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SRI LANKA SOYBEAN DEVELOPMENT PROGRAM

Report Number 19

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CONTENTS

	<u>Page</u>
PURPOSE.....	1
SUMMARY OF ACCOMPLISHMENTS.....	1
DETAILED REPORT OF ACCOMPLISHMENTS AND RECOMMENDATIONS.....	2
I. Introduction.....	2
II. Rhizobium Research.....	3
A. Present status of field research.....	3
B. Recommended field trials.....	5
1. Inoculation rates and methods.....	5
2. Nitrogen fertilizer response on nodulation and yield....	6
3. <u>Rhizobium japonicum</u> longevity in soils.....	6
4. Development of strain collection and evaluation.....	7
5. Nodulation survey of indigenous legumes.....	7
III. Inoculant Production and Use.....	8
A. Considerations concerning local inoculum production.....	8
1. Inoculum carrier evaluation.....	8
2. Inoculating method evaluation.....	9
3. Inoculum needs for other legumes.....	9
4. Personnel training.....	9
B. Establishing inoculant production capabilities.....	10
1. Culture production.....	10
2. Design of inoculum plant.....	10
3. Microbiology equipment needs.....	11
4. Inoculant storage and distribution.....	12
IV. Training.....	12

	<u>Page</u>
SUMMARY OF RECOMMENDATIONS.....	13
APPENDIXES	
A. Itinerary.....	14
B. Seminar.....	17
C. Inoculum Production Plant - Floor Plan.....	21
D. Microbiology Equipment Needs.....	22
E. Persons and Organizations Contacted.....	23
F. Acknowledgements.....	26

University of Illinois
International Soybean Program
Trip Report/Sri Lanka

NAME: R. Stewart Smith, Assistant Professor of Agronomy/
INTSOY Microbiologist.

PERIOD: 16 September-10 October, 1979.

PURPOSE:

1. To assist the Government of Sri Lanka in evaluating the need, potential and desirability of establishing a local inoculant production facility.
2. To review soybean inoculation research and utilization in Sri Lanka.
3. To plan strategies for research in the areas of inoculant production and Rhizobium japonicum research for the Sri Lanka UNDP/FAO/INTSOY Soybean Development Program.

SUMMARY OF ACCOMPLISHMENTS:

1. Visited 8 research stations and discussed R. japonicum inoculation research, observations and practices with available personnel. Inspected soybeans growing at 3 locations with either poor nodulation existing when expired inoculant was used, or no nodules when inoculation was not applied.
2. Examined available field research reports concerning R. japonicum from the period 1976 to present. Several experiments were with poor nodule establishment when seed applied inoculant was used, especially with the expired inoculant used during the last yala season. Three reports were with equally good nodulation on uninoculated and inoculated treatments, apparently because of indigenous R. japonicum. Good nodulation is recorded but without an available uninoculated control to determine if it developed from the inoculum or indigenous R. japonicum supplied with previous inoculation.
3. Reviewed present inoculation import, storage and utilization procedures.
4. Prepared an article to be printed in the SOYANEWS entitled, Rhizobium Inoculation - A Key to Successful Soyabean Production.

5. Presented seminars at the Bandarawela station, Thinneveley station, Maha Illuppallama station, and a joint C.A.R.I. - Faculty of Agriculture seminar at the University of Sri Lanka, Peradeniya Campus.
6. Visited the Giriulla Mills Coconut Processing Co. and obtained samples of the two grades of coconut shell flour (300 mesh and 100-200 mesh) which they are exporting. These materials are to be further evaluated as a potential inoculant carrier for the local production of inoculants.
7. Visited a coconut fiber processing plant and obtained samples of the pulpy waste material from the coconut husk. This material is to be further evaluated as a potential inoculant carrier.
8. Visited the Horton Plains area and sampled peat deposits. Further evaluation of this material as an inoculant carrier should be conducted.
9. Examined and recorded 11 strains of Rhizobium obtained from the Nitragin Co. These strains were stored in refrigeration and are to be maintained in the local Rhizobium culture collection.

DETAILED REPORT OF ACCOMPLISHMENTS AND RECOMMENDATIONS:

I. Introduction

Nitrogen fixation by soybeans is an essential input for high yield and protein production. Soybeans have a protein content of approximately 41% and a total nitrogen content near 10%. Therefore a soybean crop of 2500 kg/ha will remove 250 kg - N/ha. The proportion of this nitrogen supplied by fixation will be dependent on the N status of the soil, amount of N fertilizer supplied, and the abundance and effectiveness of the nodulation system. Obviously if soybeans are not nodulated the total nitrogen supply must come from added fertilizer and available soil nitrogen. With increasing costs of nitrogen fertilizer it is highly advisable to maximize nitrogen fixation, reduce or eliminate the need for nitrogen fertilizer and minimize the loss of nitrogen from the soil.

In areas where soybeans are being introduced as a new crop the symbiotic nitrogen fixing bacterium, Rhizobium japonicum, is not present. The introduction of effective strains of this Rhizobium must be accomplished during the first production season by the use of a reliable quality inoculum and satisfactory inoculating techniques. This will help assure a good yield, minimize production inputs, and contribute toward initial success and the satisfactory introduction of soybeans.

II. Rhizobium Research

A. Present status of field research.

During the two trips conducted, visits were made to the following research stations and production farms: Bandarawela, Karadian Aru, Kilinochchi, Maha Illuppallama, Mannar, Paranthan, Sita Eliya, Thinneveley and Vavuniya. Because the visiting period was between the Yala and Maha planting seasons, soybeans were observed in the fields at only three locations.

At Sita Eliya 8 varieties had been planted 25 May 1979 and treatments included an uninoculated control and the Nitragin "S" seed applied inoculant with an expiration date of November 1978. Nodulation was evaluated at one month after planting with an average of less than one nodule/plant present only on inoculated varieties Pb-1 and Local. At the 2 and 3 month samplings all uninoculated treatments were still without nodules and all varieties when inoculated had very few nodules (less than 4 nodules/plant). However, because of the poor growth of soybeans at this station and with the use of outdated inoculum of unknown quality, it is difficult to effectively evaluate the nodulation potential of this form of inoculation, or identify a possible nodulation problem in this soil.

At the Thinneveley station three soybean plantings were still in the field. A seed quality study which had not been inoculated and was without nodules, a seed increase of variety Local which had been inoculated with Nitragin "S", expiration date November 1978, and ISVEX variety trial also inoculated with the same material. Plants of lighter green color were without nodules while adjacent plants of darker green color had less than 5 lateral nodules per plant. This indicates that either the inoculant was of poor quality when used and/or soil stresses restricted nodule development. Recorded soil temperatures at 5 cm depth are frequently above 40°C and could present problems in nodule establishment.

A small observation trial of 10 varieties was inspected near Vellankulam. No inoculum had been used and all were without nodules.

Available field research reports concerning soybean inoculation from the period 1976 to present were reviewed. The M.S. thesis by Bhupan Singh, College of Agriculture, University of Sri Lanka, Peradeniya contained 7 soybean field trials, all employing Nitragin "S" inoculum at a 5 g/kg seed rate plus sucrose as a sticker. Most of the experiments had nodulation counts through the season of less than 20 nodules/plant. Two of the experiments had nodulation counts greater than 30 nodules/plant, however in one trial equally good nodulation was obtained on the uninoculated treatments. This indicates that indigenous R. japonicum were present in this trial.

Field reports at the Bandarawela station by Mrs. Aloysius (presently out of the country for advanced degree training) were examined. The effects of nitrogen and phosphorus fertilizers on nodulation had been evaluated. Significant response to inoculation was obtained in two trials. One trial was apparently on an old soybean field because good nodulation was obtained with both inoculated and uninoculated treatments.

Mrs. S. Logendra, who has received the NIFTAL Project six-week training course in Rhizobium technology, was previously at C.A.R.I. working in soybean rhizobiology but is now located at the Karadian Aru station. An experiment at Karadian Aru during the last Yala season evaluating the peat slurry and granular soil inoculation methods with and without soil mulching was being thrashed but had not been analyzed. Few treatments had more than 15 nodules/plant. Average pods/plant over the 5 replications were more than doubled from the uninoculated control treatment (21.5) to granular inoculation with mulching (45.4).

Mrs. Logendra had conducted two trials at C.A.R.I., Gannoruwa evaluating first the effect of foliar spray and split application of nitrogen on nodulation and soybean yields, and second to evaluate the effect of different methods of lime application on nodulation and soybean yield. In the experiment with low levels of nitrogen application good nodule weight per plant was recorded, but an uninoculated control was not included to evaluate the inoculation techniques nor provide the control for nitrogen effects.

The lime experiment was conducted on a field, with a pH of 5.6, which had previously grown soybeans. Nodulation was present on uninoculated treatments. No significant difference in nodule dry weight or seed yield was obtained.

At the Kilinochchi station the report of an ISVEX trial planted in May 1979 and inoculated with Nitragin "S", expiration date November 1978, was reviewed. Nodulation was evaluated at two periods with most plants obtaining less than one nodule. Pb-1 had 14.6 nodules/plant in one plot, but the other replications were very poor.

The majority of the soybean research has been conducted at the Maha Illuppallama station which is in the district presently producing the most soybeans. In the experiments reviewed Nitragin "S" had been used, but an uninoculated control had not been employed to evaluate the inoculating method and material. A SPOT trial for 1978 had uniformly good nodulation but was on a previous soybean field with indigenous R. japonicum. Last Yala season soybeans inoculated with Nitragin "S", expiration date November 1978, were grown on three soil types: well drained, moderately well drained and poorly drained. Nodules developed only on the moderately well drained soil. Uninoculated controls were not included so the presence of indigenous R. japonicum could not be evaluated.

Reports concerning research conducted with rhizobia for the grain legumes (Phaseolus mungo, black gram; Phaseolus aureus, green gram; Cajanus cajan, pigeon pea; and Arachis hypogea, peanut) and tropical pasture legumes were not very extensively found. Apparently no inoculation is used for the grain legumes and yet significant quantities of nitrogen fertilizer are projected for these crops in the Agricultural Implementation Programme - 1978/79 document (1320.7 tons of ammonium sulfate). Small quantities of inoculum have been obtained from Australia for experimental trials with some pasture legumes (Stylosanthes guianensis, stylo; Macrotyloma axillare, archer axillaris; Macroptilium autropurpureum, siratro; and Glycine wightii, glycine) by Dr. Chadhokar, FAO tropical pasture specialist. Evaluation of existing nodulation and a possible response to inoculation for these legumes must be obtained to determine the potential need for local inoculum production for legumes other than soybean.

B. Recommended field trials

Several research projects should be initiated to help develop the sound field research data concerning soybean inoculation on which to base the recommended inoculation practices. A few of those considered to be of major importance will be outlined below but are not intended to be all-inclusive of the field research trials that are important.

Because of the problems concerning nodule establishment, in the above section on present status of field research, a thorough evaluation of inoculation rates and methods of inoculating should be initiated immediately. It is recognized that a significant portion of recent nodulation problems may originate from the questionable outdated inoculum that has been used. In fact research officers have indicated that better nodulation has been obtained in previous years.

1. Inoculation methods and applied rates of rhizobia should be evaluated. The most important aspect of inoculation, with soybeans as a developing crop in Sri Lanka, is obtaining sufficient nodulation. Research conducted in the semi-tropical environment of Puerto Rico concerning soybean nodulation in R. japonicum free soil is reviewed in the seminar, Appendix B. This data indicates that the rate of applied rhizobia necessary to establish good nodulation is high. The granular soil inoculation method has been superior to the slurry seed applied technique in both near optimum and under stress conditions.

After discussion with Mrs. Logendra the following experiment was proposed and is to be conducted at the Karadian Aru station. Similar or identical trials should also be conducted at two other stations (Maha Illuppallama and Thinneveley would be recommended). This experiment would evaluate both the type of

inoculant (seed applied slurry and granular soil inoculation) and rate of rhizobia (each type of inoculant at its recommended rate and 10 times recommended rate). The treatments should include: 1) uninoculated control, 2) slurry-recommended rate, 3) granular-recommended rate, 4) slurry - 10 times rate, 5) granular - 10 times rate, and 6) 100 Kg - N/ha nitrogen fertilizer.

Such a trial should be conducted in both a Yala and Maha season at each location.

The results of this experiment should help determine the type of inoculant required, and also the form to be considered for local production. This also will indicate the response to inoculation and/or nitrogen.

2. A second experiment should be conducted to evaluate the response to nitrogen fertilizer and its effect on inoculation. The current fertilizer recommendation made in A Guide to the Cultivation of Soyabean prepared by the Department of Agriculture of Sri Lanka is to apply a fertilizer mixture containing 50 Kg of urea (20 Kg-N). This experiment should evaluate a range of nitrogen levels and should also be conducted at various stations representing the soil types in the soybean producing areas.

The Agricultural Implementation Program 1978/79 - A Working Document published by the Ministry of Agricultural Development and Research has set as a target production for the combined Maha and Yala seasons 6,253 acres of soybeans. For this production they have set as a target consumption the utilization of 44.0 metric tons of ammonium sulfate (21% - N) which converts to 23.1 metric tons of Urea (40% - N). At a fertilizer price for urea of Rs.1,638.00/metric ton as of 8.12.78 this amounts to Rs.37,837 for nitrogen fertilizer for soybeans. This expenditure should be justified by sound field research showing an increased yield response with nitrogen when good nodulation has been established, or this nitrogen fertilizer recommendation should be modified. Where nitrogen deficiency symptoms are evident in the field and good nodulation is not established, nitrogen fertilizer as a sidedress application would be recommended.

3. A third important aspect is the establishment and longevity of the R. japonicum strains in the various soils and under the different environmental conditions. This should be done in field trials to determine the persistence of R. japonicum in Sri Lanka and to help make better judgement on the need and usage of inoculation.

4. Develop a strain collection of R. japonicum and evaluate cultivar-strain interactions. Strains should be fully evaluated both for their effectiveness (ability to form a nitrogen fixing association with soybean), and infectivity (ability to form nodules with the host).

Because the field testing of many strains of rhizobia for effectiveness is both time consuming and expensive, a preliminary screening of a large number of strains should be evaluated in a greenhouse or growth chamber using a nitrogen-free growth medium. After a preliminary screening the most productive strains should be fully evaluated in field plots in the various soybean growing soils and environments.

The following eleven strains of rhizobia were brought to Sri Lanka by Dr. Carl Hittle from the Nitragin Co. These slants were examined and placed in the refrigerator for storage. This will be the initial Rhizobium culture collection to which other strains are added when obtained.

<u>Host</u>	<u>Strain No.</u>
<u>Phaseolus vulgaris</u> (bean)	127K17
<u>Phaseolus vulgaris</u> (bean)	127K80
<u>Glycine max</u> (soybean)	61A89
<u>Glycine max</u> (soybean)	61A101
<u>Crotalaria</u> Sp (Crotalaria)	3223
<u>Stylosanthes sunaica</u> (Stylo)	150B1
<u>Centrosema pubescens</u> (Centro)	25B9
<u>Vigna unguiculata</u> (cowpea)	176A22
<u>Vigna radiata</u> (mung)	127D4
<u>Vigna radiata</u> (mung)	127D5
<u>Vigna radiata</u> (mung)	127D15

5. A survey should be conducted for the existing nodulation of all legumes presently grown in Sri Lanka. Isolations of these indigenous rhizobia should be obtained. After confirming their purity local strains should be evaluated for nitrogen fixation with known productive strains. Coordination and leadership for this project should be obtained from Dr. Adam Pain a soil microbiologist on leave from England who is working for two years for the Department of Agriculture at the Postgraduate Institute of Agriculture.

III. Inoculant Production and Use

The Soybean Development Project of the Government of Sri Lanka has made a commitment to evaluate the feasibility and possible development of the local production of inoculum for the important food and forage legumes. It is the evaluation of this consultant that certain aspects must first be examined and specific research conducted before the decision concerning inoculum production can be properly determined. For the immediate soybean production the importation of soybean inoculant, with improved methods for ordering, storing and distributing, should be continued.

A. Considerations concerning local inoculum production.

The following subjects need to be examined more closely with specific research conducted as indicated. The outcome of these projects and the development of trained personnel will assist in the decision to produce local inoculant.

1. Inoculum carrier evaluation.

Research needs to be continued on the evaluation of possible carriers to be used for inoculant production. Several local sources of possible carriers were obtained during this visit and left in the custody of Mr. D. T. Weerasekera, microbiologist in the Chemistry Division of C.A.R.I. Preliminary research has been conducted with some of these carriers, however, a uniform evaluation of all sources should be established.

<u>Potential inoculum carrier</u>	<u>Source</u>
Coconut shell flour - 300 mesh	Giriulla Mills Coconut Co.
Coconut shell flour - 100 mesh to 200 mesh	Giriulla Mills Coconut Co.
Coconut fibre dust	Coconut Fibre Plant
Local peat	Horton Plains
Lignite	Neyveli Lignite Corporation Neyeli, South Arcot District, Tamil Nadu, India.
Commercial inoculant peat	The Nitragin Co., U.S.A.

Rhizobium growth and longevity trials could be established at the microbiology laboratory at C.A.R.I. With a single broth culture used to formulate inoculants with the various carriers, the Rhizobium population can be compared over time by plate counting techniques and growout Most-Probable-Number trials. Sterilization of the carriers, before the incorporation of rhizobia could also be evaluated at C.A.R.I. by utilizing the available Cobalt 60 source.

Lignite is presently being used as an inoculant carrier in India and is available in the form of grits. The lignite is pulverized to pass through a 200 mesh screen and sterilized by autoclaving at 15 lbs. pressure for 20 minutes in 1 kg lots. The raw peat should be obtained from the Nitragin Company and would serve as a positive standard against which to evaluate all other carriers. If an imported carrier is

determined to be superior the economic feasibility of importing such carriers must be considered.

It would be recommended that when local inoculant production is initiated it is very important to be assured that the quality of such inoculant be consistent. The choice of an inoculant carrier is thus an important consideration to be determined before initiating inoculum production.

2. Inoculating method evaluation.

It is also recommended that the necessary method of inoculating soybeans to consistently obtain satisfactory nodulation under various soil and environmental conditions be determined. This should be performed at various research stations representing the major soybean growing areas.

The research reported in Appendix B, which was conducted on R. japonicum-free soils in Puerto Rico, indicates that the granular form of soil inoculation is superior to the seed applied peat method. The evaluation of these two inoculating methods has been recommended in section I, B,2 of this report and is to be initiated at the Karadian Aru station this Maha season.

The necessary form of carrier (seed applied powder or soil applied granular) should be determined before the actual development of an inoculum production capability.

3. Inoculum needs for other legumes.

An inoculant production plant should produce inoculum for all legumes which respond to applied rhizobia. Soybean inoculant in Sri Lanka is required, however, the inoculation needs for the existing pulse and forage legumes has not been determined.

A survey of existing nodulation on these legumes and the evaluation of the indigenous strains by comparing nitrogen fixation to known effective strains should be initiated (see Section II. B,5 of this report). Further field inoculation trials should be conducted to determine the response to superior Rhizobium strains for each crop.

4. Personnel training.

To initiate and establish an inoculum production capability technically trained personnel are required. Short term expertise during the initial phase could be obtained, but training of local staff for conducting long range production and research

needs must be obtained. This is an important aspect for both rhizobia research and production capabilities and will be discussed in more detail later in this report.

B. Establishing inoculant production capabilities.

When the decision has been made to establish an inoculant production plant in Sri Lanka the following outline should be of assistance in the planning and implementation stages.

The present inoculum needs appear at most to be for 10,000 acres. The following inoculum production facility should be designed to facilitate expansion as needed.

1. Culture production.

Large scale fermentation equipment is not required to produce sufficient culture for the projected production needs. The gyratory bench top shaker with twelve 1 liter working capacity Fernbach flasks (described in the report by Dr. Joe Burton, Sri Lanka Soybean Development Program - Report Number 15, 1977) is an acceptable system. This type of system does not require a compressed air system with filters and is thus more dependable. When increased production needs warrant larger fermentation tanks these shakers are useful in producing the required starter cultures for the larger fermenter.

An alternate culture production system with a larger capacity (10-50 liters) can provide good serviceability when fabricated from steel drums fitted with brass inlet, outlet, inoculating, and sampling ports. Such a system is described by Date, R.A. and R.J. Roughley, Preparation of Legume Seed Inoculants, IN A Treatise on Dinitrogen Fixation. Section IV: Agronomy and Ecology. Hardy and Gibson (eds.). John Wiley and Sons, N.Y. This system uses cotton wool packed metal cylinder filters to sterilize the air from a compressed air supply. The vessel with filter and outlet tube attached and two-thirds filled with medium must be sterilized by autoclaving.

2. Design of inoculum plant.

The floor plan of the inoculum plant is presented in Appendix C. Minor alterations to the design recommended by Dr. Joe Burton (Sri Lanka Soybean Development Program-Report Number 15, 1977) has been suggested with the following justifications:

- a. The plating room has been eliminated with the intention that the plating and transfers will be done in a laminar flow hood placed in the transfer room. Proper maintenance of a laminar flow hood provides a reliable area in which to do all aseptic operations.
- b. The elimination of the plating room provides more space in that office. This could be sufficient for at least two people. Therefore, the second office would be vacant and is suitable for a glassware and chemical storage room.
- c. Moving the storage room to the second office allows the lab bench along that wall to be maintained. The area designated for the microscope table could also be kept as lab bench.
- d. The walk through area serving as an entrance to the two cold rooms has been partitioned, but still allowing access to the cold rooms. A door has been provided from this new room into the culture production room. The new space would be suitable for the microscope and other laboratory instruments.

These few alterations provide a larger laboratory working space and maintains considerably more laboratory bench space.

3. Microbiology equipment needs.

To initiate the recommended rhizobia laboratory research and to better equip a soil microbiology research laboratory, necessary equipment acquisition is desirable. This would not necessitate purchasing duplicate equipment, for when the inoculum production plant is established this equipment would be available for both research and production needs.

It is recommended that a microbiology research laboratory be improved at C.A.R.I. in the near future. When inoculum production is initiated the equipment can be moved to the designated inoculum plant adjacent to the Food Processing Pilot Plant. Reasons for locating the research lab at C.A.R.I. are that considerable physical alterations will be required to convert the existing inoculum plant space into the functional laboratory. This includes plumbing installation, wall removal, plus wall installation in the new section designated as an instrument room. Such improvements would be justified when the space is converted into inoculum production. Also, greenhouse space which is necessary for rhizobia - plant growout tests is presently available at C.A.R.I.

Therefore equipment needs will be separated into first equipment necessary for the microbiology research lab and then additional equipment when inoculum production is initiated (Appendix D).

4. Inoculant storage and distribution.

Inoculum is a perishable product and one should emphasize the fact that care should be exercised in maintaining the best storage and handling conditions. Inoculum quality is best maintained in refrigeration. A cold room is functioning at the designated inoculant plant and would be satisfactory for inoculum storage. Presently the imported inoculant is stored in an airconditioned room. It is recommended that in the future all inoculum be stored in the cold room at C.A.R.I. or the Food Processing Pilot Plant.

Care should be exercised in expediting shipment of inoculum to the user. The cold room at Maha Illuppallama, being in the area with concentration soybean production, may be a convenient alternate location for inoculum storage.

Expiration dates on the inoculum package should be strictly adhered to. Future ordering of imported inoculants should include sufficient material for the Yala and Maha planting seasons, but should not accumulate excess material which is carried beyond the expiration date.

IV. Training

Training is a key element in the strengthening of indigenous competence in soil microbiology. A flexible training program featuring degree and nondegree training is needed. A doctoral candidate should be selected and trained in rhizobiology to direct and coordinate the total laboratory and field research program. Additional personnel should be trained in Rhizobium technology.

The possibility of training Sri Lankan rhizobiologists at ICRISAT has been discussed with Dr. P. J. Dart, Microbiologist. Even though no soybean rhizobia work is being conducted at ICRISAT effective training would be obtained by working with one of ICRISAT's pulses.

The University of Hawaii NITAL Project periodically offers a six-week training course in Rhizobium technology. The objectives of the course are to provide training in the techniques of applied Rhizobium research and to prepare workers for participation in a cooperative research network.

SUMMARY OF RECOMMENDATIONS:

1. Continue to import soybean inoculum for the immediate future.
2. Promote training of Sri Lanka personnel in microbiology with special emphasis on rhizobiology. Obtain a qualified person to direct and coordinate the total rhizobia program.
3. Initiate needed research to assist in the decision making process concerning the production of inoculum.
 - a. Inoculum carrier evaluations.
 - b. Inoculating methods evaluations.
 - c. Inoculum needs for other legumes.
4. Additional recommended field trials.
 - a. Evaluate the response to nitrogen fertilizer and its effect on nodulation.
 - b. Establish R. japonicum soil longevity trials under different environmental conditions.
 - c. Develop a strain collection and evaluate cultivar - strain interactions.
 - d. Survey existing nodulation on indigenous legumes in Sri Lanka.
5. Develop the microbiology research laboratory at C.A.R.I. in the near future (recommended equipment acquisition - Appendix D).
6. When the inoculant production plant is to be established alter the existing floor plan as indicated in Appendix C.
7. Combine the microbiology research and inoculant production activities and equipment in the improved inoculant plant.
8. Purchase the additional equipment for inoculum production (listed in Appendix D) after firm commitments for production are made.
9. Improve existing inoculum purchasing and storage.
 - a. Only purchase sufficient inoculant for one year. Do not use material past the expiration date.
 - b. Store all inoculum in a cold room for as long as possible.

APPENDIX A

Itinerary

- September 16 - Arrived in Colombo. By car to Kandy.
- September 17 - Attended the Maha Soybean Planning Meeting at C.A.R.I., Gannoruwa. Read background reports.
- September 18 - Discussed with Mr. D. T. Weerasekera, Microbiologist in the Chemistry Division of C.A.R.I., inoculant production, inoculant research and research needs in Sri Lanka. Examined his lab in which he produces small quantities of rhizobia culture for experimental inoculant production. Toured the Cobalt radiation building (1972-2036 Curries, presently 900 Curries) which has been used to sterilize small quantities of inoculant carrier. Toured the greenhouse in which Leonard jars, glass tubes and crocks are used for rhizobia testing. Met Miss N. Gunapala, microbiology assistant, who is conducting greenhouse Azolla-Anabaena trials.
- September 19 - Accompanied by Carl Hittle. Visited Dr. Walter G. Fernando, Deputy Director Research, Department of Agriculture, Peradeniya; Dr. Hugh Doggett, Regional Coordinator Food Legumes, I.D.R.C.; Dr. Henry E. Fernando, Deputy Director Research/Head C.A.R.I.; Mr. W. B. Medagama, Deputy Director, Seed and Planting Materials, Department of Agriculture; Dr. T. B. Ratnayake, Director Agriculture Development, Ministry of Agriculture Development and Research, Colombo; and Dr. C. R. Panabokke, Director, Department of Agriculture, Peradeniya.
- September 20 - Visited with Dr. Mike Chan and Mr. Wilmot Wijeratne and toured Soybean Food Processing Pilot Plant, Gannoruwa. Discussed options for better utilization of the inoculant production plant before and during the period of its development. Visited Dr. S. Nagarajah, Head of Agriculture Chemistry Division, C.A.R.I. Accompanied by Carl Hittle, visited Dr. H. P. M. Gunasena, Head of Agronomy Department, Faculty of Agriculture, University of Sri Lanka, Peradeniya.
- September 21 - Traveled from Kandy to Giriulla and was given a tour of the Giriulla Mills Coconut Plant by Mr. Andrews, Plant Manager. Obtained samples of both 300 and 100-200 mesh coconut shell flour for continued examination as a possible inoculant carrier. Also visited a coconut fibre plant and obtained samples of the pulpy

- waste material from the coconut husk. This material accumulates in large quantities and should also be evaluated as an inoculant carrier. Continued to Colombo and with Carl Hittle visited Mr. Garvey Laurent, Resident Representative, FAO. Returned to Kandy.
- September 22 - With Elaine Brewer and Barbara Worthy (Canadian Cross Roads Volunteers) and Carl Hittle to the Soybean Food Processing Pilot Plant to discuss with Mr. Wilmot Wijeratne the possible service of the two volunteers in the Home Food Training Program. Toured Peradeniya Botanical Gardens.
- September 23 - Sightseeing in Kandy. Prepared an article for the SOYANEWS, "Rhizobium Inoculation - A Key to Successful Soyabean Production".
- September 24 - At C.A.R.I. Visited in more detail with Miss N. Gunapala, microbiology assistant about the the Azolla-Anabaena research. Accompanied by Mr. D. T. Weerasekera and Miss N. Gunapala to the Veterinary Vaccine Production Center, Peradeniya where Mr. Weerasekera makes occasional use of their laminar flow hood and freeze driers. Visited air conditioned room at C.A.R.I. with Mr. M. Z. Caffoor where the imported Nitiagin "S" inoculant is stored,
- September 25 - Accompanied by Carl Hittle, D. T. Weerasekera, Elaine Brewer and Barbara Worthy from Kandy to Nuwara Eliya. Visited the Sita Eliya Research Station where we discussed soybean and inoculation trials with Dr. V. Yogaratnam and Miss C. R. Arasaratnam. Night at Hill Club in Nuwara Eliya.
- September 26 - From Nuwara Eliya to Horton Plains where we searched for and sampled peat for continued evaluation as a possible inoculant carrier. Traveled to Bandarawela Research Station where we discussed their soybean and inoculation research trials and I presented a seminar. Night at Ella Resthouse.
- September 27 - Traveled from Ella to Batticaloa where we met Mr. and Mrs. Logendra and arranged our visit of the Karadian Aru Research Station for the next day. Night at Suntan Hotel, Kalkudah.

- September 28 - Visited Karadiya Aru Research Station and discussed past, present and future soybean inoculation field research with Mrs. S. Logendra. Discussed my soybean inoculation research in Puerto Rico and International Inoculation Trials. Returned to Kandy.
- September 29-30 - Rest and worked on report.
- October 1 - Accompanied by Carl Hittle. Visited Dr. P. A. Chadhokar, FAO Tropical Pasture Specialist at the Veterinary Production and Pasture Development Center and discussed current and past research with tropical forage legumes. Visited Dr. Jogaratnam, Director Postgraduate Institute of Agriculture. Visited Dr. R. Kumerasinghe, microbiologist in the Faculty of Science, Department of Botany. Dr. Kumerasinghe had her training at Rothamsted working on R. trifolii root infection process.
- October 2 - Accompanied by M. Z. Caffoor and Duncan Brown, (VSO) traveled from Kandy and visited the following stations on the way to Jaffna: Vavuniya, Kilinochchi, Paranthan. Night at Subhas Hotel, Jaffna.
- October 3 - Visited the Thinneveley station, gave a seminar and traveled to Mannar. Night at Mannar Guesthouse.
- October 4 - Visited Mannar D.A.E.O Officers and traveled to Vellankulam to inspect an INTSOY soybean observation trial. Traveled to Maha Illuppallama, met with soybean Research Officers and presented a seminar. Night at Maha Illuppallama Guesthouse.
- October 5 - Official holiday in Sri Lanka. Sightseeing in Anuradhapura and Sigiriya then return to Kandy.
- October 6 - Worked on report. Met with Dr. Adam Pain a soil microbiologist on leave from England working for two years at the Postgraduate Institute of Agriculture. Discussed mutual interests, contacts and observations concerning soil microbiology and rhizobiology in Sri Lanka.
- October 7 - Worked on report. Accompanied by Carl Hittle. Visited Dr. Chris Panabokke, Director of Agriculture, to review my observations and recommendations.
- October 8 - Worked on report. Presented a seminar (see Appendix B) at the Faculty of Agriculture, University of Sri Lanka Peradeniya, jointly sponsored by the Postgraduate Institute of Agriculture and Central Agricultural Research Institute, Gannoruwa.
- October 9 - Worked on report.
- October 10 - Traveled by car from Kandy to Colombo. Depart Sri Lanka for Bangkok.

APPENDIX B

INTSOY RHIZOBIUM RESEARCH AND INTERNATIONAL INOCULANT TRIALS

by R. S. Smith, Dept. Agronomy, Univ. of Illinois

Seminar

Presented at: Bandarawela Research Station, September 26, 1979.

Tinneveley Research Station, October 3, 1979.

Maha Illuppallama Research Station, October 4, 1979.

University of Sri Lanka, Peradeniya, October 8, 1979.

The objective of INTSOY Rhizobium japonicum research conducted in Puerto Rico during the period from July 1977 to present has been to determine required rates of rhizobia and to evaluate methods of inoculation necessary to establish successful soybean nodulation in R. japonicum - free tropical soils environments. Trials have been conducted under field conditions considered to be near optimum for rhizobial establishment and survival, and also under stress conditions of low soil pH - high aluminium content, and also in a δ soil with high soil temperature.

The first field experiment investigated the number of R. japonicum necessary to establish satisfactory nodulation in a subtropical soil free of indigenous soybean rhizobia. The treatments included R. japonicum applied as a liquid in the seed furrow from concentrations of 3.9×10^2 R./cm (390 R./cm) with 10 fold increases to include the rate of 3.9×10^9 R./cm (3,900,000,000 R./cm). The treatment delivering 3.9×10^6 R./cm is approximately equal to the quantity of rhizobia supplied with the granular soil inoculant. The 3.9×10^4 R./cm rate approximates the commonly used seed applied peat inoculant.

The number of tap root nodules, total number of nodules and nodule weight per plant were evaluated at 25 and 53 days after planting. The number of tap root nodules and total number of nodules continued to increase with increasing rates of applied rhizobia. The rate equivalent to seed applied inoculant (3.9×10^4 R./cm) produced less than 2 tap root nodules at both sampling periods, whereas the rate equivalent to soil applied inoculation (3.9×10^6 R./cm) produced 6.4 and 8.7 tap root nodules/plant at 25 and 53 days respectively. The total number of nodules/plant with 3.9×10^4 R./cm was 5.9 and 2.5, while the 3.9×10^6 R./cm rate produced 12.5 and 21.1 nodules/plant at 25 and 53 days respectively. Comparing the nodule weight/plant at these two rates of inoculation shows an increase from .0012 to .0058 g at 25 days and an increase from .0204 to .0818g at 53 days with the higher rate of rhizobia.

This experiment suggests that improved nodule establishment may be obtained with the rate of rhizobia contained in a soil inoculation method when compared to the rate supplied in the standard peat seed applied inoculant.

The second field experiment was conducted on a R. japonicum - free soil with a pH of 4.5 and 4.0 meg of available aluminium. Davis variety of soybean was selected as being aluminium sensitive and Lee-74 as being aluminium tolerant. Hydrated lime ($\text{Ca}(\text{OH})_2$) was applied before planting at the following rates: 0, 1770, 3540, 5300 and 7080 kg/ha. All of the variety and liming treatment combinations were planted both without inoculation and with granular soil inoculation. Tap root nodulation, total nodule number and nodule dry weight were mainly effected only by the inoculation treatment. The only nodulation parameter significantly influenced by the liming treatment was the total number of nodules with the Lee variety. In this instance the first increment of lime provided a significant increase in nodule number. The yield value with Davis was increased 2.8 times and Lee-74 2.4 times with inoculation while the liming and variety treatments did not influence yield.

This study indicates that on this acid soil with a high level of available aluminium the only significant yield response was with the nitrogen supplied with symbiotic fixation. But more important is that granular soil inoculation was successful in establishing sufficient nodulation on this acid soil without lime addition.

In the third experiment high soil temperatures and low soil moisture stresses for seven days following planting were utilized to evaluate inoculant types and rates on soybeans grown in a Rhizobium japonicum free soil in Puerto Rico. All inoculant treatments were evaluated under stress conditions in one-half of the experiment which did not receive rain or irrigation and had a moisture level below wilting point for the first seven days. During this period in the dry section the soil temperature at 2.5 cm reached a daily maximum of between 38 to 40°C. All inoculant treatments were also evaluated in one-half of the experiment which was irrigated to provide a moisture level near optimum. This moisture reduced the maximum soil temperature at 2.5 cm to between 31 and 35°C.

Granular soil inoculant was the best treatment in producing tap root nodules, total number of nodules, and nodule dry weight/plant after both 32 and 98 days. Liquid soil inoculant in the seed furrow, and liquid placed 2.5 cm below the seed were generally better than all other treatments (liquid 5.0 cm below the seed, liquid 7.5 cm below the seed, and peat powder inoculant applied to the seed).

Applying all inoculants at 10 times rate produced a consistent but non-significant increase in nodulation parameters when comparing each inoculant treatment to its standard x rate, except for granular soil inoculant where significant nodulation increases were observed in 8 of the 12 evaluations with the higher 10X rate.

Nodulation between dry and irrigated conditions was compared by totaling the values for all treatments in both moisture regimes. Number of tap root nodules, total number of nodules, and nodule dry weight/plant were significantly increased ($P = 1\%$) at both sampling dates under irrigation when compared to the dry conditions. All nodulation values were at least 3 times better under irrigation. The number of tap root nodules was 6 times larger under irrigation than in the dry section at the 98 day sampling, due to the lack of increase in tap root nodules under the dry condition between the 32 and 98 day sampling, whereas with irrigation the tap root nodulation had doubled during this period.

The results of these field trials in Puerto Rico are useful in making recommendations concerning inoculation and nodulation establishment under various soil conditions. In all trials conducted a significant improvement in nodule establishment was observed with the granular soil inoculation, which is supplying more rhizobia, than with the standard peat seed applied inoculum. I would suggest to research scientists in countries introducing or expanding soybean production to evaluate inoculation rates and types of inoculants under their various soil and environmental conditions.

The excellent INTSOY international cooperators network, established with the ISVEX trials sent from the University of Illinois, was utilized to incorporate two INTSOY international inoculant trials this year.

The International Inoculant Shipping Evaluation (IISE) is a trial included with all ISVEX shipments which has the objectives of evaluating soybean inoculant quality after exposure to international shipping and storage conditions, and to estimate the quality of the granular inoculant used with the ISVEX trials as an aid in the interpretation of ISVEX nodulation data.

Included in all ISVEX trials sent from the University of Illinois to cooperators in various countries was a 50 g sample of granular soybean inoculant, stapled to a preaddressed envelope to be sent by the cooperator to the INTSOY microbiology laboratory in Puerto Rico. A Temperature Monitoring Template, which changes color when each of the following temperatures are exceeded (38° , 43° , 49° and 54°C), was attached to the inoculant sample to determine the maximum temperature under which the inoculant was exposed during shipment. Upon receipt in Puerto Rico the following determinations will be made with each sample:

- a. The maximum temperature to which the sample was exposed.
- b. The moisture content of the inoculant sample (initial moisture was 35%).
- c. Plate count determination of viable R. japonicum on YEM + Congo red agar.

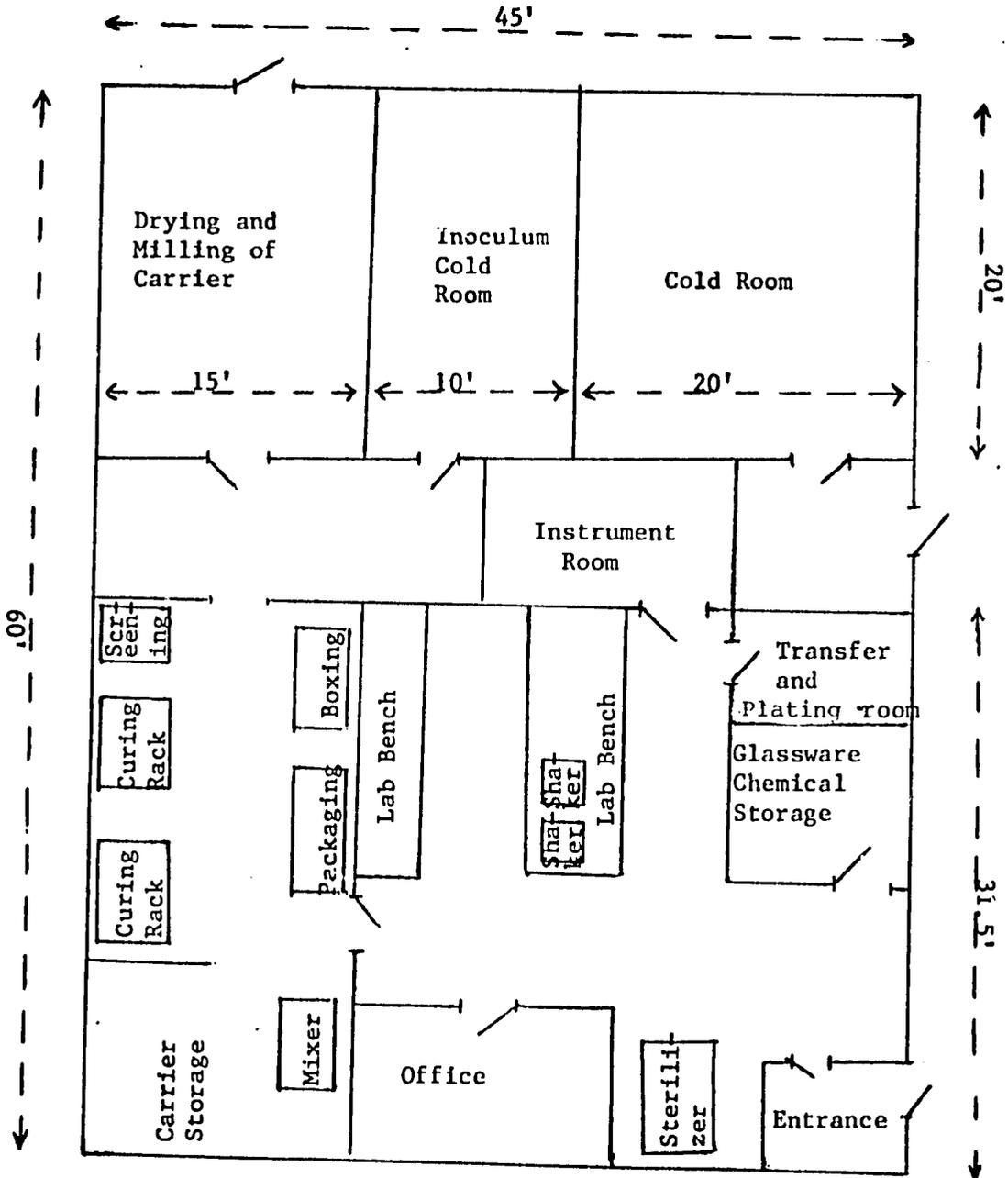
The viable R. japonicum count obtained will be correlated with the maximum temperature of exposure, inoculant moisture loss and length of time in shipment to determine their effect on the inoculant quality during international shipping conditions.

The second inoculant trial, sent only to those ISVEX cooperators that requested it, is the International Soybean Rhizobium Inoculant Experiment (ISRIE). The objectives of ISRIE are to (1) determine the soybean yield response to inoculation with Rhizobium japonicum; (2) evaluate the granular soil inoculation method; and (3) to assist in better interpretation of the nodulation data obtained from the ISVEX trials. ISRIE has four treatments: (1) non-inoculated control, (2) granular soil inoculation, (3) granular soil inoculation plus 25 Kg-N/ha at planting, and (4) no inoculation but 25 Kg-N/ha at planting plus 75 Kg-N/ha 5 weeks after planting. Shipped to each cooperator from the University of Illinois were sufficient Davis variety soybeans, granular soybean inoculant, and a booklet with recommended management practices and recommended observations. The experiment is a randomized complete block design with plots of 4 rows, 5 meters long, and 4 replications.

Parameters to be determined, at both 4 weeks after emergence and at beginning pod fill, are nodule abundance, percent active nodule (as determined by presence of leghaemoglobin), and a relative visual rating of the relative plant color. Grain yield is to be determined at maturity.

APPENDIX C

Inoculum Production Plant - Floor Plan



Scale: 1 inch = 10'

APPENDIX D

Microbiology Equipment Needs:

A. Microbiology research laboratory

Gyratory Shaker (New Brunswick Model G-33 or equivalent)	---	\$ 1,000
Sterilizer, large, horizontal 24" x 36" x 48"	---	\$ 20,000
Research microscope, binocular with 10X, 40X, 100X dry and 100X oil immersion objectives	---	\$ 2,500
LaminaP Flow Hood	---	\$ 2,000
pH meter	---	\$ 900
Sterilizing oven 24" x 20" x 20"	---	\$ 600
Moisture balance	---	\$ 650
Torsion balance, 500g capacity	---	\$ 600
Water still or deionizer	---	\$ 1,000
Miscellaneous laboratory glassware and supplies	---	\$ 2,500

B. Inoculum production equipment

Custom made steel drum for culture production or Gyratory shaker (New Brunswick Model G10-21 or equivalent)- Two at \$1,800.	---	\$ 3,600
Paddle type horizontal mixer	---	\$ 1,300
Air conditioners (1800 BTU's) 1 office, 1 transfer room, 3 laboratory 5@ \$ 600	---	\$ 3,000
Balance- top loading with 1200 g capacity	---	\$ 300
Sealer for polyethylene packages - foot operated	---	\$ 300
Aging pans, fiberglass	---	\$ 2,000
Scales, 0-200 lbs. 3@ \$400	---	\$ 1,200

APPENDIX E

PERSONS AND ORGANIZATIONS CONTACTED

Department of Agriculture, Peradeniya and Colombo

Dr. Christopher R. Panabokke, Director of Agriculture
Dr. Walter G. Fernando, Deputy Director of Research
Mr. W. B. Medagama, Deputy Director, Seed and Planting Materials (Farms)
Mr. T. B. Ratnayake, Director Agriculture Development, Ministry of
Agricultural Development and Research, Colombo

Central Agricultural Research Institute (C.A.R.I.), Peradeniya

Dr. Henry E. Fernando, Deputy Director of Research
Dr. S. Nagarajah, Head Chemistry Division
Mr. B. N. Emerson, Research Officer
Mr. D. T. Weerasekera, Research Officer
Mr. M. Z. Caffoor, Research Assistant
Dr. Carl N. Hittle, INTSOY Project Leader
Dr. Mike S. Chan, INTSOY Food Processing Specialist
Miss N. Gunapala, Experimental Officer
Mr. Wilmot Wijeratne, Research Officer

University of Sri Lanka, Peradeniya:

Faculty of Agriculture

Dr. H. P. M. Gunasena, Head Department of Agronomy
Dr. M. W. Thenabadu

Faculty of Science

Dr. R. Kumerasingh, Botany Microbiologist

Postgraduate Institute of Agriculture

Dr. Jogaratnam, Director
Dr. Howard Ray
Dr. Adam Pain, Microbiologist

Agricultural Research Stations:

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Mr. W. S. Manokaran, R.O.I.C.
Mr. K. D. Vasabal, Research Officer
Mr. S. Dharmalingam, Research Officer

Karadian Aru

Mrs. S. Logendra, Research Officer
Mr. Logendra, Experimental Officer

Kilinochchi

Mrs. B. Regunathan, Research Officer

Maha Illuppallama

Dr. M. H. J. P. Fernando, Deputy Director of Research, Dry Zone
Mrs. M. Samarasinghe, Research Officer
Mr. J. Handawela, Research Officer
Mr. V. Arulnandhy, Research Officer
Mr. H. Gamage, Research Officer

Mannar

Mr. S. Theiviyathan, Research Officer

Paranthan

Mr. P. Yogaratnam, R.O.I.C.

Sita Eliya

Dr. V. Yogaratnam, R.O.I.C.
Miss C. R. Arasaratnam, Experimental Officer

Thinneveley

Mr. J. A. Lewis, Research Officer

Vavuniya

Mr. T. S. Pathmanathan, Research Officer

District Agricultural Extension Officers

Mr. Sivamuthulingam, Jaffna

FAO

Mr. Garvey Laurent, Resident Representative, Colombo
Dr. P. A. Chadhokar, Tropical Pasture Specialist, Peradeniya.

IRRI

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Dr. Russell Freed

Giriulla Mills Coconut Processing Co.

Mr. Andrews, Plant Manager

C.D.R.C.

Dr. Hugh Doggett, Regional Coordinator Food Legumes

Veterinary Vaccine Production Center

Dr. Arawawela

APPENDIX F

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My profound thanks to Carl Hittle for organizing the schedule of interviews and travel and for accompanying me during the majority of my visits. Finally I wish to express my gratitude to Carl and Grace Hittle for the welcome I received in their home and for their generous time provided as host and hostess.