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ANNUAL PROGRESS REPORT

1984

RESOURCES SUPPORT SERVICES AGREEMENT
BETWEEN
U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT
AND
U.S. DEPARTMENT OF AGRICULTURE,
AGRICULTURAL RESEARCH SERVICE

RSSA BST - 4109-R-AG - 3164

'World Rhizobium Study and Collection Center'
Nitrogen Fixation and Soybean Genetics Laboratory
Beltsville Agricultural Research Center
Beltsville, Maryland

Annual Progress Report - CY 1984
RSSA BST - 4109-R-AG-3164

Current Staff

Deane F. Weber, Microbiologist
Harold H. Keyser, Microbiologist
Richard F. Griffin, Biological Laboratory Technician
Martin Reinhart, Biological Aid (half time)

I. OVERALL OBJECTIVES

1. Collection, characterization, documentation and preservation of *Rhizobium*.

In CY 1984 the lyophilization and freezing (in glycerin) of the entire assessioned collection was completed. The composition of that collection follows:

Group	Number of strains
Rhizobium sp.	403
R. japonicum	290
R. trifolii	127
R. leguminosarum	68
R. meliloti	67
R. phaseoli	19
TOTAL	974

Nodule collections were made from *Robinia psuedoacacia* and *Albizzia julibrissan* in Maryland. Both of these trees are considered potentially important as sources of wood, forage or for land stabilization. The rhizobia isolated from *Albizzia* nodulated and fixed N_2 with soybean cv. Williams, and several isolates reacted with antiserum of *R. japonicum* USDA 94. The *Robinia* isolates did not nodulate soybean, and *Robinia* plants were very specific in their nodulation with homologous isolates.

Nodule isolates were also recovered from *Apios americana* in Maryland. This plant produces a very high protein tuber, and is well adapted to wet and acidic soils. In order to determine the rhizobial requirements and specificity of *A. americana*, a good seed source is required as well. Unfortunately, this is not the case. However, Dr. W. J. Blackmon of Louisiana State University, has an active program on improvement of *Apios*, including a diversity of genotypes, and he is eager to cooperate on studies of N_2-fixation and rhizobial requirements. This will be undertaken in 1985 and 1986.

Further evaluation of the fast-growing soybean rhizobia revealed some marked symbiotic preferences with Glycine soja genotypes. P.I. 468397 fixed three times as much N_2 with fast growers than it did with slow-growing R. japonicum. Also an extreme incompatibility was found between this P.I. and strain USDA 123. Crosses to G. max Williams have been made to evaluate hybrids which may exclude USDA 123 in a competitive situation.

2. Distribution of cultures of Rhizobium for research and inoculum production in DC's and LDC's.

In CY 1984 the collection distributed 614 cultures of Rhizobium for 102 requests, serving 41 countries. A summary of the 1984 requests follows:

R. japonicum	354 cultures	58%
R. sp.	133 cultures	22%
R. phaseoli	45 cultures	7%
R. leguminosarum	19 cultures	3%
R. trifolii	24 cultures	4%
R. meliloti	39 cultures	6%

Universities accounted for 61% of the requests, 34% by Research Institutes, and 6% from private companies. We also filled 12 requests for seed, antiserum, or peat inocula.

Below is a summary of Rhizobium requests serviced by the collection over the last nine years:

Year	Requests	Cultures
1976	31	193
1977	61	320
1978	47	227
1979	69	515
1980	57	508
1981	117	765
1982	124	842
1983	127	786
1984	102	614

3. Serve as a depository for microbial germplasm.

No germplasm was deposited by outside sources in CY 1984.

4. Train researchers and technicians in techniques and subject matter for applied research and technology of Rhizobium biology and consult with and provide technical assistance to those who work with rhizobia in LDC's.

In CY 1984 we did not have a trainee on the project.

Dr. Lucien Bordeleau was again hired as a consultant to represent our project in efforts to assist the development of the West African MIRCEN in Senegal. His report of that mission is attached to the report, as is the Plan of Action for the MIRCEN.

Dr. Keyser attended the 1st Conference of the African Association of Biological Nitrogen Fixation in Nairobi, Kenya from July 19-27. Several prospective trainees and collaborators were identified. A commitment was made to provide training to Beatrice Anyango from the University of Nairobi. This training would begin in CY 1985.

Dr. Keyser attended the coordination meeting for grantees on the BOSTID Research Grants Program in Chiang Mai, Thailand, July 30-August 4. He presented the seminar "Field application of serologically marked rhizobial strains", and consulted individually with grantees on their BNF programs.

Dr. Weber attended the V. International Culture Collection Congress in Bangkok, Thailand, December 2-6, 1984. He presented talks on Nitrogen Fixation in Agriculture and on the Rhizobium MIRCEN at Beltsville.

Report on the ~~second~~ THIRD mission of Dr Lucien M. Bordeleau on the establishment of a MIRCEN in Senegal for the West African Region

February 4 to 28, 1984

To: National Academy of Sciences, Washington

Purpose of the mission

Senegal: To assist in setting up Mircen as a regional center for West African Region: (1) follow up on NAS (BOSTID) and AGFUND research projects, (2) revision of culture collection, (3) meeting with West African Microbiologists to regionalize the MIRCEN, (4) preparation of the 1st conference of African Association for B.N.F., (5) training on manufacturation and quality control of legume inoculant, (6) planning field plots for inoculant trials.

France: To confer with officials of UNESCO in Paris on the functioning of MIRCEN network.

Upon my arrival at Dakar, Senegal, I conferred with Dr Moctar TOURÉ, DRAI, concerning the situation of the MIRCEN at Bambey and I informed him of my proposed schedule of work. We agreed on having a round-up meeting on February 18, 1984 to establish a plan of action for the future.

The first part of my mission was at CNRA-Bambey. I revised the Bainbarra groundnut project sumbitted to BOSTID and I prepared the guidelines for an answer to the BOSTID granting committee to meet their requirements in the approval of the project. Mamadou GUEYE is responsible to finalize and to document the response to the committee.

As outlined in the meeting report of 18/2/84, I was disappointed of the overall situation concerning the functioning of Bambey's MIRCEN. Mamadou GUEYE has not done much since my last mission in September 1983, either by lack of initiative from his part, or difficulties in getting the proper logistic for functioning. Facing this situation, I had therefore to change my schedule of work in order to make my mission profitable to this MIRCEN. After reviewing the culture collection of this MIRCEN, I decided to organize a sampling mission in South Senegal to collect native legumes and soil samples. With the help of ORSTOM-DAKAR colleagues, we spent four days and collected several samples that will be used to isolate new strains of Rhizobium. Mamadou GUEYE should process the samples in ORSTOM laboratory at Dakar.

The second part of my mission was at Dakar and I used ORSTOM facilities to give a training on inoculant manufacturation techniques and quality control, as well as on methods for field application. Dr Y. DOMMERGUES and his colleagues were very helpful in providing me the facilities. I gave 15 hours lecture and an equal time on practical laboratory work, but much more is needed to assure a good transfer of those biotechnologies. We desperately need French textbook, both on basic and applied techniques of soil microbiology for West African countries. Dr DOMMERGUES and I agreed on preparing first a textbook on the subject, and we will look for financial support for publication; this will be a great contribution because no text exists.

I enclose a letter that I send to professor P. Viaud of Ecole Nationale Supérieure Universitaire de Technologie (ENSUT) Dakar, that illustrate the willing collaboration of this institution to help the West African MIRCEN. I am very pleased by this willingness and I believe they should be encouraged to pursue their initiatives.

The last part of my mission was spent in France. I conferred several hours with Dr E. DASILVA of UNESCO, Paris, to explore ways of having the West African MIRCEN located at Bambey more profitable for the region. Because ISRA cannot assume all the responsibilities attached with the MIRCEN, it is necessary to collaborate with the existing institutions in Senegal. Dr Moctor TOURÉ is very supportive of the idea and he gave a specific mandate to Mamadou GUEYE to explore all the possibilities and prepare documents for collaboration with ENSUT and ORSTOM. M. GUEYE should report before or on March 30, 1984. on the subject. We all realize that the actual MIRCEN at Bambey is precarious

and short term solutions need to be applied. Otherwise, there is no need to pursue consulting mission, because nothing is done. Specific points of collaboration are outlined in my letter to P. VIAUD. I am confident that this collaboration (ISRA/INSUT) will be a success. I also travelled to Dijon to confer with researchers of INRA (Institut National de la Recherche Agronomique, France) that has experience in Africa; first to evaluate their strains of Rhizobia for semi-arid land and second to explore the techniques used for inoculating soils in dry land. The "Laboratoire de Microbiologie des Sols" of INRA-Dijon has in collection several Rhizobium cultures that were isolated from Senegalese soils while Mamadou NDIAYE of ISRA-Bambey was doing his DEA training with Dr N. Amarger at Dijon. For reasons that I do not know, nobody mentioned this work in Senegal and I believe these Rhizobium strains and the competition studies that were done will be useful for the MIRCEN at Bambey. Dr Amarger of Dijon will send these cultures to Mamadou GUEYE. Of interest was the information that was given to me concerning the collaboration of FAO with researchers of Montpellier (IRAT) for the establishment of pilot plants inoculant manufacture in developing countries. On a next trip, I should spend sometime in Montpellier before my next mission to Senegal, to explore fully this opportunity and possibly to get FAO help to Senegal in establishing an inoculant manufacture, outside the ISRA structure.

As outlined in my second mission report of September 1983, my next mission is scheduled in August-September 1984. I will pursue this schedule unless ISRA and the MIRCEN of A.O. do not progress with the collaboration with ORSTOM and ENSUT. Of course, NAS has to approve on the proposals I made. Meanwhile, I will be frequently in touch with Mamadou GUEYE to support and encourage him in doing his work.

I am thankful to Dr Moctar TOURÉ (DRAI) for his courtesy and help in conducting my mission in Senegal. I should also mention the everwillingness of Dr Y. DOMMERGUES (ORSTOM) and his colleagues in assisting and helping as much as possible the West African MIRCEN.

Lucien M. Bordeleau, Ph.D.
Microbiologist
NAS Consultant for W.A. MIRCEN

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Enclosed: Lettter to professor P. Viaud
Report MIRCEN meeting 18/2/84

Agriculture Canada

Research Branch Direction de la Recherche

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Sainte-Foy, le 6 mars 1984

Your file Votre référence

Our file Notre référence

Professeur P. Viaud
École Nationale Supérieure
Universitaire de Technologie (ENSUT)
Dakar, Sénégal
Afrique de l'Ouest

Cher Professeur Viaud,

Je fus très impressionné par le programme de l'atelier d'échanges et de réflexion sur la filière biogaz-compost ENSUT 20-23 février 1984. Malheureusement, je n'ai pu assister à cet atelier étant retenu par d'autres activités dans le cadre de ma mission pour le MIRCEN de l'A.O. Je suis aussi très enthousiasmé par les cours de biofermentation et de biologie des sols que l'ENSUT organise à compter de cette année et en collaboration avec l'ORSTOM. Déjà votre laboratoire est en préparation et sera fonctionnel d'ici peu. Je constate donc l'implication sérieuse de l'ENSUT dans l'enseignement et la vulgarisation des biotechnologies utiles à l'Afrique de l'Ouest.

À la réunion sur le MIRCEN de l'A.O. tenue le 18 février 1984 à la Direction générale de l'ISRA et présidée par le Dr Moctar Touré, monsieur Vincent Jacq a présenté la proposition de l'ENSUT pour abriter temporairement le MIRCEN. M. Touré a accueilli avec enthousiasme cette proposition; il a mandaté spécifiquement Mamadou Gueye d'élaborer sous forme de protocole d'accord entre l'ISRA et l'ENSUT pour cette collaboration sur le MIRCEN, son rapport doit être remis au plus tard le 30 mars 1984.

À l'UNESCO, j'ai discuté longuement avec le Dr E. DaSilva de la situation actuelle du MIRCEN à Bambey, de l'implication très visible de l'ENSUT dans des activités qui cadrent avec les objectifs des MIRCEN et de la collaboration possible ENSUT/ISRA. D'abord le Dr DaSilva m'a assuré qu'il vous obtiendrait un support financier pour publier les délibérations sur l'atelier Biogaz-Compost qui vient de se tenir. Nous considérons que cette activité cadre bien dans les objectifs du MIRCEN. Vous n'avez qu'à lui faire directement la demande avec documents à l'appui.

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En deuxième lieu, afin d'accélérer la signature d'un protocole d'entente ISRA/MIRCEN pour la participation de l'ENSUT et en même temps permettre à l'ENSUT de recevoir quelques supports financiers, je vous demanderais de faire pression auprès de votre directeur, Dr S. Seck, afin qu'il écrive au Dr DaSilva de l'UNESCO pour l'informer qu'un accord a été pris entre l'ISRA et l'ENSUT. Copie de l'accord devrait être jointe à la lettre du Dr Seck et l'accord devrait porter les signatures de Seck et Touré (question de bonne politique vis-à-vis l'UNESCO). Nous avons convenu que l'accord devrait porter sur les points suivants, à savoir: 1 - Que l'ENSUT, dans le cadre des activités du MIRCEN, prenne en charge l'enseignement dans les domaines de la fixation biologique de l'azote et des biofermentations, car l'ISRA ne peut assurer cette mission. 2 - Que l'ISRA et le CNRA de Bambey garde la responsabilité administrative du MIRCEN de l'A.O. et assure le volet d'appui aux expérimentations au champ, la collection des cultures de microorganismes et la production d'un bulletin de nouvelles. 3 - Que les éditeurs du bulletin de nouvelles soient Mamadou Gueye et P. Viaud, afin de couvrir toutes les activités du MIRCEN. 4 - Que la technologie de production d'inoculants biofertilisants soit initiée conjointement avec l'ENSUT et le CNRA de Bambey (l'ENSUT n'étant pas gouvernementale pourrait recevoir une aide financière de la FAO pour une usine pilote). 5 - Que l'ENSUT produise un film vidéo en français sur les techniques d'application de la fixation biologique d'azote et des biofermentations (biogaz) pour le bénéfice du réseau MIRCEN.

J'aime à croire que vous pourrez faire accélérer la signature de cet accord. D'autre part, Mamadou Gueye doit travailler dans le même sens et arriver à un accord d'ici le 30 mars 1984, mais je doute de sa diligence en la matière.

Le Dr DaSilva me prie de vous signaler qu'il vous enverra toute copie de courrier MIRCEN dans le futur, afin que vous puissiez intervenir à l'occasion pour accélérer les procédures.

Croyez, professeur Viaud, à ma sincère considération et à mon entière collaboration pour le succès du MIRCEN de l'Afrique de l'Ouest.

Vôtre,

Dr Lucien M. Bordeleau
Consultant UNESCO/NAS

P.j. Procès verbal réunion MIRCEN du 18/2/84

Compte rendu de la réunion sur le MIRCEN du 18 février 1984

Présidée par Dr Moctar TOURÉ, DRAAI. Mamadou GUEYE est désigné rapporteur de la réunion.

Ordre du jour:

1 - Rapport de la mission du Dr Lucien BORDELEAU, consultant UNESCO/NAS pour le MIRCEN

2 - Discussions du rapport

Présents:

- M. TOURÉ	DRAAI
- M. SONKO	Directeur scientifique/ISRA
- M. NDOYE	D/AGRO/ISRA
- M. L. BORDELEAU	Consultant UNESCO/NAS
- M. GUEYE	Coordonnateur MIRCEN/ISRA
- M. CISSE	SR/NIEBE/BAMBEY
- M. D.F. MBAYE	SR/Patho mil/ISRA
- V. JACQ	ORSTOM

1 - Rapport du Dr L. BORDELEAU

Le rapport de la mission du Dr BORDELEAU a comporté 4 points majeurs:

- situation du MIRCEN au CNRA de Bambey
- régionalisation du MIRCEN
- formation
- collection des souches de *Rhizobium*

Situation du MIRCEN au CNRA

À la suite de ses précédentes missions, Dr L. BORDELEAU avait recommandé qu'il fallait prendre des mesures pour une bonne intégration du MIRCEN dans le laboratoire de Rhizobiologie pour que le MIRCEN puisse pleinement jouer son rôle d'appui.

Dr L. BORDELEAU a constaté que rien n'a été fait pour permettre cette intégration et que Mamadou GUEYE, coordonnateur du programme MIRCEN, n'est pas encore intégré à l'équipe de rhizobiologie.

Régionalisation du MIRCEN

Dr L. BORDELEAU a également constaté qu'il n'y a pas de progrès dans l'effort de régionalisation du MIRCEN à travers l'Afrique de l'Ouest. En effet, lors de sa deuxième mission, Dr L. BORDELEAU et Mamadou GUEYE avaient rédigé ensemble une fiche technique du MIRCEN destinée à vulgariser les objectifs du MIRCEN. Dr L. BORDELEAU regrette que cette fiche technique n'ait jamais été envoyée.

Dr L. BORDELEAU et Mamadou GUEYE ont réactualisé cette fiche technique qu'ils ont remise au directeur du département de recherches sur les productions végétales avec les adresses de tous les chercheurs susceptibles d'être des correspondants du MIRCEN à travers l'Afrique occidentale.

Formation

Il n'a pas été possible de donner le cours sur les techniques de manufacturation et de contrôle des inoculants Rhizobium à Bambey à cause du manque de moyens.

L'École Nationale Supérieure Universitaire de Technologie (ENSUT) organise du 20 au 22 février 1984 une série de conférences sur le biogaz. Dr Lucien BORDELEAU regrette que le MIRCEN n'y participe qu'à titre d'invité. Il aurait été souhaitable que le MIRCEN soit l'organisateur ou le co-organisateur d'une pareille conférence conformément à ses objectifs.

Collection de cultures

Dr BORDELEAU déplore le très faible nombre de Rhizobium dans la collection du MIRCEN qui, en fait, n'est qu'un catalogue de souches ORSTOM. Très peu de souches de Rhizobium ont été isolées depuis la création du MIRCEN.

Dr BORDELEAU a alors rappelé que la collection de souches de Rhizobium est une activité permanente du MIRCEN. À cet effet, il a rappelé qu'il a effectué avec Mamadou GUEYE une mission de collecte d'échantillons de sol en Casamance pour isoler des souches de Rhizobium.

Dr Lucien BORDELEAU a conclu qu'après une année de fonctionnement, le MIRCEN est encore très éloigné de ses véritables objectifs. Il faudrait alors des mesures urgentes pour éviter un échec.

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2 - Discussions du rapport

Les discussions ont porté essentiellement sur l'intégration du MIRCEN dans le laboratoire de Rhizobiologie du CNRA. De l'avis unanime, cette intégration est nécessaire pour que le MIRCEN puisse jouer son rôle d'appui.

À la suite de ces discussions, le DRAAI a fait les propositions suivantes:

1° Le coordonnateur du programme MIRCEN doit réétudier avec le Directeur du département productions végétales les plans de réfection qu'il avait proposés. Ceci doit se faire dans les plus brefs délais, compte tenu du début des travaux de réfection du CNRA prévu vers le 25 février selon le Directeur scientifique ISRA;

2° Rappelant que Mamadou GUEYE est un agent ISRA dont la responsabilité est de coordonner les activités du MIRCEN qui est définitivement et irréversiblement domicilié au CNRA de Bambey, il lui a donné un mandat de faire des prospections à l'ISRA, à l'ORSTOM, à l'ENSUT et à tout autre laboratoire ou institut susceptible de participer au programme MIRCEN.

Ces prospections doivent aboutir à l'élaboration sous forme de document de propositions voire de protocoles d'accord entre l'ISRA et les autres instituts dans le cadre des activités du MIRCEN.

Ce document doit être remis au plus tard le 30 mars 1984. Il servira de support de discussions lors de la prochaine réunion sur le MIRCEN.

Après ces discussions, V. JACQ, chercheur à l'ORSTOM, a rappelé les programmes ORSTOM de microbiologie au Sénégal, programme 601: biofermentation et programme 602: fixation biologique de l'azote. Il a ensuite informé l'assistance que l'ENSUT en accord avec l'ORSTOM organise à compter de cette année deux types de cours

- cours en biofermentation

- cours de biologie des sols.

Ceci a fait l'objet d'un protocole d'accord entre l'ORSTOM et l'ENSUT. V. JACQ a exprimé le voeu des organisateurs de ces cours d'y faire participer le MIRCEN conformément à ces objectifs. Il a en outre déclaré que l'ENSUT est tout à fait disposé à entretenir des relations de collaboration avec le MIRCEN. Il a lu la proposition de l'ENSUT écrite par le professeur P. VIAUD qui comporte les points suivants:

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- structure d'accueil temporaire à l'ENSUT pour recevoir Mamadou GUEYE
- structure de recherche immédiate
- possibilité d'utiliser le compost d'écorce d'arachide pour la production d'inoculum
- programme de formation déjà en cours y inclus les travaux pratiques
- possibilité d'utiliser les structures d'information

Le Directeur des Recherches agricoles et agro-industrielles (DRAAI) a trouvé que cette proposition est bonne et acceptable.

L'assemblée a convenu que le contenu scientifique du programme de Mamadou GUEYE dans le cadre du MIRCEN se résume ainsi:

- apporter le support scientifique au programme de Rhizobiologie à Bambey
- identifier les souches de Rhizobium
- faire une collection de nouvelles souches de Rhizobium des légumineuses du Sénégal

À la fin de la réunion, le DRAAI a rassuré Dr L. BORDELEAU, quant à ses inquiétudes sur l'avenir du MIRCEN. Le Directeur du département des recherches sur les productions végétales a annoncé la parution très prochaine du rapport sur la réunion de microbiologie tenue à Dakar en mai 1983.

L'ordre du jour de la prochaine réunion sur le MIRCEN sera:

1 - Information sur MIRCEN: étude du document de M. GUEYE

2 - Biotechnologie

Dakar, le 18 février 1984

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REPUBLIQUE DU SENEGAL

MINISTERE DE LA RECHERCHE
SCIENTIFIQUE ET TECHNIQUE

INSTITUT SENEGALAIS
DE RECHERCHES AGRICOLES

DEPARTEMENT DE RECHERCHES
SUR LES PRODUCTIONS VEGETALES

PROGRAMME ET PLAN D'ACTIONS DU MIRCEN DE L'AFRIQUE DE L'OUEST

par

Mamadou Guèye

Mars 1984

Centre National de Recherches Agronomiques
de Bambey

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INTRODUCTION

Le MIRCEN de l'Afrique de l'Ouest, installé en 1983 au CNRA de Bambey-Sénégal a orienté son programme sur la Fixation Biologique de l'Azote. Il centralise pour leur conservation et leur utilisation des Rhizobium, des Mycorhizes et des Frankia pour la production d'inocula biofertilisants. Les activités du MIRCEN de l'A.O. sont :

- Collection de culture :

. Collection et utilisation des souches de microorganismes : Rhizobium, Frankia, Mycorhizes et autres souches d'intérêt économique.

- Formation :

. Stage de perfectionnement pour le personnel local et régional ayant déjà une formation en microbiologie pour l'application de la fixation biologique de l'azote. Ces stages sont intensifs et durent 2 à 4 semaines.

- Information

. Une "Revue du MIRCEN de l'A.O" sera publiée semestriellement et traitera des activités nationales et régionales du MIRCEN : technologies d'inoculation, essais au champ, production d'inoculum, compost, production de biogaz.

Ce document indique le programme et le plan d'actions définis par l'équipe du MIRCEN pour atteindre les objectifs fixés.

1 - ISOLEMENT DE NOUVELLES SOUCHES DE MICROORGANISMES

1.1 - Isolement de nouvelles souches de Rhizobium

1.1.1 - A partir des sols de la région de la Casamance

Lors de la dernière mission de consultation de Dr. L.M. BORDELEAU, plusieurs types de sol ont été prélevés à différents endroits de la région de Casamance : Séfa, Affinian et Djibélor. Au total 10 sols ont été prélevés. Ces sols sont indiqués au tableau 1.

L'isolement des souches des Rhizobium se fera à partir des légumineuses suivantes : arachide, niébé, soja et voandzou. D'autre part, des Rhizobium doivent être isolés à partir de Stylosanthes poussant dans la région de Kolda.

1.1.2 - A partir des sols de Bambey

Le MIRCEN de l'Afrique de l'Ouest étant domicilié au CNRA de Bambey, il est judicieux d'isoler des souches de Rhizobium à partir des sols de la station et sur les légumineuses décrites en 1.1.1.

1.1.3 - A partir des sols de la vallée du Sénégal

Actuellement, au Nord du Sénégal, le projet forestier allemand est en cours de réalisation. En collaboration avec un chercheur de ce projet, Simon BADJI, des souches de Rhizobium peuvent être isolées sur tous les arbres fixateurs d'azote poussant dans ces régions et sur les légumineuses annuelles décrites en 1.1.1.

2 - APPUI SCIENTIFIQUE AU LABORATOIRE DE RHIZOBIOLOGIE

Le laboratoire de Rhizobiologie abrite deux opérations : Rhizobiologie du Niébé et Rhizobiologie du Soja. Cette dernière opération sera très prochainement transférée à Djibélor, lieu d'affectation du programme Soja. Une partie du personnel exécutant affecté à ce laboratoire n'a pas les connaissances de base requises en rhizobiologie et les responsables de ces opérations ont souhaité une initiation et un approfondissement des connaissances microbiologiques. C'est pourquoi le MIRCEN dont un des principaux rôles est de diffuser les connaissances microbiologiques par l'organisation de petits cours et stages de perfectionnement, doit prendre en charge la formation et le recyclage du personnel exécutant du laboratoire de Rhizobiologie. Cette formation devra principalement être axée sur :

- l'isolement des souches de Rhizobium ;
- les techniques de purification des Rhizobium ;
- la conservation des souches de Rhizobium ;
- la production d'inoculum ;
- le contrôle de la qualité des inoculum.

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Tableau 1 : Sols prélevés en Casamance

Numéro	Date de prélèvement	Lieu de prélèvement	Observations
1	Février 84	Séfa	précédent maïs (station)
2	"	"	" mil (station)
3	"	"	" sorgho (station)
4	"	"	rhizosphère de crotalaire
5	"	Nioro	précédent maïs (station)
6	"	Affinian	précédent niébé
7	"	"	verger de citrus
8	"	Djibélor	champ paysan . précédent arachide
9	"	"	champ paysan : précédent niébé
10	"	"	précédent niébé (station)

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D'autre part l'opération "Rhizobiologie du Niébé" comprend certaines actions irréalisées compte tenu de la priorité d'autres actions qui sont en cours. Les actions non réalisées sont :

a/- l'étude du nombre et de l'efficience des souches de Rhizobium cowpea dans le bassin arachidier, puis dans d'autres régions du Sénégal.

b/- l'étude de la dynamique des populations de Rhizobium sur une année complète (saison sèche et saison des pluies).

c/- la mise en place d'une collection de souches de Rhizobium (souches locales et souches d'origine étrangère).

d/- l'étude des légumineuses dites "sauvages" en collaboration avec le laboratoire national d'élevage et de recherches vétérinaires (LNERV) avec l'appui d'un botaniste.

e/- l'étude de l'importance de la mycorhization du niébé et de l'arachide.

Cette opération souhaite l'appui du MIRCEN pour la réalisation de ces actions. Quant à l'opération soja qui a déjà une certaine expérience sur la production d'inoculum pour le soja, elle souhaite l'appui du MIRCEN pour la collection de souches de Rhizobium pour le soja et le contrôle de la qualité de l'inoculum produit.

Le MIRCEN de l'Afrique de l'Ouest qui intègre actuellement le laboratoire de Rhizobiologie devra apporter toutes l'aide et l'assistance scientifiques et techniques à ces deux opérations, particulièrement à l'opération soja en raison de la situation particulière qu'elle traverse (départ du rhizobiologiste et arrivée d'un stagiaire) et aux actions de l'opération Niébé, particulièrement les actions c, d et e.

3 - COLLABORATION AVEC LES INSTITUTS DE RECHERCHE

Compte tenu de l'urgence à dynamiser et à vulgariser les activités du MIRCEN, il convient de développer une fructueuse collaboration avec des instituts de recherche travaillant dans le domaine de la microbiologie. Considérant les objectifs prioritaires du MIRCEN (collection et utilisation des souches de microorganismes) deux instituts semblent les mieux indiqués à une telle collaboration : l'ORSTOM et l'Ecole Nationale Supérieure Universitaire de Technologie (ENSUT).

3.1 - Collaboration avec l'ORSTOM

La collaboration avec l'ORSTOM a existé depuis la création du MIRCEN. Il convient de la renforcer.

3.2 - Collaboration avec l'ENSUT

Dans le but d'une future collaboration avec le MIRCEN et en considérant les objectifs du MIRCEN (recherche et formation), l'ENSUT a formulé des propositions qui sont résumées comme suit :

3.2.1 - Recherche

Le MIRCEN admis à temps partiel au laboratoire de microbiologie de l'ENSUT, peut étudier la possibilité de produire un inoculum sur un support solide constitué par l'inclusion de souches efficientes de Rhizobium ou de mycorhize dans des composts obtenus par voie fermentaire.

3.2.2 - Formation

L'ENSUT possède une infrastructure et un équipement particulièrement adaptés à l'organisation de stages ou de séminaires. L'ENSUT propose :

1/- Le MIRCEN peut organiser dans les locaux de l'ENSUT, si possible en collaboration avec l'ENSUT, des stages de formation ou des séminaires.

2/- L'ENSUT dispense actuellement des cours sur la fixation biologique de l'azote. L'ENSUT souhaite que le MIRCEN prenne en charge les volets de l'application de la fixation biologique : production d'inoculum, contrôle de la qualité de l'inoculum, techniques d'inoculation.

Le MIRCEN, est tout à fait favorable à ces propositions. Le MIRCEN organisera donc un stage de formation à l'intention des ressortissants de l'Afrique de l'Ouest, en Août 1985 à l'ENSUT. Le nombre de candidatures sera limitée à 15 et une lettre devra être adressée à Dr. E.J. DASILVA pour une première annonce officielle. Monsieur DOMMERGUES assistera scientifiquement l'organisation de ces cours auxquels Dr. L.M. BORDELEAU prendra part.

D'autre part à la rentrée Octobre 1984, et dans le cadre de l'enseignement dispensé par l'ENSUT, le MIRCEN prendra en charge le volet "production d'inoculum et contrôle de la qualité d'un inoculum" et étudiera la possibilité d'inclure des souches de Rhizobium dans les composts produits par l'ENSUT.

Le Directeur de l'ENSUT est également favorable à ces propositions.

4 - ESSAIS D'INOCULATION AU CHAMP

4.1 - Essai à Affinian

Lors d'une mission en Casamance effectuée en compagnie de Dr. BURDELEAU, nous avons visité le Centre d'Animation Rurale de Affinian (CARA) dirigé par un curé : le frère RICHARD dont la principale activité est d'encadrer de jeunes paysans et de les initier aux techniques agricoles. C'est donc un centre important pour le MIRCEN et il est nécessaire de collaborer très étroitement avec le frère RICHARD pour la vulgarisation de la fixation biologique de l'azote. C'est pourquoi après discussion avec frère RICHARD, il a été proposé au MIRCEN :

- la mise en place au CARA d'un essai d'inoculation d'une petite parcelle de niébé. L'inoculum sera produit par le MIRCEN. Il s'agira de faire une démonstration de la technique d'inoculation.

- l'inoculation d'une pépinière de citrons avec un champignon endomycorhizien.

Ceci est confirmé aux programmes du MIRCEN et du CARA. Ces opérations seront conduites pendant l'hivernage 1984.

4.2 - Essai à Bambey

Le MIRCEN étant domicilié au CNRA de Bambey, le même inoculum destiné à Affinian sera utilisé à Bambey pour un essai d'inoculation de niébé sur une parcelle de 0,5 ha pendant l'hivernage 1984. Il s'agira également de vulgariser les techniques d'inoculation.

5 - ACTIVITES DE RECHERCHE

La proposition de recherche du MIRCEN sur Voandzeia subterranea adressée à l'Académie américaine des sciences est acceptée. Le MIRCEN coordonnera toutes les opérations de ce programme axées sur la sélection, la phytopathologie et la rhizobiologie de V. subterranea.

Ce programme débutera très probablement en juillet 1984.

6 - REGIONALISATION DU MIRCEN

Actuellement, la fiche technique du MIRCEN est envoyée à tous les pays où il y a un correspondant probable du MIRCEN.

- Dr. Mathiar ZENGBE : Côte-d'Ivoire
- Mr. Saihou TAAL : Gambie
- Dr. Salawu ASIMI : Haute-Volta
- Dr. Blaise KABORE : Haute-Volta
- Pr. Messaoud LAHBIB : Mali
- Dr. Kalemani MULONGOY : Nigéria
- Dr. Denis S. AMARA : Sierra Léone

Il faudra, en accord avec l'UNESCO (Dr. DASILVA) convoquer la première réunion régionale du MIRCEN. La première réunion ~~de ce genre~~ peut être tenue en Février 1985, coincidant ainsi avec la dernière mission de Dr. BORDELEAU. Le compte rendu de ces réunions et les publications qui y seront présentées pouvent faire l'objet d'un numéro spécial de la "Revue du MIRCEN" (voir infra).

Les rencontres annuelles des microbiologistes sont un exemple de ce genre de réunion. C'est pourquoi le MIRCEN propose que toutes les publications présentées lors de telles rencontres fassent l'objet d'un numéro de la Revue du MIRCEN.

7 - LA REVUE DU MIRCEN

Ce bulletin du MIRCEN doit paraître deux fois par an. Le premier numéro est prévu pour Avril 1984. Cette revue traitera des activités du MIRCEN et celles de tous ses collaborateurs à qui le MIRCEN devra nécessairement demander les contributions deux mois avant la parution d'un numéro.

CONCLUSION

Ce plan d'actions est actuellement en application avec l'intégration progressive du MIRCEN dans le laboratoire de rhizobiologie du CNRA de Bambey.

La réalisation effective de ce programme, particulièrement le volet formation, nécessite des accords entre l'ISRA auquel est rattaché le MIRCEN et les différents instituts "collaborateurs" du MIRCEN.

REPUBLIQUE DU SENEGAL
MINISTERE DE LA RECHERCHE SCIENTIFIQUE
ET TECHNIQUE

INSTITUT SENEGALAIS DE
RECHERCHES AGRICOLES

DEPARTEMENT DE RECHERCHES SU
LES PRODUCTIONS VEGETALES

RAPPORT DE MISSION EFFECTUEE
DU 7 AU 14 AVRIL 1984
A NOUAKCHOTT (R.I.M)

par

Mamadou GUEYE

Mai 1984

Centre National de Recherches Agronomiques
de Bambey

7λ

I - INTRODUCTION

1) - Généralités

La mortalité des peuplements de Acacia senegal (gommiers) a annulé la production de gomme arabique en Mauritanie (la Mauritanie produisait encore ver 1970, 7000 à 8000 tonnes de gomme arabique par an). Cette mortalité des gommiers arrivés au terme de leur longivité a été accentuée par le cycle de sécheresse des années 70 et a favorisé localement la désertification et la privation de pâturage aérien. Par contre, les jeunes gommiers (ou les gommiers d'âge moyen) ont résisté au cycle de sécheresse.

Il est par conséquent opportun de réunir beaucoup de facteurs de production pour un reboisement correct des zones de production de gommiers. Le Fond Europeen de Developpement (FED) a financé un projet de reboisement en Acacia senegal dans la région du TRARZA qui fut la principale zone productive de gomme arabique. Ce projet est intitulé Projet Pilote de Régénération des Gommiers (PPRG).

D'autre part, en Mauritanie, principalement autour de la ville de Nouakchott (zone nord et nord-est), un système de dunes vives s'est formé après un déboisement causé par un surpâturage, une surcharge du bétail et un long cycle de sécheresse. Ces dunes vives menacent d'ensabler la ville de Nouakchott. Pour stabiliser ces dunes et protéger la ville, un projet de reboisement en Acacia raddiana autour de Nouakchott dénommé Projet de la Ceinture Verte (PCV) a été financé par la Fédération Luthérienne Mondiale.

Ces deux projets (PPRG et PCV) ont conjointement sollicité une mission d'appui du MIRCEN de l'Afrique pour inoculer en pépinière de jeunes plantes de Acacia senegal et Acacia raddiana qui seront par la suite transplantées dans les zones de reboisement.

2) Objectifs de la mission

Les objectifs de cette mission étaient :

(i) l'inoculation de jeunes plantes de Acacia senegal et Acacia raddiana avec une souche de Rhizobium efficiente sur ces légumineuses.

(ii) l'initiation des agents du PPRG à la production d'un inoculum liquide de Rhizobium.

(iii) la collecte de nodules d'arbres forestiers et le prélèvement de sols.

II - TRAVAUX EFFECTUES

1) Inoculation des Acacia

Au cours de notre mission, nous avons inoculé des plantes de Acacia senegal et Acacia raddiana avec un inoculum liquide de la souche de Rhizobium MAO 30 (ORS 911). Cet inoculum préparé au laboratoire de rhizobiologie du CNRA de Bambey, contenait 10^8 Rhizobium par ml.

Les plantes d'Acacia sont cultivées dans des gaines en plastique contenant du sable. L'inoculation consistait à introduire 10 ml d'inoculum dans un trou de 2 cm de profondeur aménagé le long de la racine de la plante.

Le tableau 1 résume toutes les inoculations que nous avons effectuées au cours de la mission. A la fin de notre séjour, il nous restait encore environ 5 litres d'inoculum que les agents du PPRG et du PCV devaient utiliser pour les Acacia raddiana de la pépinière de TEVRAGHZEINA à Nouakchott.

2) Initiation à la préparation d'un inoculum liquide

Le PPRG a installé à travers le TRARZA beaucoup de pépinières de Acaci senegal qui sont entretenues par les communautés rurales. Le personnel technique du PPRG doit alors effectuer plusieurs missions en vue d'inoculer les jeunes plantes de Acacia senegal.

Pour satisfaire cette demande d'inoculum, nous avons initié le personnel technique du PPRG à la production en grande quantité d'inoculum liquide de l souche MAO 30. Ce travail a été facilité par la collaboration effective du laboratoire de microbiologie du Centre National de l'Elevage et de la Recherche Vétérinaire (CNERV) de Nouakchott. Ce laboratoire dispose d'un équipement et de produits nécessaires et suffisants pour la préparation d'un inoculum liquide de Rhizobium (annexe I).

Ce stage de très courte durée consistait à :

- la préparation d'un milieu de culture pour Rhizobium (milieu YEM) ;
- la production de 5 l (2 x 2,5 l) de suspension de Rhizobium, souche MAO 30 (ORS 911) à partir de l'inoculum que nous avions préparé à Bambey.

A la fin de notre mission, le personnel technique du PPRG, particulièrement B. DELAITE, ingénieur agronome nouvellement recruté au projet, avec l'assistance scientifique des chercheurs du CNERV pourra produire l'inoculum liquide de Rhizobium MAO 30 (ORS 911) nécessaire aux pépinières de Acacia senegal. Il faut noter également que de retour au Sénégal, nous avons donné à A. CASTAN, expert forestier consultant au PPRG des souches de Rhizobium efficientes sur Acacia raddiana et Acacia senegal (Annexe II). Ces souches étaient conservées à + 4°C dans des tubes contenant du milieu YEM gélosé incliné.

3) Collecte de nodules et prélèvements de sol

Lors de la visite de la pépinière de ROSSO, nous avons observé la présence de nodules sur les racines de jeunes plantes de Acacia nilotica nilotica et de Leucaena leucocephala que nous avons montrés aux contrôleurs d'économie rurale chargés du suivi de la pépinière.

Les contrôleurs d'économie rurale avaient fait d'abord pousser les plantes d'Acacia et de Leucaena dans le sol de la pépinière avant de les transférer dans de petits sacs en plastique contenant du sol prélevés à la périphérie de ROSSO.

Nous avons prélevé des nodules de Acacia nilotica nilotica et de Leucaena leucocephala, du sol de la pépinière et de la périphérie de ROSSO pour un isolement de Rhizobium à Bambey.

Tableau 1 : Inoculation de Acacia senegal et Acacia reddiana par un inoculum liquide de Rhizobium : souche MAO 30 (ORS 911).

Date	Lieu	Légumineuse	Age	Nombre de plante inoculées
1 Avril	Siège du PPRG Nouakchott	A. senegal	5 mois	90
-"-	-"-	A. senegal	1 an	50
-"-	-"-	A. senegal	8 mois	15
-"-	Pépinière du désert. Nouakchott	A. senegal	2 mois	350
9 Avril	Pépinière du désert. Nouakchott	A. senegal	1 semaine	340
-"-	-"-	A. raddiana	1 semaine	60
-"-	Pépinière forestière expérimentale de TENRAGH-ZEINA Nouakchott	A. senegal	1 an	50
12 Avril	Station forestière de MEDERDRA	A. senegal	2 semaines	11
13 Avril	Pépinière du désert. Nouakchott	A. raddiana	1 semaine	100

III - CONCLUSION

1) Pour cette mission, nous avons préparé uniquement un inoculum de Rhizobium. L'inoculation des Acacia raddiana et A. senegal avec les champignons endomycorhiziens à vésicules et à arbuscules est à envisager comme le souhaite le consultant A. CASTAN.

2) N'ayant rencontré aucun rhizobiologiste mauritanien, nous pensons qu'il est très urgent pour les autorités mauritaniennes d'envoyer quelques agents en formation en vue de promouvoir et de vulgariser la Fixation Biologique de l'Azote, particulièrement dans le domaine de la reforestation. Cela est nécessaire pour atteindre les objectifs nationaux que sont la fixation des dunes et la régénération des pâturages aériens.

   Nous avons à cet effet informé les autorités mauritaniennes (Direction de la Protection de la Nature) de l'organisation des cours de formation sur la Fixation Biologique de l'Azote à l'Ecole Nationale Supérieure Universitaire de Technologie (ENSUT) de Dakar. Déjà deux (2) bourses FE de formation sont disponibles au PPRG.

3) En ce qui concerne la régionalisation du MIRCEN de l'Afrique de l'Ouest nous pensons que Dr. Boubacar Cissé DIALLO, responsable du laboratoire de microbiologie du CNERV pourrait être un correspondant du MIRCEN en République Islamique de Mauritanie. Bien que ses activités ne correspondent pas actuellement à celles du MIRCEN, son équipe (Dr. CHAMOISEAU et Dr. Le JAN) pourrait assister B. DELAITE et le personnel du PPRG et du PCV à la production d'inoculum et à la conservation des souches de Rhizobium.

   Cependant des informations complémentaires et de la documentation sur les activités des MIRCENS doivent être envoyées à Dr. B.C. DIALLO.

Annexe I - Equipement du laboratoire de microbiologie du CNERV

Matériel	Nombre
- Hotte à flux laminaire	1
- Autoclave	1
- Lyophilisateur	1
- Incubateur	2
- Appareil eau distillée	1
- Cartouches pour eau déminéralisée	1
- Armoire réfrigérée (conservation des produits)	2
- Congélateur	1
- Petit matériel de laboratoire	nombreux
- Verrerie	nombreux
- Produits chimiques	nombreux
- Chambre stérile	

Annexe II - Souches de *Rhizobium* données à A. CASTAN expert forestier au PPRC

1ère Référence	2è Référence	Effectif sur	Croissance sur YEM
MAO 30	ORS 911	Acacia senegal	Rapide
MAO 31	ORS 920	Acacia reddiana	"
MAO 32	ORS 921	-"-	"
MAO 33	ORS 922	-"-	"
MAO 34	ORS 923	-"-	"
MAO 35	ORS 924	-"-	"
MAO 36	ORS 925	-"-	"

ANNEXE III - Personnes rencontrées

Mouhamed Ould ABBY	Directeur du projet Ceinture Verte
Mamadou Issa BARRY	Contrôleur des Eaux et Forêts - Coordinateur au PPRG
Mamadou Ibra BASS	Contrôleur d'économie rurale - Chef du cantonnement forestier de MEDERDRA - PPRG.
Augustin CASTAN	Expert forestier - Consultant au PPRG
Georges CHAMOISEAU	Docteur Vétérinaire - CNRV, laboratoire de microbiologie
Benoit DELAITE	Ingénieur forestier - PPRG
Boubacar Cissé DIALLO	Docteur vétérinaire - CNERV, laboratoire du microbiologie
Ousseynou GAYE	Contrôleur d'économie rurale - Chef du cantonnement forestier de R'KIZ - PPRG
Ahmed HAIDARA	Directeur du PPRG
Hadya KANE	Directeur de la protection de la nature
Christian Le JAN	Docteur vétérinaire-CNERV, laboratoire de microbiologie.
Malick MBODJ	Contrôleur d'économie rurale - Chef du cantonnement forestier de ROSSO - PPRG.
Memine Ould SALECK	Contrôleur d'économie rurale - chef de la division Pépinières et Recherche Forestière du Projet Ceinture Verte.
Monsieur TAIMAN	Représentant de la Fédération Luthérienne Mondiale.

FIELD APPLICATION OF SEROLOGICALLY MARKED RHIZOBIAL STRAINS

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Field research is inherently time consuming, and more variable than laboratory or greenhouse research. It often represents the final stage of testing in research on biological nitrogen fixation (BNF) and is surely the most relevant in putting BNF to work. It can also be expensive and usually very labor intensive. The successful field application of a biological inoculum requires careful preparation and execution. The importance of attention to the details of a field trial is no less critical than with sophisticated laboratory procedures. The use of serologically (or otherwise) marked strains of Rhizobium in field trials allows the investigator to assess strain effects both qualitatively and quantitatively. Studies of environmental, host genotype and microbrial effects on nodulation, and rhizobial populations in the soil and rhizosphere require the use of marked strains.

This brief overview focuses on common techniques used in field research with marked Rhizobium inoculants.

Attached to this paper are the protocols from three international Rhizobium inoculation trials: (1) International Bean Inoculation Trial (IBIT), formerly

sponsored by Dr. P. Graham at CIAT; (2) International Soybean *Rhizobium* Inoculation Experiment (ISRIE), formerly sponsored by INTSOY; and (3) International Network of Legume Inoculation Trials (INLIT), sponsored by NifTal. They provide clear examples of techniques and procedures mentioned in this paper and are useful references for anyone initiating a field trial involving *Rhizobium*.

STATISTICAL DESIGN

Critical to the proper interpretation of data from a field trial is the use of a statistical design that is best for the purpose and conditions of that trial. The large variability inherent in the field requires a design that best detects the sources of variability and allows the separation of environmental and real treatment effects. A review of applied agricultural statistics is not intended here, and the reader is directed for such to Pearce (1983), Little and Hills (1972), Steel and Torrie (1960), and LeClerg, Leonard and Clark (1962).

It is important to emphasize that the choice of statistical design needs to be considered in the planning stage of a trial. It is recommended that the investigator consult a statistical expert in their institute regarding proper design. They can be very helpful in the selection of randomization, replication, and local control for the purpose at hand.

As listed in the Appendix, the IBIT, ISRIE, and INLIT experiments all suggest a randomized complete block design. The strength of this popular design is its ability to remove variation due to a gradient (block effect) in a field, thereby increasing precision in detection of treatment effects. The selection of a given design can be influenced by the size, shape, history, and general nature of the field site. A minimum of three and preferably four or more replicates is recommended, along with a provision for keeping out grazing animals.

FIELD SELECTION, PREPARATION, AND PRELIMINARY DETERMINATIONS

As noted by Brockwell (1980) (in a chapter concerning methods for field use), the selection of a field for an experiment with Rhizobium may be determined or limited by practical considerations, but hopefully, it will meet the requirements for the purpose of the trial. These requirements, of course, will be quite different if one is investigating N_2-fixing effectiveness of strains versus an assessment of competitiveness and persistence. In the former case, a field should be chosen which has very little to no indigenous competing rhizobia, whereas, indigenous rhizobia would be required for the latter case. Regardless, the best of possibilities would be the selection of a field that is uniform in soil type and aspect, is level, free of drainage problems, and low in available N. Since this is seldom found, the investigator must check for observable gradients or other variations, as this will influence choice of statistical design and the actual plot layout. Knowledge of previous crops, soil amendments, and pesticide additions can also be useful in field site selection. (Was a legume in the same cross inoculation group grown before? Was 300 kg NH_4NO_3/ha added last year? Was atrizine applied recently?) In some areas of the world a field with access to irrigation water may be prefered or essential.

Once a site is selected, the field will usually be prepared in some fashion (plowing, discing, or rototilling). Also, if herbicides are not used, one or two cultivations may be necessary. Such operations, especially on a new field, usually result in increased mineralization of organic nitrogen and this may affect the amount of N_2 fixation by the legume-Rhizobium symbiosis. If the available N level in the field is judged to be too high, it can be lowered by growing a cereal as wheat or corn prior to the trial (with removal of top growth), or incorporation of a highly carbonaceous material such as sugarcane bagasse (see Cassman, Whitney and Fox, 1981).

Subsequent to working the field, amendments as lime or fertilizer are added, taking care that the machinery used is relatively clean so as to prevent introduction of rhizobial contamination.

Certain determinations are desirable on a field that is being used in a test with rhizobia are desirable. They include: Rhizobium (specific or total) population level, soil pH, available soil N status (extractable NH_4+NO_3, mineralizable N), and soil test levels of certain nutrients (e.g. P, Ca, K, S, Mo & Zn). If indigenous rhizobia are present, their number should be determined (see Vincent, 1970), and it should be determined if they are distinguishable from the marked strains that are being applied in the experiment. If the soil pH is below 5.5 most legumes will respond to lime, and a soil buffer curve will need to be determined. The nutrients listed above are those that legumes have been shown to respond to, and a basal application to the site may be necessary. Also, note that the INLIT design provides for a high fertility treatment, which emphasizes the importance of having good plant growth in obtaining a response to N_2 fixation.

INOCULANT PREPARATION AND USE

Most researchers in BNF use one of three types of inoculant for field trials: (1) a peat base powder applied to seed, (2) a peat base granular inoculant applied to the soil in the seed row, and (3) a liquid inoculant applied to the seed and soil in the seed row. While several other forms of incoulants have been used (see Burton, 1979) they are not discussed here.

While researchers often take advantage of the generosity of commerical inoculant producers in supplying custom peat powder inoculant, it is fairly easy to make with a good quality peat.

In the author's laboratory, the peat powder inoculant is prepared by adding 100 ml of a stationary phase rhizobial broth culture to 180 g air dry peat. The addition of neutralizing base may also be required, as many peats are acidic. The peat is incubated (up to 3 weeks for slow growers) at room temperature (25° C) in polyethylene bags provided with aeration. At the time of use, a population of 10^8 to 10^9 cells/g peat should be attained. In the U.S., the normal rate of use of inoculant is 4.4 g/kg seed regardless of seed size (Burton, 1979). Seed retention of the peat is easily obtained by coating the seed with a 35-40% (w/v) solution of gum arabric (gum acacia) which has been neutralized.

The peat granular inoculant is useful in situations requiring the delivery of a high number of cells to the soil (such as acidic, hot, sandy soils, etc.). It can deliver much more inoculant per area (5-30 kg/ha) than obtained by regular seed applied peat. Preparation of this form of inoculant is best done by a generous commercial inoculant manufacturer. See Burton (1979) for a more complete description.

The liquid broth inoculant is easily grown and applied. The rate of application in the seed row can readily be adjusted to 10^6-10^9 cells per cm of row.

Rhizobia do not survive well on seed, and therefore, the inoculum should only be applied within a few hours of planting. Neither of the two peat forms of inoculant should be exposed to the sun or high temperatures for very long. The liquid and the granular forms should be applied to the row simultaneously with the seed or after the seed has been placed in the forrow.

Cross contamination of applied rhizobia is always a possibility in field plots. This can be reduced by careful hygiene (for operators and their

equipment), the provision of access alleys in the field, and planting the uninoculated and nitrogen controls before the strain treatments.

Planting should be done by replicates (or blocks), and should be accomplished in as little time as possible due to the relative fragility of biological inoculants. A concise summary of inoculant preparation and application is given by Brockwell (1982).

MAINTENANCE, SAMPLING, AND HARVEST DETERMINATIONS

Plant damage by grazing animals can be eliminated by a fence around the area. If this is not possible, several rows of 'feed' border should surround the plot. If torrential rains are likely, cross contamination between individual plots can be minimized by digging drainage ditches around plots, though this requires much labor. Critical to the maintenance of a field plot is, of course, the control of weeds and insects through a variety of standard procedures.

Table 1 summarizes the essentials of the IBIT, ISRIE, and INLIT field experiments, including the various sample and harvest components. A comparison of these three trials shows the kind of data most commonly obtained in field trials of the *Rhizobium*-legume symbiosis. Their details are covered sufficiently in the Appendix, and I will focus here on nodule sampling of the marked strains.

Field nodule samples are commonly taken for measurements of mass, number, volume, visual rating, cross-sectional pigmentation, nitrogenase activity, and for identification of the nodule occupant(s). These assessments are frequently made twice during the growing season of an annual crop species to detect differences in early and late nodulation. Within a replicate, nodule samples for subsequent identification can be taken from a given number of plants (IBIT;

Roughley, Blowes and Herridge, 1976; Moawad and Bohlool, 1984), a given length of row (Caldwell and Weber, 1970; Caldwell and Vest, 1968), or randomly until a sufficient number is obtained (Bezdicek, 1972; Johnson and Means, 1964; Boonkerd, Weber and Bezdicek, 1978). In most cases 50 nodules per replicate per treatement are tested for their serological or antibiotic markers, though some investigators sample as low as 25 nodules per replicate. In fields containing background rhizobia, Vest, Caldwell and Peterson (1971) caution that more than one sample per replicate should be taken, due to population variation. They found R. japonicum variability to be greater within than between areas (blocks) in the field.

In most legume field trials the harvest parameters of seed yield (grain legumes) or total dry matter production (forages) are determined. Nitrogen analysis of that harvest component is recommended since this information can be used in calculating N_2-fixation values (see discussion by Drs. Fried and Herridge). Other determinations that can be made on grains (e.g. soybean) include oil and protein content and 100-seed weight. See Nutman (1976) for a good discussion of results of the IBP field experiments with nodulated legumes.

Experimental unit:	4.5 x 1.0 m single row (minimum)	5.0 x 0.6 m row, 4 row plots	7.5 x 0.6 m row, 4 row plots
Design:	Randomized complete block, 4 replications	Randomized complete block, 4 replications	Randomized complete block, 4 replications
Sample and yield data:	At 25 days after planting; nodule no. - 5 plants/rep, nodule fresh weight, nodule color	At 4 weeks after emergence and at pod fill; nodule no. and color, plant color	No. rhizobia/g peat at application, age of plants when nodules appear, nodule abundance, distribution, and color at thinning and at 50% flowering
	Plant dry weight at flowering (5 plants)	Plant height, lodging at maturity	Total plant dry matter (10 plants) and fresh weight of nodules/plant at 50% flowering
	Seed yield - 10 plants/rep	Pod per plant, grain yield, 100 seed weight	Seed yield of grain legumes or dry matter of cut forage legumes
Optional data:	Acetylene reduction activity at 25 days and at flowering. Percent N in seeds. Chemical analysis of soil. Strain competition for nodule sites.		Acetylene reduction activity and chlorophyll content at 50% flowering. Strain competition for nodule sites.

1. UC = uninoculated control
2. NC = nitrogen control
3. I = inoculum

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FAST-GROWING SOYBEAN RHIZOBIA

H. H. Keyser, M. J. Sadowsky, and B. B. Bohlool

Rhizobium are the bacteria capable of forming a nitrogen-fixing symbiosis with leguminous plants. Traditionally, their classification has been based on specific host infectivity with the recognition of two broad groups of fast- and slow-growing strains (Jordan and Allen, 1974). In addition to growth rate, the two groups are distinguished by several important differences, which has led to the proposed establishment of a separate genus for slow growers (*Bradyrhizobium* gen. nov.), while retaining fast growers in the genus *Rhizobium* (Jordan, 1982).

The soybean-nodulating bacteria, *R. japonicum*, have been classified as slow growers. Recently, however, Keyser et al. (1982) reported the existence of fast-growing isolates obtained from soil and nodules collected in China. Examination of the 11 fast-growing isolates for microbiological, genetic, and symbiotic properties has shown them to be distinct from the slow growers. They represent a unique and valuable addition of *Rhizobium* germplasm. In this article we present an overview of microbiological and symbiotic properties of the fast-growing soybean rhizobia. Results from genetic studies are presented elsewhere in this volume by Atherly et al. Several investigators are currently examining these fast growers for various attributes, and knowledge of them should increase rapidly.

ISOLATION

The 11 fast-growing strains were obtained from three separate collection expeditions in China (Table 1). Strain USDA 191 was isolated by J. C. Burton (Nitragin Co., Milwaukee) and the others by T. S. Hu and S. N. May in the USDA Nitrogen Fixation and Soybean Genetics Laboratory at Beltsville, Md. The isolates were authenticated by three cycles of plant passage through *G. max* cv. Peking and reisolation of single colonies. Careful examination showed no slow-growing colonies that could have been masked by the fast-growing type. Subsequent use of fluorescent antibodies confirmed their purity.

CULTURAL CHARACTERISTICS

The fast growers have generation (doubling) times of 2.9 to 4.8 h at 28 C in yeast extract-mannitol-salts (YEM) broth with a concomitant acidic reaction (Keyser et al., 1982). This is in contrast to slow-growing *R. japonicum*, which have generation times of 6 or more hours and a neutral to alkaline reaction in YEM media (Vincent, 1974). On YEM agar plates the fast growers gave 1 to 5 mm diameter colonies after 6 to 7

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Table 1. Strain history of fast-growing soybean rhizobia from China.

USDA accession no. and synonyms	Geographic origin of isolate	Source material from which isolation was made
191 (=440)	Shanghai, Jiangsu Province	Soil: isolated from nodule of trap host *G. max* cv. Jupiter[a]
192 (=0B2=PRC 192)	Tsinan, Shantung Province	Desiccated root system of *G. soja*: isolated from nodule of trap host *G. max* cv. Peking[b]
193 (=0B3=PRC 193)	Taiyuanchen, Shansi Province	Desiccated root system of *G. soja* PI 468397: isolated from nodule of trap host *G. max* cv. Peking[b]
194 (=PRC 194)	Chengchou, Honan Province	Desiccated nodule of *G. max*: direct isolation[c]
201 (= PRC 201)	Same as 194	Same as 194
205 (=PRC 205)	Same as 194	Same as 194
206 (=PRC 206)	Sinsiang, Honan Province	Same as 194
208 (=PRC 208)	Same as 206	Same as 194
214 (=PRC 214)	Same as 206	Same as 194
217 (=PRC 217)	Same as 206	Same as 194
257 (=PRC 257)	Wukung, Shensi Province	Same as 194

[a]Collected by J. Tanner, Univ. of Guelph, 1979.
[b]Collected by T. S. Hu, Chinese Academy of Agric. Sciences, Peking, 1980.
[c]Collected by W. Fehr, Iowa State Univ., and K. Hinson, Univ. of Florida, 1979.

days, whereas colonies of slow growers are only 0.5 to 1 mm. The fast growers produce typical circular, convex, white colonies on YEM agar, with little production of extracellular polysaccharide (Sadowsky et al., 1983). In liquid media, cells from late-log-phase to stationary-phase became enlarged and exhibited marked pleomorphism, while slow growers remained as typical rods. The growth rate difference between the two types of soybean rhizobia is maintained in sterile soil (Sadowsky, 1983). This information indicates that the cultural characteristics of the fast-growing soybean rhizobia are similar to other fast-growing *Rhizobium* species (e.g., *R. leguminosarum* and *R. trifolii*).

GROWTH RESPONSES

The fast-growing soybean rhizobia do not require vitamins (biotin, thiamine, pantothenate) for growth in a defined medium (Stowers and Eaglesham, 1984), a characteristic shared by slow-growing rhizobia. However, unlike slow growers they are tolerant of pH 9.5 and sensitive to pH 4.5 (Sadowsky et al., 1983). As with other fast growers, they are more sensitive to antibiotics than are the slow growers (Sadowsky, 1983; Stowers and Eaglesham, 1984). Also, in contrast to slow-growing *R. japonicum*, the fast growers are tolerant of high levels of NaCl (Sadowsky et al., 1983; Yelton et al., 1983; Stowers and Eaglesham, 1984). Likewise, such tolerance is also found

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among the fast-growing *R. meliloti* (Graham and Parker, 1964). Yelton et al. (1983) demonstrated that tolerance of 0.4 M NaCl is accompanied by increased levels of K^+ and glutamate in USDA 191. Stowers and Eaglesham (1984) found the fast growers, with one exception, to be tolerant of 0.17 M NaCl, but completely sensitive to 0.34 M NaCl. Sadowsky et al. (1983) found that all 11 of the fast growers made growth at 0.34 M NaCl, although some strains had growth limited to a few tolerant colonies after 14 days. None of the slow-growing *R. japonicum* tested by Sadowsky et al. (1983) produced any colonies at 0.34 M NaCl.

CARBOHYDRATE UTILIZATION

Fast-growing species of *Rhizobium* can use many sugars and organic acids for growth, whereas the slow growers are more specialized and prefer pentoses (Fred et al., 1932). Studies of the carbohydrate utilization by the fast-growing soybean rhizobia have shown they match the pattern of other fast-growing rhizobia (Sadowsky et al., 1983; Yelton et al., 1983; Stowers and Eaglesham, 1984). Unlike the slow-growing *R. japonicum* the fast growers utilized cellobiose, inositol, lactose, maltose, raffinose, glucitol, sucrose, and lactose. The ability to use lactose for growth requires sufficient activity of β-Galactosidase. Sadowsky et al. (1983) demonstrated large differences between the fast- and slow-growing soybean rhizobia in levels of β-Galactosidase activity (Table 2).

Consistent with the division of the rhizobia into fast- and slow-growing groups based on carbohydrate utilization is the division of these organisms based on the

Table 2. Enzyme activities of fast- and slow-growing soybean rhizobia (Sadowsky et al., 1983).

Strain	Enzyme activities	
	6-PGD[a]	β-Galactosidase[b]
Slow-growing rhizobia		
USDA 31	<0.5	7.1
USDA 110	<0.5	3.8
USDA 123	<0.5	3.0
USDA 136	<0.5	7.6
USDA 138	<0.5	3.4
PRC 005	<0.5	4.2
PRC 113-2	<0.5	3.3
PRC 121-6	<0.5	4.8
PRC B15	<0.5	4.8
Fast-growing rhizobia		
USDA 191	97	–
USDA 192	181	459
USDA 193	140	183
USDA 194	126	101
USDA 201	50	345
USDA 205	159	97
USDA 206	59	261
USDA 208	147	383
USDA 214	160	245

[a]6-phosphogluconate dehydrogenase, expressed as nanomoles of reduced NADP produced per min per mg of protein.

[b]Expressed as micromoles of o-nitrophenol produced per min per mg of protein.

presence and absence of enzymes of the pentose phosphate pathway. Only fast-growing rhizobia have NADP-linked 6-phosphogluconate dehydrogenase (6-PGD) activity, a key enzyme of that pathway (Keele et al., 1969; Martinez-de Drets and Arias, 1972). Table 2 shows the clear differences between the two types of soybean rhizobia in 6-PGD activity, again linking these fast growers to other fast-growing rhizobia. Studies by Yelton et al. (1983) and Stowers and Eaglesham (1984) also showed the presence of 6-PGD activity in these fast-growers.

Of the 30 strains examined from 6 species of *Rhizobium*, only the fast-growing soybean rhizobia were capable of substantial growth in ethanol (Sadowsky, 1983). While the fast growers grew to final cell densities of 10^8-10^9 cells/mL on 0.2% ethanol, the slow-growing *R. japonicum* never reached densities greater than 10^7 cells/mL on any concentration of ethanol. No activity of NAD- or NADP-linked alcohol dehydrogenase was found.

BIOCHEMICAL ATTRIBUTES

In comparing the 11 fast-growing soybean rhizobia with 7 isolates of slow-growing *R. japonicum*, Sadowsky et al. (1983) found both groups to be positive for catalase, oxidase, urease, penicillinase, and nitrate reductase. Neither group produced H_2S or 3-Ketolactose. Bernaerts and DeLey (1963) found that production of 3-Ketolactose from lactose is limited to species of *Agrobacterium*, a genus closely related to the fast-growing *Rhizobium*. Graham and Parker (1964) found that production of penicillinase was more common in slow-growing rhizobia, though some isolates of the fast-growing *R. leguminosarum* possessed this attribute.

Gelatinase activity clearly differentiated the two groups. The slow growers were negative while the fast growers and an isolate from *Leucaena* were positive (Sadowsky et al., 1983).

In a test of litmus milk reactions, 20 of 21 slow growing *R. japonicum* produced an alkaline pH change with no peptonization (Sadowsky et al., 1983). By comparison, 10 of 11 fast growers produced peptonization of the medium, though pH changes were variable. This response of the fast-growing soybean isolates is typical of other fast-growing *Rhizobium* species (Graham and Parker, 1964).

The fast growers apparently lack hydrogenase activity, as determined in culture and in bacteroid preparations. All seven of the fast growers tested for hydrogenase in free-living culture were negative (S. Uratsu, pers. commun.). Yelton et al. (1983) found USDA 191 to lack hydrogenase activity in culture as well as in symbiosis with *G. max* cv. Clark and cowpea (*Vigna unguiculata* [L.] Walp.) cv. Calif. Blackeye 5. We have found all 11 of the fast growers also to be negative in symbiosis with *G. max* cv. Peking and *G. soja* PI 468398.

SEROLOGICAL RELATIONSHIPS

The fast-growing soybean rhizobia are serologically related (Sadowsky, 1983). Tests with whole-cell antisera indicated that the fast growers shared at least one heat-labile antigen in common. Examination with the more specific somatic antigens showed at least three serological groups among the fast growers; USDA 192 (3 strains), USDA 194 (2 strains), and USDA 205 (6 strains).

In agglutination tests with somatic antisera from 14 non-cross-reacting strains of slow-growing *R. japonicum*, the only cross reaction we found with the fast growers occurred between the USDA 194 group and the USDA 122 group (representing the

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slow growers USDA 122, USDA 136, CB 1809, and others). Sadowsky (1983) found the same degree of difference between the fast and slow growers. He also found that while USDA 136 cross-reacted with USDA 194 in immunofluorescence reactions, this was not detected in immunodiffusion reactions using somatic antisera.

Immunodiffusion tests with species of *Rhizobium* other than *R. japonicum* with antisera from three of the fast growers showed some very interesting relationships. Fast-growing isolates of *R. meliloti*, an isolate from *Sesbania*, and an isolate from *Lablab purpureus* (L.) Sweet had whole-cell and somatic antigens in common with the fast-growing soybean rhizobia (Sadowsky, 1983). Slow growers from *R. lupini* and the 'cowpea' groups did not cross react. The fast-growing soybean rhizobia appear to be fairly distinct among *Rhizobium* in their serological relatedness to both slow growers (*R. japonicum*) and fast growers (*R. meliloti* and 'cowpea' types), though Trinick (1980) reported the same for isolates of *Leucaena*.

SYMBIOSIS WITH LEGUMES

The fast-growing soybean rhizobia nodulate and fix N_2, to varying degrees of effectiveness, with *G. max* and *G. soja* genotypes and with some legumes of the cowpea cross inoculation group. Similar host range is found with the slow-growing *R. japonicum* (Leonard, 1923; Van Rensburg et al., 1976). The fast growers do not nodulate hosts of other fast-growing species (*R. meliloti; R. trifolii; R. phaseoli; R. leguminosarum*) (Keyser et al., 1982; unpublished data). Many adapted genotypes of North American soybeans form ineffective symbioses with the fast growers (Keyser et al., 1982). Table 3 shows the response of four cultivars. In most cases the fast-growers

Table 3. Top dry weight of soybean cultivars inoculated with fast- and slow-growing soybean rhizobia.[a]

Strain	Cultivars of *G. max*			
	Wilson-6	Bedford	Clark	Hardee
		– g/jar[b] –		
Slow growers				
USDA 110		4.8 r		6.1 r
USDA 122	6.4 r		4.6 r	
Fast growers				
USDA 191		1.9 t		2.4 s
USDA 192	1.9 u		2.7 s	2.4 s
USDA 193	1.5 uv		2.2 s	
USDA 194	1.7 uv	3.0 s	2.9 s	
USDA 201			2.8 s	2.5 s
USDA 205	2.6 t	1.9 t	2.8 s	2.2 s
USDA 206		2.0 t		
USDA 208	3.3 s		3.1 s	2.4 s
USDA 217	1.8 uv		2.3 s	2.6 s
Uninoculated	1.3 v	1.8 t	2.6 s	1.9 s

[a]Tests conducted in vermiculite-Leonard jar assemblies, grown for 5-6 weeks in a growth chamber.

[b]Each value is a mean of 3 replicates, 3 plants per replicate. Values followed by the same letter within a column are not significantly different ($P < .05$) according to Duncan's New Multiple Range Test.

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were not significantly better than an uninoculated control, as well as being significantly inferior to the reference slow grower. Nodule production on these cultivars ranges from normal (with cultivars Bedford and Hardee) to sparse and atypical (with cultivars Clark, Kent, and Lee). Similar ineffective responses by North American soybeans have been reported by Van Rensburg et al. (1983) and Stowers and Eaglesham (1984). This general ineffective response is not without exception as Yelton et al. (1983) reported USDA 191 to be effective with Clark, and Dowdle (pers. commun.) found three of the fast growers to be effective with other cultivars, based on acetylene-reduction.

Peking forms an effective symbiosis with the fast growers (Table 4). Peking is an unimproved selection of a line from China. Also, Stowers and Eaglesham (1984) found the fast growers to be very effective with three "Asian-type" soybeans. Van Rensburg et al. (1983) found an effective symbiosis between the fast growers and two South African soybeans (Table 5). Cultivar Geduld is a selection from a cross of Dixie and a line from China, while Usutu was selected from crosses in which Geduld was involved. Perhaps, the low frequence of effectiveness with the U.S. cultivars is atypical of soybean germplasm in general and simply results from the narrow germplasm base of U.S. cultivars, which may be built upon accessions with a very low frequency of effectiveness with the fast growers.

Cowpea forms an effective symbiosis with the fast growers (Table 4). This has been confirmed by Stowers and Eaglesham (1984) and Yelton et al. (1983). While they are

Table 4. Shoot nitrogen content in three legumes inoculated with fast- and slow-growing soybean rhizobia.[a]

Strain	Host legume		
	G. max cv. Peking	*G. soja* PI 468398	*Vigna unguniculata* cv. Calif. Blackeye-5
		– mg N/jar[b] –	
Fast growers			
USDA 191	65.3 r	6.9 rs	97.6 rstu
USDA 192	37.7 u	8.5 r	88.8 rstuv
USDA 193		6.8 rs	103.0 rst
USDA 194	46.3 stu	7.1 rs	63.5 vw
USDA 201	63.8 r	7.0 rs	78.3 tuv
USDA 205	50.4 s	6.0 rs	93.6 rstu
USDA 206	38.7 tu	5.8 s	49.5 w
USDA 208	45.9 stu	6.9 rs	78.8 tuv
USDA 214	45.3 stu	7.6 rs	73.2 uvw
USDA 217	49.1 st	7.0 rs	82.9 stuv
USDA 257		7.0 rs	104.9 rs
Slow growers			
USDA 122	52.6 s	6.2 rs	
176A22[c]			116.1 r
Uninoculated	6.9 v	1.1 t	8.7 x

[a]See footnote a, Table 3.
[b]See footnote b, Table 3.
[c]A slow-growing reference strain of *R. sp.* 'cowpea miscellany'.

Table 5. Dry mass and nodulation of cultivars of *G. max* inoculated with slow- and fast-growing strains of *R. japonicum* (from Van Rensburg et al., 1983).

Strain	Cultivar of American origin	Cultivars of South African origin		
	Forrest	SSS3	Geduld	Usutu
	– g/plant[a] –			
Slow grower				
WB1	2.72	2.66	2.11	1.87
Fast growers				
USDA 193	0.56[b]	0.58	2.14	1.46
USDA 194	0.64[b]	0.52	1.59	1.33
USDA 201	0.67[b]	0.49	2.07	1.79
USDA 205	0.67[b]	0.80	2.29	1.38
USDA 206	0.71[b]	0.52	1.57	1.47
Uninoculated	0.82[b]	0.68[b]	0.56[b]	0.54[b]

[a]Average of 3 replicates LSD (P (0.01) = 0.52; c.v. = 28.4%.
[b]No nodules formed.

also effective with pigeon pea (*Cajanus cajan* [L.] Huth) the fast growers are ineffective with mung bean (*Vigna radiata* [L.]) and species of *Sesbania* and *Macroptilium* (Keyser et al., 1982; Stowers and Eaglesham, 1984).

The most interesting comparison of host preference between the fast- and slow-growing soybean rhizobia is found in their reaction with different genotypes of wild soybean (*G. soja*). Keyser et al. (1982) reported the fast growers to be effective at N_2-fixation with a line from China (Table 4). Subsequent tests with seven other *G. soja* genotypes demonstrated significant effectiveness interactions with both rhizobia groups (Keyser and Cregan, 1984a). The response of two genotypes with opposite group preferences is shown in Figure 1. PI 468397 exhibits a general specificity for effectiveness with the fast growers as opposed to slow growers. This very unusual reaction has not yet been found in *G. max*, and indicates there is a large range of symbiotic adaptations in *G. soja* germplasm.

CONCLUSIONS

The fast-growing soybean rhizobia from China are distinct from the slow-growing *R. japonicum*. Several distinguishing bacteriological properties used to separate the slow-growing *Bradyrhizobium* from the fast-growing *Rhizobium* place these new isolates in the *Rhizobium* genus. However, the similar host range with the slow growers points out the difficulty of including both the bacteriological and the host related properties in the classification system of root nodule bacteria.

China is considered to be the gene center of the *G. max* and *G. soja*, and apparently it is also the gene center of two types of soybean rhizobia. The occurrence of the fast growers in China is not rare, as they were found in five provinces. In the collection made by Fehr and Hinson, 8 of 69 nodules contained fast-growing isolates. In contrast, the occurrence of the fast growers in U.S. soils is apparently very low at best, though most workers would probably discard such isolates as suspected contaminants.

Genetic studies of the fast growers have also shown them to be distinct from the

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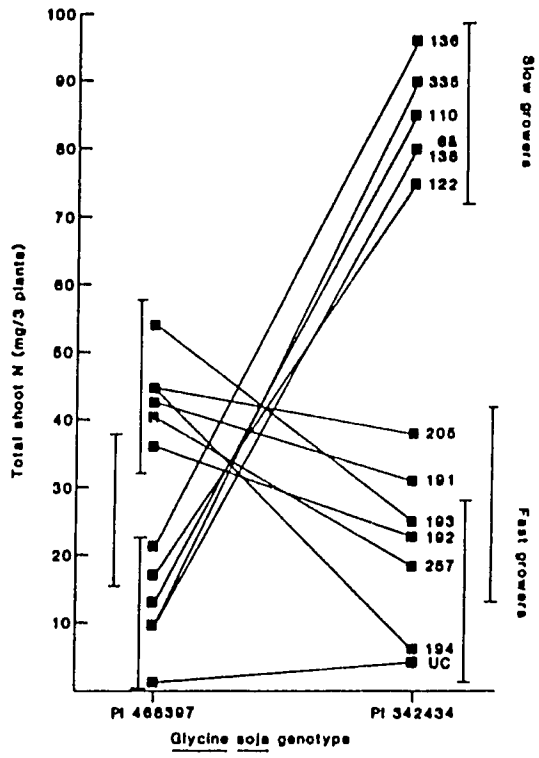

Figure 1. Total shoot N in two *G. soja* genotypes in symbiosis with fast- and slow-growing strains of rhizobia. Strains are listed by USDA number, UC = uninoculated control. Values within a *G. soja* genotype encompassed by the vertical bar are not significantly different ($P < 0.05$) as tested by Duncan's New Multiple Range Test from Keyser and Cregan, 1984b).

slow growers (see the chapter by Atherly et al., this volume). Their potential for genetic manipulation, combined with their differential symbiotic reactions with *G. max* and *G. soja* genotypes, provides researchers with valuable organisms for use in increasing our understanding of the soybean-rhizobia symbiosis.

NOTES

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Interactions of Selected *Glycine soja* Sieb. & Zucc. Genotypes with Fast- and Slow-Growing Soybean Rhizobia[1]

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ABSTRACT

Seven genotypes of *Glycine soja* Sieb. & Zucc. (wild soybean) were examined for effectiveness of N_2-fixing symbioses with fast- and slow-growing soybean rhizobia. Some genotypes were identified which demonstrated differential effectiveness with the two rhizobia groups, while other lines had no apparent preference. Two genotypes with opposite preferences were tested with six strains of each rhizobia group. *Glycine soja* PI 342434 formed very effective symbioses with the slow growers, fixing over three times as much N with this group of strains as with the fast-growing strains. Conversely, PI 468397 formed the most effective symbioses with the fast growers, fixing about three times as much N as with the slow growers. The demonstration of greater symbiotic effectiveness with the fast-growing rather than the slow-growing rhizobia has not previously been reported in *Glycine* species. PI 468397 was the genotype from which one of the fast-growing strains was originally isolated. Thus, differential rhizobia group (fast or slow) interactions found in *G. soja* genotypes indicate that *G. soja* possesses a greater diversity of reaction to the two rhizobia groups for this characteristic than common North American *G. max* genotypes.

Additional index words: **Symbiotic N_2-fixation, Nodulation, Nodule function, *Glycine max* (L.) Merr.**

RHIZOBIUM JAPONICUM, the microsymbiont of soybean [*Glycine max* (L.) Merr.], is a slow-growing group of bacteria with generation times of 6 or more hours (21). In contrast, the fast-growing rhizobia for alfalfa (*Medicago sativa* L.), pea (*Pisum sativum* L.), and bean (*Phaseolus vulgaris* L.) have generation times of 2 to 4 h, and have some distinct physiological differences from slow-growing soybean rhizobia (9, 21). The differences between the fast- and slow-growing rhizobia have led to the proposal to create two genera, *Rhizobium* and *Bradyrhizobium*, respectively (9). Recently, Keyser et al. (10) described a new group of fast-growing soybean rhizobia that is similar in physiological reactions to other fast-growing species of *Rhizobium* (16), but shared the legume host range of the slow-growing soybean rhizobia. Nodulation of a number of North American soybean cultivars by these fast growers resulted in ineffective N_2-fixation. However, the fast growers were effective with 'Peking' soybean and a *G. soja* Sieb. & Zucc. line from China (10).

Although *G. soja* is suggested to be the wild ancestor of the cultivated soybean (8), few studies have been conducted on its association with rhizobia. The seed lectin properties (13, 15), polar attachment of *R. japonicum* to roots and root hairs (18), and the formation of infection threads (14) in *G. soja* are similar to that in *G. max*. Brockwell et al. (3) found that N_2-fixing capabilities of slow-growing rhizobia with three *G. soja* genotypes were very similar to that in *G. max* genotypes.

Given the unique symbiotic effectiveness pattern of fast growers with *G. max* genotypes, we investigated further the symbiotic N_2-fixation capability of the fast growers with several *G. soja* genotypes. The purpose of this study was to determine if such effectiveness interactions with fast- or slow-growing soybean rhizobia exist in *G. soja* germplasm.

MATERIALS AND METHODS

Bacteria. The fast- and slow-growing rhizobia are maintained in our laboratory, within the USDA-Beltsville *Rhizobium* Culture Collection. The slow growers, USDA 6, 110, 122, 136, and 138, generally are considered to be among the most effective N_2-fixing symbionts of *G. max* (11). USDA 335 (PRC 113-2) is also an effective slow-growing isolate which was recently obtained from China. The fast growers used were USDA 191, 192, 193, 194, 205 and 257. Within each rhizobia group the strains represent three distinct serogroups based on specific somatic antigens (2, 11). Inoculum strains were grown in a yeast-mannitol-salts solution (20) to a population density of 10^9 cells/ml, and one ml of this culture was used to inoculate each seedling.

Plants. Seed of *G. soja* were obtained courtesy of Dr. R. L. Bernard, USDA-ARS, University of Illinois, Urbana, with the exception of PI 468396 and PI 468397 which were obtained from T. S. Hu, Soils and Fertilizer Institute, Beijing, China. A brief description of the *G. soja* genotypes is given in Table 1. Seeds were surface sterilized by immersion in acidified $HgCl_2$ (20) for 3 min followed by 5 rinses in distilled water. Seeds were germinated on soft agar (10g/L), and uniform 3-day-old seedlings were placed in modified Leonard jar assemblies (12) containing sterile vermiculite. Each assembly was supplied with an N-free nutrient solution, and inoculated.

Experiment I. In the initial trial, four control *G. max* and five test *G. soja* genotypes were compared for symbiotic effectiveness with one slow-growing (USDA 122) and two fast-growing (USDA 191 and 192) rhizobia in the greenhouse. A randomized complete block design was used with four replications and three plants per Leonard jar. Plants were grown with natural sunlight supplemented with incandescent light to extend the photoperiod to 18 h. Day and night temperatures were maintained at 25 ± 5° C. At 35 days after inoculation, plants were harvested and dry weight of the shoots and nodules was determined. To estimate N_2-fixation, the total shoot N accumulation was determined.

Experiment II. In the second trial, one control *G. max* and six *G. soja* genotypes were tested as described in Exp. I, with the additional treatment of an uninoculated control

Table 1. Origin and maturity group of *G. soja* genotypes used in symbiotic tests.

Plant introduction	Country of origin	Maturity group
PI 342434	Japan	V
PI 378693B	Japan	VII
PI 407095	Japan	VII
PI 407287	Japan	V
PI 424005	Korea	IV
PI 468396	China	Not determined
PI 468397	China	Not determined

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for each genotype. With the exception of PI 378693B, the *G. soja* genotypes from Exp. I were included and two additional *G. soja* genotypes were tested (PI 468396 and PI 468397). Experimental design, light and temperature regimes, length of trial, and harvest procedures were as described for Exp. I.

Experiment III. Based upon shoot N accumulation in Exp. I and II, two *G. soja* genotypes were chosen for a more detailed trial. PI 342434 was selected as a genotype displaying effective N_2-fixation with a slow-growing strain (USDA 122), and PI 468397 as a genotype having more effective N_2-fixation with fast growers (USDA 191 and 192) than with the slow grower. The two *G. soja* genotypes were inoculated individually with the six fast-growing and six slow-growing rhizobia. The experiments were conducted in a growth chamber with a 14 h photoperiod, 25°C day temperature, 20°C night temperature, and a photon flux density of 392 μmol m^{-2} s^{-1} at seedling level. At 42 days after inoculation, plants were removed and shoot and nodule dry weights determined. Total N accumulation in the shoot was determined by the Kjeldahl procedure. A randomized complete block design was used and the treatments, including uninoculated controls, were replicated four times; each replicate contained three plants. Because of space restrictions in the growth chamber, the first two replications were grown and harvested and were immediately followed in the same growth chamber by replications three and four. A preliminary analysis indicated no statistically significant interactions with time, and the data from the four replications were therefore analyzed as one experiment.

RESULTS AND DISCUSSION

Experiment I

In Exp. I (Table 2), the four *G. max* genotypes exhibited a symbiotic response with the fast- and slow-growing rhizobia as previously reported (10). The commercial soybean cultivars 'Bedford', 'Essex', and 'Williams' demonstrated significantly greater nodulation and N accumulation with a standard slow growing strain (USDA 122) than with either fast grower. In contrast, the N accumulation in Peking was similar with USDA 122 and with the fast grower, USDA 191. The *G. soja* genotypes PI 342434, PI 378693B, PI 407095 and PI 407287 accumulated significantly more shoot N with the slow grower, thereby reacting similarly to the commercial *G. max* cultivars, Bedford, Essex, and Williams. A contrasting N accumulation pattern was demonstrated by PI 424005 in that N accumulation with the slow grower did not exceed that which occurred with the fast growers. Indeed, with the fast grower, USDA 191, numerically, although nonsignificantly, more N was accumulated than with USDA 122. Thus, the response of PI 424005 was similar to that of the *G. max* genotype Peking. The ranking and statistical significance of effectiveness (total shoot N) of strains within *G. max* genotypes, with the exception of Peking, agreed with the nodule dry weight data (Table 2). Among the *G. soja* genotypes PI342434 and PI 424005 showed agreement between nodulation and effectiveness, however, for the remaining three *G. soja* genotypes significant differences in effectiveness between strains were not paralleled by similar differences in nodulation.

Table 2. Mean shoot N accumulation and nodulation response of *G. max* and *G. soja* genotypes in symbiosis with fast-growing (USDA 191 and USDA 192) and slow-growing (USDA 122) rhizobia (Exp. I).

Glycine genotype	Rhizobium strain	Total shoot N	Nodule dry wt.
		mg jar^{-1}	
G. max			
Bedford	122	95.7 a†	208 a
	191	24.2 b	128 b
	192	28.2 b	129 b
Essex	122	106.1 a	278 a
	191	31.9 b	142 b
	192	13.7 c	10 c
Williams	122	120.3 a	315 a
	191	49.0 b	265 b
	192	21.9 c	1 c
Peking	122	70.5 a	186 a
	191	78.7 a	181 a
	192	54.3 b	150 a
G. soja			
PI 342434	122	53.2 a	142 a
	191	19.9 b	85 b
	192	20.8 b	80 b
PI 378693B	122	48.0 a	200 a
	191	14.6 b	63 c
	192	19.4 b	121 b
PI 407095	122	29.3 a	86 a
	191	5.4 b	39 ab
	192	5.3 b	30 b
PI 407287	122	42.9 a	117 a
	191	20.4 b	97 a
	192	17.9 b	94 a
PI 424005	122	18.9 ab	74 ab
	191	30.0 a	116 a
	192	12.8 b	48 b

† Means within a column within a *Glycine* genotype not followed by the same letter differ significantly at the 0.05 probability level as tested by Duncan's Multiple Range Test.

Experiment II

A previously undocumented response was demonstrated by PI 468397 in that significantly greater shoot N accumulation occurred when inoculated with the fast growers than with the slow grower (Table 3). In addition, with PI 468397 the normally effective slow grower, USDA 122, was completely ineffective, i.e., accumulated no more N than the uninoculated control. In PI 424005, the trend detected in Exp. I was confirmed, as N accumulation and nodulation were significantly greater with USDA 191 than with USDA 122 and USDA 192. A similar result was obtained with PI 468396. PI 342434, PI 407095, and PI 407287 produced results like those in Exp. I in which significantly more nitrogen was fixed with USDA 122 than with either of the fast-growing strains. When PI 407095 was inoculated with USDA 191 or 192, shoot N accumulation from N_2-fixation was not significantly greater than the uninoculated control. PI 407095 was thus the only genotype which was completely ineffective with the fast-growing rhizobia. Comparison of nodulation and effectiveness data in Exp. II suggested that nodule function rather than nodule formation was limiting in several cases. This was demonstrated in PI 468396 and PI 468397. In each genotype nodulation was similar with the three strains but the N accumulation attributable to the strains differed significantly.

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Table 3. Mean shoot N accumulation and nodulation response of *G. max* and *G. soja* genotypes in symbiosis with fast-growing (USDA 191 and USDA 192) and slow-growing (USDA 122) rhizobia (Exp. II).

Glycine genotype	Rhizobium strain	Total shoot N	Nodule dry wt.
		mg jar⁻¹	
G. max			
Bedford	122	63.4 a†	115 ab
	191	26.3 b	133 a
	192	25.4 b	97 b
	UC‡	17.0 c	0
G. soja			
PI 342434	122	91.0 a	164 a
	191	27.7 b	147 a
	192	22.4 c	100 b
	UC	8.6 d	0
PI 407095	122	20.5 a	49 a
	191	3.0 b	30 ab
	192	3.1 b	22 b
	UC	1.6 b	0
PI 407287	122	45.2 a	108 a
	191	16.1 b	93 a
	192	11.4 b	68 b
	UC	5.1 c	0
PI 424005	122	20.2 b	77 b
	191	34.0 a	129 a
	192	19.7 b	80 b
	UC	6.4 c	0
PI 468396	122	7.0 b	32 a
	191	13.1 a	34 a
	192	7.8 b	21 a
	UC	1.2 c	0
PI 468397	122	8.6 c	49 a
	191	30.2 a	65 a
	192	21.5 b	48 a
	UC	1.7 c	0

† See footnote in Table 2.
‡ UC = uninoculated control.

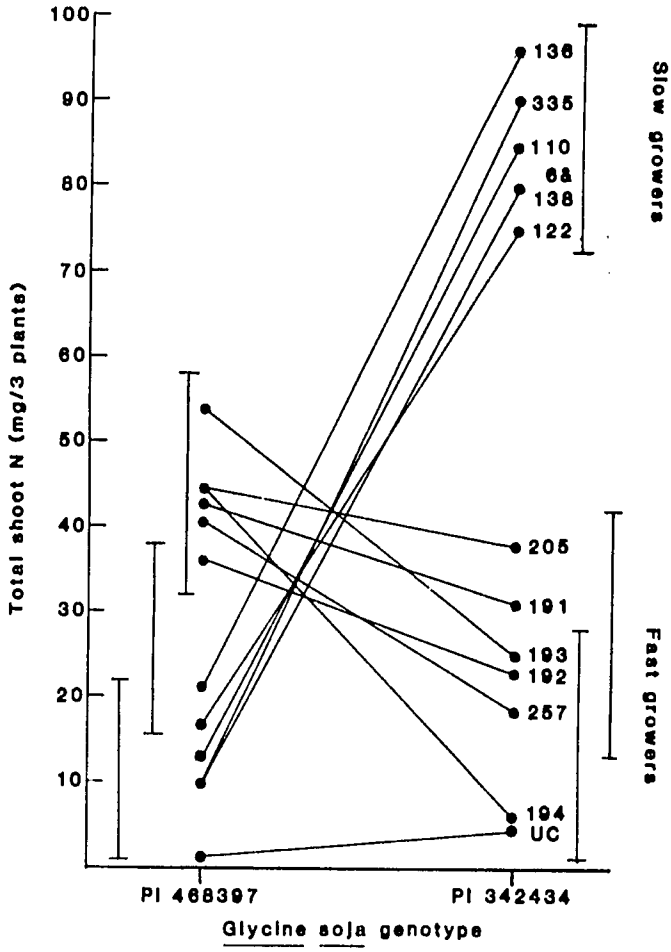

Fig. 1. Total shoot N in two *G. soja* genotypes in symbiosis with fast- and slow-growing strains of rhizobia (Exp. III). Strains are listed by USDA number, UC = uninoculated control. Values within a *G. soja* genotype encompassed by the same vertical bar are not significantly different ($P \leq 0.05$) as tested by Duncan's Multiple Range Test.

Results of Exp. I and II demonstrated that effectiveness interactions exist in *G. soja* which are similar to those previously found in *G. max*. Furthermore, the response of PI 468397 was clearly different from that found previously in *G. max*. Therefore, we conducted a more extensive experiment to further examine the interaction of PI 468397 with a series of fast- and slow-growing strains of the soybean rhizobia. In addition, we included PI 342434 because this genotype reacts with slow- and fast-growing rhizobia in a manner similar to common *G. max* genotypes.

Experiment III

The N accumulation by the two *G. soja* genotypes with the six fast- and six slow-growing rhizobia indicated firstly, that the symbiotic effectiveness reaction of each genotype was markedly consistent for all strains within the two rhizobia groups and, secondly, that a rhizobia group × *G. soja* genotype interaction was very apparent (Fig. 1). The total shoot N of PI 342434 was significantly greater with all of the slow growers than with any of the fast growers. Of the plants inoculated with the fast growers only those inoculated with USDA 191 and USDA 205 accumulated significantly more shoot N than the uninoculated control. The same analysis for PI 468397 showed that none of the plants inoculated with slow growers accumulated significantly more N than the uninoculated control while all of the plants inoculated with fast growers did. Although some strains within the two rhizobia groups were not significantly different from each other, a very striking reversal in the effectiveness of the groups was apparent in the two *G. soja* genotypes.

The partitioning of the rhizobia strain × *G. soja* genotype interaction into fast-growing rhizobia × *G. soja* genotype, slow-growing rhizobia × *G. soja* genotype, and rhizobia group × *G. soja* genotype indicated that 95.7% of the interaction sums of squares was attributable to the last effect. This substantiates the obvious differences of the reactions of the *G. soja* lines to the two rhizobia groups rather than to dissimilar reactions to strains within each group. A comparison of the rhizobia group means for symbiotic parameters in the two *G. soja* genotypes showed that genotype-rhizobia group means were significantly different for shoot dry weight, percentage N in the shoots, as well as total shoot N (Table 4). Only nodule dry weight was not significantly related to the genotype-rhizobia group.

While the effective rhizobia group fixed about three times as much N_2 as the ineffective group within each

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Table 4. Comparison of group means of fast-growing and slow-growing rhizobia for symbiotic parameters with two *G. soja* genotypes (Exp. III).

G. soja genotype	Rhizobia group	Shoot dry wt.	% N in shoot	Total shoot N	Nodule dry wt.
		g jar^{-1}		mg jar^{-1}	mg jar^{-1}
PI 342434	FG†	1.73 b‡	1.28 d	23.1 c	125 a
PI 342434	SG	3.34 a	2.52 a	84.3 a	128 a
PI 468397	FG	2.02 b	2.15 b	44.1 b	71 b
PI 468397	SG	0.85 c	1.79 c	15.2 d	65 b

† Each group mean represents 24 observations (6 strains × 4 reps); FG = fast growers, SG = slow growers.

‡ Means within a column not followed by the same letter differ significantly at the 0.05 probability level as tested by Duncan's Multiple Range Test.

genotype, the two groups produced essentially the same nodule mass in each genotype. The single exception was USDA 194 with PI 342434, which produced only 49.9% of the mean nodule mass of the other 11 strains. Also, the intact nodule and cross-sectional appearance was normal for all strains, and fluorescent antibody typing of ineffective nodules (see 17) indicated the presence of abundant bacteroids. Therefore, the events conferring ineffectiveness in these symbioses presumably occur between the sequential symbiotic steps of bacteroid development and nodule function (22).

Differential rhizobia group interactions with other species of fast- and slow-growing rhizobia have been reported for species of *Acacia* (6), *Lotus* (7), and *Lupinus* (1). Several cases of specific genotype-strain and genotype-serogroup interactions for ineffectiveness in *G. max* and the slow-growing rhizobia are known (19). Our results demonstrate similar interactions in the wild soybean, *G. soja*. Also, no *G. max* genotype has been identified that demonstrates superior effectiveness with the fast growers in comparison to the slow growers, as found here with *G. soja* PI 468397. Conversely, this PI demonstrated a unique ineffectiveness with the six slow growers. These slow growers represent three serogroups (6, 110, 122) and ineffectiveness with all of them has not been found in *G. max* genotypes. The typical symbiotic response of *G. max* genotypes to the two rhizobia groups is that seen in PI 342434. Our results demonstrate that the wild soybean germplasm has a greater diversity of reaction to the two rhizobia groups than previously reported in *G. max* cultivars common to North America (10). This may be because the latter were developed from a narrow germplasm base (4).

Glycine soja PI 468397 is the genotype from which the fast grower USDA 193 was isolated (collected from Taiyuanchen, Shansi Province, China, 1979), and the results presented here indicate the usefulness of such paired (seed and nodule) collection in subsequent evaluation of that plant genotype with its corresponding rhizobia isolate as proposed by Date and Halliday (5). Also, while China is considered the gene center of *G. max* and *G. soja* (8), it apparently is part of the gene center of two very different groups of soybean rhizobia. Comparative studies with these two groups may further our understanding of the soybean-rhizobia symbiosis.

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