | 2 2 $10^{8}$ | 6 6 | 6 6 |
| | B | | $> 10^{6}$ | | 4 9 |
| | 861 A | 1 8 $10^{4}$ | $> 10^{1}$ | | 4 9 |
| | B | | 1 $10^{3}$ | | 4 8 |
| | 907 A | 3 5 $10^{3}$ | 4 6 $10^{7}$ | | 6 5 |
| | B | | 3 2 $10^{7}$ | | 6 6 |

1 K y to media 1 = Key er with glycerol no pH nd cato 2 = Keyser s w th glycerol with b omo e ol gr en pH ind cator 3 = Keyser s w th a ab nose no pH ind cato 4 Keyse s w th a ab no e with bromoc esol green pH nd cato 5 = Yeast mann tol med um no pH nd ato

2 Repl cat s A and B

Table 34  Current inoculation recommendations developed for several promising forage legumes

| Species | Accession CIAT No | Rhizobium strain CIAT No | Technology |
|---|---|---|---|
| Category 4 | | | |
| *Desmodium ovalifolium* | 350 | 299 | Rock phosphate pellet |
| *Zornia latifolia* | 728 | 71 | Rock phosphate pellet |
| *Stylosanthes capitata* | 1019 1315 | 71 + 1238 | Rock phosphate pellet |
| *Pueraria phaseoloides* | 9900 | 79 | Rock phosphate pellet |
| Category 3 | | | |
| *Stylosanthes capitata* | 1318 1323 1325 1342 1405 1728 1943 | 71 + 1238 | Rock phosphate pellet |
| *Zornia* spp | 9179 9220 9245 9258 9260 9270 9286 9295 9 48 | 71 | Rock phosphate pellet |
| *Aeschynomene brasiliana* | 9681 9684 | 71 | Rock phosphate pellet |
| *A histrix* | | 71 | Rock phosphate pellet |
| *Desmodium heterophyllum* | 349 | 31 | Rock phosphate pellet |
| *Stylosanthes hamata* | 147 | 71 | Rock phosphate pellet |
| *Codariocalyx gyroides* | 3001 | 299 | Rock phosphate pellet |

The acid medium was slightly modified and two carbon sources (arabinose and glycerol) were tested for ability to support rhizobial growth without a change in the pH Bromocresol green a pH indicator with an equivalence point in the acid range (pH = 4 5) was also tested for possible adverse effects on rhizobial growth Preliminary results indicated that both media permitted rhizobial growth while final reaction was dependent on the strain (Table 33) Glycerol was chosen as a suitable yet less expensive C source (US$461 90/kg and $9 55/kg for arabinose and glycerol respectively)

Strains have been isolated from nodules collected from *Z latifolia* 728 and *S capitata* 1315 grown under greenhouse conditions in a non inoculated Carimagua soil The cultures were obtained by streaking rich and acidified rhizobial media with the cell suspension from a single nodule The paired isolates efficiencies will be compared under defined conditions in Leonard jars This study will be periodically repeated to determine if there are adverse effects of storage on either media over time

## Inoculant Recommendations

The inoculant recommendations for promising legume accessions are given in Table 34 During 1979 63 kg of peat based inoculum were produced 36 kg were used by CIAT and the Instituto Colombiano Agropecuario (ICA) while 12 and 8 kg were sent to national and international agencies respectively and 7 kg to private entities

# SOIL FERTILITY AND PLANT NUTRITION

The overall objective of the Soil Fertility and Plant Nutrition Section is to identify and correct mineral deficiencies and toxicities during the pasture establishment period on the acid soils with low native fertility of the Tropical Pastures Program target area The research strategy takes into account the soil plant