IICA

Relatórios de Consultoria
IICA/EMBRAPA-PROCENSUL II

SIMPÓSIO INTERNACIONAL DE FIXAÇÃO DE NITROGENIO DAS NÃO-LEGUMINOSAS

ESCRITÓRIO NO BRASIL

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SIMPÓSIO INTERNACIONAL DE FIXAçÃO DE NITROGEÑIO DAS NÃO-LEGUMINOSAS

Relatórios de Consultoria IICA/EMBRAPA-PROCENSUL II

Claudino Rodriguez Barrueco
David A. Zuberer
Frederick Skinner
Okon Yacob

Brasília, janeiro de 1989

INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA
EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA

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APRESENTAÇÃO

A reprodução e difusão dos Relatórios de Consultores, no âmbito restrito das Diretorias das Unidades do Sistema Nacional de Pesquisa Agropecuária, vinculado à EMBRAPA, tem como objetivo principal o de divulgar as atividades desenvolvidas pelos consultores e as opiniões e recomendações geradas sobre os problemas de interesse para a pesquisa agropecuária.

As atividades de consultoría são realizadas no âmbito do Projeto de Desenvolvimento da Pesquisa Agrtopecuária e Difusão de Tecnologia na Região Centro-Sul do Brasil - PROCENSUL II, financiado parcialmente pelo Banco Interamericano de Desenvolvimento - BID e a EMBRAPA conforme os contratos de Empréstimo 139/IC-BR e 760/SF-BR, assinados em 14 de março de 1985 entre o Governo Brasileiro e o BID.

As opiniões dos consultores são inteiramente pessoais e não refletem, necessariamente, o ponto de vista do IICA ou da EMBRAPA.

A coordenação dos Contratos IICA/EMBRAPA agradeceria receber comentários sobre estes relatórios.

Horacio H. Stagno
Coordenador Contratos IICA/EMBRAPA

INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA
CONVÊNIO IICA/EMBRAPA

RELATÓRIO FINAL DE CONSULTORIA

1. Nome do consultor: Claudino RODRIGUEZ BARRUECO

2. Especialista em: BIOLOGIA DO SOLO

3. Nome do Projeto do IICA: 2.SB.3

4. Especificar qual o Programa da EMBRAPA em que a consultoria está sendo prestada:

PROG : PROCENSUL II

SUBPROG: V INVESTIGACION EM SOLOS

Código Atividade Projeto IICA: 2.SB.306	Código contábil: A4874B1B03106-8
Título da Atividade do Projeto do IICA correspondente a esta consultoria	Cooperaçao com EMBRAPA en programa de pesquisa em solos y analisis do solos.

PERÍODO DE CONTRATAÇÃO	SEDE DA CONSULTORIA
Agosto 22 a Setembro 5,1987	SEROPEDICA, RIO DE JANEIRO
PERÍODO DE PRORROGAÇÃO	SEDE DA PRORROGAÇÃO

5. Fonte financiadora: PROCENSUL II

6 - OBJETIVO

- Participar juntamente con o Chefe do Programa de Biologia do Solo da EMBRAPA em Atividades no sentido de fortalecer o Programa de Biologia do Solo, planejando, treinando, avaliando e assessorando na pesquisa e também, participar do Simpósio Internacional de Fixação de Nitrogênio das Não-Leguminosas, de 23 a 28 de agosoto de 1987.

7 - ORGANISMOS BENEFICÍARIOS

- EMBRAPA e Institutos Agropecuários do Brasil.

8 - TÉCNICOS COLABORADORES

- Grupos de trabalho da EMBRAPA - UAPNPBS, Seropedica, Km 47, Rio de Janeiro , Ciêntistas: R.Boddey, S. Manhaes Sento, S.S. Urquiaga Caballero, J.A.R. Pereira, M. Penteado Stephan

9 - PESSOAS ENTRVISTADAS COM RELAÇÃO AS ATIVIDADES DESENVOLVIDAS

- As mencionadas no ponto 7, assim como membros do Departamento de Bioquimica da Universidade Federal do Parana, em Curitiba.

10 - ATIVIDADES DESENVOLVIDAS

Participou-se ativamente da exposição de resultados experimentais do abaixo firmado, assim como na descrição dos trabalhos cientificos desenvolvidos dentro do Programa de Biologia de Solo pelos grupos do Brasil e de outras nacionalidades na semana do IV Simpósio Internacional, incluindo formulação de projetos de que possam consuzir a um fortalecimento do Programa dentro dos sistemas biológicos fixadores de nitrogênio que não são leguminosas, principalmente actimorrizas, azolla, e sobre a associação bactéria-gramineas. Visitou-se a instalação da EMBRAPA no Km 47, proposto possíveis colaborações num futuro próximo com Centros de Investigação da referida matéria em diversos países, incluindo provisoriamente a continuidade do presente IV Simpósio em um país europeu.

Assim mesmo se faz trocas sobre futura experimentação neste campo, especialmente nos novos cultivos de não-leguminosas, assim como apoio a revistas a trabalhos expostos no IV Simpósio.

11 - RESULTADOS DAS ATIVIDADES

- Tem se procurado extreitar os laços de colaboração científica entre o grupo reprsentado pelo abaixo afirmado e grupos brasileiros que sem dúvida beneficiarão amabas as partes. Temos consciência da relevância do trabalho deselvolvido no Brasil, e de seu impacto potencial no aumento da produção agrícola, assim como do interesse que a instituição Brasileira tem em potencial do aspecto atual ou investigação ad hoc em consonância com as tendências internacionais científicas. A valorização que tem merecido esta consultoria sobre eta área científica no Brasil é altamente positiva.

12 - CIRCUNSTÂNCIAS OU ACONTECIMENTOS EXTERNOS AO IICA QUE AFETARAM A EXECUÇÃO DE SUAS ATIVIDADES

- Nenhuma

13 - PUBLICAÇÕES

- Do Simpósio celebrado se publicará o correspondente número na revista científica "Plant an Soil", de conhecida categoria internacional e que supõe a difusão dos resultados atuais na presente linha de investigação.

14 - RECOMENDAÇÕES TÉCNICAS E SUGESTÕES PARA SE ALCANÇAREM OS OBJETIVOS ESTABELECIDOS PARA A MISSÃO

- PoteNcial humano e instrumental para um desenvolvimento científico de acordo com as necessidades planejadas na exposição ou termos científicos de interesse' comum no IV Simpósio.

15 - CONCLUSÕES

- A continuidade de contatos é necessaria a fim de cristalizar a confirmação de esforços para a evolução de investigações até um objetivo comum incluindo aspectos básicos e aplicados das mesmas, cujo interesse é comum a todos os países de vocação agrícola,com grande parte de seu produto nacional proveniente da agropecuária.

INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE
IICA/EMBRAPA CONTRACT

CONSULTANT FINAL REPORT

1. Consultant's full name: David A. ZUBERER

2. Specialist in: SOIL BIOLOGY

3. Title of IICA Project: 2.SB.3

4. EMBRAPA Program for which consultancy is provided:

SUBPROG V : PESQUISA EM SOLOS
PROG : PROCENSUL II

IICA Project Activity Code: 2.SB3.06	Administrative Code: A4874B1B03106
Title of Activity of IICA Project corresponding to this consultancy	Cooperation with EMBRAPA on research on soil managemet and soil analysis.

CONSULTANT CONTRACT PERIOD	DUTY LOCATION (Center)
August 19th to 29th, 1987	SEROPEDICA, RIO DE JANEIRO
CONTRACT EXTENTION PERIOD (If any)	DUTY LOCATION (Center)

5. Financial support: PROCENSUL II

ACTIVITIES UNDERTAKEN BY THE CONSULTANT AND RESULTS

I conferred with several members of the Soil Biology Program of EMBRAPA and discussed ways in which we might enhance the development of members of the S.B. Program currently on leave from Brazil to Texas A&M University, College Station, Texas.

In addition I met with students and discussed their current research efforts with the S.B. Program and shared ideas regarding their research problems (projects).

Dr. Johanna Dobereiner and I discussed in some detail what I may be able to do at Texas A&M University to facilitate the scientific development of the members of the soil biology unit currently on leave there. I will take back several ideas for research projects of mutual interest to our respective programs.

SUPPORT TO RESEARCH UNDERTAKEN BY OTHER EMBRAPA RESEARCHERS

The consultant will work with members of the soil biology group.,currently on leave to Texas A& University. At least one member will work in the laboratory where research regarding several aspects of grass/bacteria associations is ongoing. It is anticipated that both of our organizations will benefit from the exchange of ideas, laboratory practices, etc.

SUPPORT PROVIDED TO EMBRAPA RESEARCHERS IN THESIS AND DISSERTATION WORK

Consultant will instruct members of the Soil Biology Program in an upper level course in soil microbiology at Texas A&M University.

OTHER ACTIVITIES DEVELOPED BY THE CONSULTANT

I visited the EMBRAPA research station at Km.47 - Rio de Janeiro and toured laboratories and experimental sites in the field. We discussed on-going research programs with the Soil Biology Program. I found this visit most helpful in understanding the full scale of the research effort ethin the program and the discussions with scientists were useful in identifying areas of mutual interest for possible future collaboration.

CONSULTANTS SUGGESTIONS AND TECHNICAL OR INSTITUTIONAL RECOMMENDATIONS FOR THE IMPROVEMENT OF THE RESEARCH SERVICE

Dr. Dobereiner indicated that the soil biology program in in the process of obtaining an electronic microscope´ic. Anything which can be done within EMBRAPA to facilitate the development of such a facility and the training of personnel in the skill necessary to operate and maintain such a facility, would assist the further strengthening of the already well recognized program. The development of this facility will allow members of this

research group to extend their investigations into root/bacteria interactions to a level that is simply not possible without such tool.

The research project dealing with the quantification of N-fixation in grass/bacteria systems using15N methods should be continued and if possible expanded as it is crucial to the development of a complete understanding of the utility of these associations in current and future agricultural production systems.

AGREEMENTS OR COMMITMENTS ESTABLISHED WITH EMBRAPA RESEARCHERS

I believe that we have possibly opened up new avenues for the exchange of information between the soil microbiology program of Texas A&M University (Department of Soil and Crop Sciences) and the soil biology program of EMBRAPA under the direction of Dr. Johanna Dobereiner.

CONSULTANT'S COMMENTS ON CIRCUMSTANCES WHICH AFFECTED HIS WORK

I regret that because of professional commitments and teaching schedules al Texas A&M I was not able to spend more time with scientists at the Research Center of Km.47 My impression is that Drs. Dobereiner and Boddey as well as others, have established an impressive, highly active research program which will provide answers to many of the unresolved questions or controversies in the area of associated N-fixation.

August 28, 1987 David A.Zuberer

INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE
IICA/EMBRAPA CONTRACT

CONSULTANT FINAL REPORT

1. Consultant's full name: Frederick SKINNER

2. Specialist in: SOIL BIOLOGY

3. Title of IICA Project: 2.SB.3

4. EMBRAPA Program for which consultancy is provided:

PROGRAM : PROCENSUL II

SUBPROGRAM : V SOIL RESEARCH AND SOIL ANALYSIS

IICA Project Activity Code: 2.SB.306	Administrative Code: R4874B1B03106-8
Title of Activity of IICA Project corresponding to this consultancy	Cooperation with EMBRAPA on research on soil management and soil analysis

CONSULTANT CONTRACT PERIOD	DUTY LOCATION (Center)
August 22nd to September 12th, 1987	SEROPEDICA - RIO DE JANEIRO
CONTRACT EXTENTION PERIOD (If any)	DUTY LOCATION (Center)

5. Financial support: PROCENSUL II

6. Institutions assisted:

EMBRAPA/UAPNPBS, Km 47, Seropédica 23851 RJ.

7. Cooperating staff:

Dr Johanna Döbereiner (EMBRAPA) Symposium organiser;

Dr R.M.Boddey (EMBRAPA) Symposium organiser and collaborating editor;

Dr I. Fendrik (Universität Hannover, FRG) Symposium organiser and collaborating editor;

Dr E-G. Niemann (Universität Hannover, FRG) Symposium organiser.

8. Not relevant.

9 - 15. Report of activities.

(a) I attended the Fourth International Symposium on Nitrogen Fixation with Non-Legumes, held in Rio de Janeiro, 23-28 August 1987 as Editor of the Symposium Proceedings. My collaborating editors for the publication are Dr R.M. Boddey (EMBRAPA) and Dr I. Fendrik (Hannover).

(b) Of the 28 invited papers listed, 27 were presented by their authors and 24 typescripts submitted for publication. Of the 26 contributed papers listed, only 17 were presented and 12 typescripts submitted to the editors. It is possible that 8 more papers will be submitted before the deadline set for the middle of September. In general, the papers were of high quality and the editors are confident that a very satisfactory volume of proceedings will be published.

(c) Topics covered in the symposium.
The occurrence of N-fixing symbionts in the root nodules of non-legumes, such as the Frankia-Alnus and Rhizobium -Parasponia symbioses, and the mechanisms of infection of higher plants by such organisms formed the subject of the first major section. Some comparisons with the mechanisms of infecton of legumes by rhizobia were made.

The N-fixing cyanobacteria were considered from the points of view of isolation, characterization of species and metabolism, and the role of Anabaena spp. in the Azolla-Anabaena symbiosis. The agronomic importance of the Azolla-Anabaena association in wetland rice production was discussed.

The majority of papers presented dealt with the species of Azospirillum and related N-fixing organisms found on or just within the roots of many crop plants and which can enhance growth of such crops, especially in tropical areas. Although there is evidence for the direct transfer of fixed nitrogen from such bacteria to the host plant, these associations are complex and other mechanisms such

as the production of phytohormones and the making available of soil nutrients can evidently play their part in improving plant growth. The isolation, identification, physiology and genetics of these bacteria and the mechanisms of their association with higher plants were discussed in detail.

The quantification of plant-associated biological nitrogen fixation remains a major problem which it is essential to solve in order to confirm the reality of this association and to influence agricultural practice.

(d) During the Symposium and later, at EMBRAPA, Km 47 from 28 August until 8 September, the papers received were examined by the editors and advice on their scientific content sought from other specialists. Those papers considered worthy of publication, and it is expected that most of them will reach this standard, will be edited in detail later and submitted to our publishers, Martinus Nijhoff, Dordrecht, The Netherlands by the end of 1987 for publication as a special volume of Plant and Soil.

16. F.A. Skinner.

F.A. Skinner

8 September 1987

INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE
IICA/EMBRAPA CONTRACT

CONSULTANT FINAL REPORT

1. Consultant's full name: Okon Yacob

2. Specialist in: SOIL BIOLOGY

3. Title of IICA Project: 2.SB.3

4. EMBRAPA Program for which consultancy is provided:

PROGRAM : PROCENSUL II

SUBPROGRAM : V RESEARCH ON SOIL AND SOIL ANALYSIS

IICA Project Activity Code: 2.SB.3.06	Administrative Code: R4874B1E03106-8
Title of Activity of IICA Project corresponding to this consultancy	Cooperation with EMBRAPA on research on soil management and soil analysis

CONSULTANT CONTRACT PERIOD	DUTY LOCATION (Center)
July 15th to August 31st, 1987	SEROPEDICA - RIO DE JANEIRO
CONTRACT EXTENTION PERIOD (If any)	DUTY LOCATION (Center)
Modified to July 19th to August 29th 1987, according communication of Dra. Johanna Dobereiner	SAME

5. Financial support: PROCENSUL II

August 28 1987

F I N A L R E P O R T T O

1) Inter-American Institute for Cooperation on Agriculture (IICA)

2) by YAACOV OKON

3) Project:

Strengthening Agriculture Research and Technology Diffusion

4) Activity:

Technical cooperation to strengthen the Soil Biology Program

5) Objective:

To participate together with the Head of the Soil Biology Program and Conservation Service of EMBRAPA in activities to strengthen the soil microbiology research by planning, training, evaluating, advising on research and to participate in the IV International Symposium on Nitrogen Fixation of Non-Leguminous Plants (August 23-28, 1987).

6) Period: July 20 1987 - August 29 1987

7,8) Duty Station and Institution Assisted

Soil Biology Program and Conservation Service of EMBRAPA UAPNPBS, Seropedica Rio de Janeiro-R.J.

9) Cooperating Staff

Dr. Johanna Dobereiner, Director, UAPNPBS-EMBRAPA
Dr. Robert M. Boddey
Dr. Segundo Urquiaga
Dr. Marilia Penteado Stephan

Biologist Jose Antonio Ramos Pereira
Ing. Agron. Ricardo Rocha, Fellow of CNPq
Ing. Agron. Veronica Mazur, Researcher
Ing, Agron. Jaime O. Mandel, Fellow of CNPq
Lic. Microbiol. Monica Fulchieri, Researcher (Visitor from Universidad de Rio Cuarto, Cordoba, Argentina)

10) Personal Interviewed for the development of activities

I gave two formal lectures in UAPNPBS-EMBRAPA to the staff and students on Azospirillum and plant growth promoting Rhizobacteria, and another one in the Department of Biochemistry, University of Parana, Curitiba (14th August 1987).

Dr. Johanna Dobereiner, Director UAPNPBS-EMBRAPA:

General discussion on the project of plant growth promotion by bacteria of the genus Azospirillum, including evaluation of the state of the art on the subject, working hypotheses on the mode of action of bacteria on roots, such as "hormonal" effect, improved plant nutrition, biological nitrogen fixation, importance of inoculum size and selection of bacterial type. Further cooperation, exchange of information and bacterial strains and future visits were discussed.

Dr. Robert Boddey

General discussion on the state of the art of associative biological nitrogen fixation, especially evaluation techniques.

Dr. Segundo Urquiaga

Discussed in detail techniques and experiments for the N-15 dilution technique and the N-15 natural abundance techniques for studying biological nitrogen fixation in sugar cane, rice and other gramminaeae.

Dr. M. Christina Neves, Dr. M. Angela Hungria and Dr. Avilio Franco

Held discussions on their work related to nitrogen fixation in the Rhizobium-legume symbiosis.

Dr. Norma Rumjanek

Discussed the GS-GOGAT pathway in nodules and methodology to measure enzyme activities on roots.

Dr. Marilia Penteado Stephan

Discussed physiological properties of Sacarobacter nitrocaptans. Specially possible fate of glucose catabolism, products that may reduce the pH drastically after 10 h growth, and suggested to follow morphology of cells during different growth stages to check for capsule (gum) formation.

Veronica Mazur

Discussed possible way of studying ecology of Sacarobacter in sugar cane roots, and methodology that can be used for demonstrating infection through the vascular system of sugar cane.

Ricardo Rocha, Jose Antonio Ramos Pereira, Jaime O. Mandel and Monica Fulchieri

Carried out joint experimental work and discussions of the current aspects and techniques on Azospirillum associations with roots (see report on experiments carried out).

On Friday, August 14th, visited The Universidad Federal du Parana, Curitiba and discussed Azospirillum systems with Dr. Fabio O. Pedrosa.

11,12) Activities developed, research report and results of activities

Subject: Effect of inoculation of wheat and sorghum on root development.

BACKGROUND

Extensive work during the last year has shown that inoculation of cereals and forage grasses with Azospirillum brasilense improved plant growth and productivity in many cases (Okon, 1985). Enhancement of plant growth by A. brasilense does not seem to be limited to its role in the N fixation by the Azospirillum plant association. Therefore, other mechanisms have been proposed to explain its beneficial effects. It has frequently been observed that inoculation with Azospirillum enhances root development, improving mineral and water uptake by the root system (Okon and Kapulnik, 1986). Several morphological parameters that determine the charactgeristics of the root system are affected by Azospirillum e.g., root elongation, initiation of adventitious roots, root branching or root-hair differentation (Okon and Kapulnik, 1986).

In Brazil consistent increases in plant yield have been obtained in field experiments with wheat by using A. brasilense strain 245 but not strains Cd (Boddey et al., 1986, Baldani et al., 1987), whereas in Israel favourable effects have been obtained in various plant species with strain Cd ATCC 29729 (Okon, 1985).

Questions asked:

1) Is the inoculum size used, important for affecting root development as has been previously observed (Okon and Kapulnik, 1986).

2) Is the Azospirillum strain used important for affecting root development as has been previously observed for wheat and sorghum, root colonization and effects on yield (Baldani et al., 1987).

N O T E All experiments that were carried out are preliminary, their main purpose was to learn techniques, methods of evaluation and general trend effects.

a) Three experiments were carried out in petri dishes, to study the effects of Azospirillum inoculation on wheat and sorghum root morphology of 48 and 72 h seedlings (Okon and Kapulnik, 1986).

b) Experiment in greenhouse. Azospirillum inoculation of wheat.

Materials and Methods

Wheat (Triticum aestivum) cv..... and Sorghum bicolor cv Br 300 were used. Seeds were surface sterilized by dipping the seeds in a one 1% solution of chloramine T for 15 min. Then the seeds were thoroughly washed with sterile water to remove the chloramine T.

It is recommended to sterilize in a flask with a side arm to exert a vacuum, release of the vacuum, several times to aid penetration of the disinfectant. The seeds (5 seeds per plate) were sown in petri dishes with a filter paper and 2 ml of sterile water. The seeds were inoculated with 1 ml per plate suspension of Azospirillum brasilense and incubated in the dark at 30 C for

48 and 72 h.

The treatments in the first experiment were non-inoculated control (a suspension of autoclaved cells 10^7 /ml). A. brasilense strain Cd, strain 245, strain 246, and strain 245 NR from the collection of UAPNPBS-EMBRAPA. The second and third experiment treatments were non-inoculated control and inoculation with strain Cd as compared to 245. Each treatment consisted of 6 replicates (6 plates with 5 seeds each).

Preparation of inoculum. Bacteria were grown in liquid NF b medium with NH_4Cl (1 gr per liter) and without indicator for 48 h at 30 C in a shaker. The cells were collected by centrifugation (6,000 rpm for 10 min) and resuspended with 0.05 M phosphate buffer pH 7.0. The number of cells in the suspension was evaluated by a Hauser Scientific Batch Counting Chamber Cat. No. 700 (Hauser Scientific Blue Bell, Pa), absorbance at 540 nm with a Perkin Elmer III spectrophotometer and counts by the most probable number method. Ten fold dilutions were made in phosphate buffer to bring cell suspension of approximately 1-2 x 10^9 - 10^5 .

Calibration of inoculum size

Counts of *Azospirillum brasilense*

First batch: Culture 200 ml suspension was centrifuged and resuspended in 30 ml buffer phosphate

Strain	Absorbance 540 nm	Counting Chamber	MPN*
Cd	1.05	2×10^9	-
245	1.1	3×10^9	9×10^9
245 NR	1.14	2×10^9	6×10^9
246	1.23	2.5×10^9	1.3×10^8

* One replicate, data not reliable

Second batch: Culture 300 ml suspension was centrifuged and resuspended in 40 ml buffer phosphate

Strain	Absorbance 540 nm		dilution 10^{-1} , 10^{-2}	Counting Chamber
Cd	1.103	0.2	0.222	2.5×10^9
245	1.08	0.2	0.02	2.4×10^9
245 NR	1.11	0.25	0.025	3.0×10^9
246	1.09	0.21	0.025	2.55×10^9

Third batch: Culture of 300 ml suspension was centrifuged and resuspended in 40 ml buffer phosphate

Strain	Absorbance 540 nm dilution 10 , 10			Counting Chamber
Cd	1.03	0.19	0.020	2.14×10^9
245	1.12	0.22	0.025	2.35×10^9

Characteristics checked in Petri dish experiments:

1. Root length
2. Root tip morphology with a binocular
3. Respiration rate of root tips

Experiment in greenhouse

Surface sterilized seeds one seed per pot were sown 27/7/1987 in 2 l pots, containing washed sand and irrigated with tap water. Once a week the plant received 50 ml of complete Hoagland solution (2 g/liter NH_4NO_3). The seeds were inoculated at sowing with a one ml suspension of Azospirillum brasilense strain Cd, 245, 245 NR and 246 at a cell concentration of approximately 2×10^5, 2×10^7, and 2×10^9 cell/ml. The plants were grown in a greenhouse with temperature, light intensities and photoperiod normal for Seropedica, Km 47 in July-August (17 C night, 30 C day 12 photoperiod). Each treatment consisted of 12 replicates (12 pots). The plants were harvested 19/8/87 for bacterial counts (2 pots per treatment) and

19,20,/8/1987 for determining root-surface area, plant measurements and root and shoot dry weight.

Measurements

1. Root morphology (visual), root volume appearance of adventitious roots.
2. Root surface area (Titration method, gravimetric method [Carley and Watson, 1976).
3. Root and shoot dry weight
4. MPN counts of crushed roots.

Root surface area

Plant roots were thoroughly washed with tap water nd dried in air to remove excess water. The roots were dipped in 1.0 M HCl solution for 15 sec. Then the roots were dried (blotted) in between filter paper for 5 min to blott excess acid that remained in a film between the roots.

The roots were transferred to 500 ml flasks with 200 ml distilled water and shaken 2 min at 250 rpm. (The time that the roots are wshed in distilled water must be the same for each plant (time of introduction and removal). The amount of HCl adsorbed to the roots is proportional to the root surface area. The 200 ml solutions from washed roots were titrated with 0.1 M NaOH with 0.5 ml phenolphtalein as pH indicator. Results are expressed as ml NaOH 0.1 M per plant.

The gravimetric method consisting of dipping the root system in a saturated solution of $Ca(NO_3)_2$ for 30 sec, dripping the excess solution adsorbed to roots for 1 min, and measuring the

differential weight before and after adsorption, in an electronic balance. This method was not sensitive enough to show differences in three week old wheat root systems.

MPN counts of crushed roots (2 plants per treatment were measured)

A root system was dipped in 100 ml 0.05 M phosphate buffer, and the roots were crushed with a Steril mortar and piston to give an homogenous extract. Serial ten-fold dilutions were carried out in 0.05M phosphate buffer immediately after crushing and 0.1 ml samples were sown in semi-solid NFb medium for MPN counts with 3 replicates.

Experiment with petri dishes for respiration in roots

Twenty five 1 cm long root tips were cut from 3-day-old seedling grown in moist filter paper at 30 C in the dark. The respiration rate of the root tips was measured in a plant nutrition medium at 25 C with an O_2 electrode (the one used by Marilia, P. Stephan) and are expressed as umol O_2 per min taken up per segment or per g dry weight of sample, or per protein content. The response time of the electrode to changes in O_2 concentration was to slow to measure respiration rates of root segments. But the technique was learned by the participating researchers.

Table 1:

CFU/ml	Average root length cm	No. of seeds that germinated out of 15	No. of roots per seed Average	Average index of root hairs
CONTROL	0.98	7	2.4	1.1
Cd 10^6	2.9	7	3.3	1.55
Cd 10^7	2.2	12	2.6	2.00
Cd 10^8	2.7	10	2.8	1.40
Cd 10^9	2.2	9	2.6	1.30
245 10^6	2.1	10	3.0	1.63
245 10^7	2.6	13	2.9	1.73
245 10^8	3.2	9	3.0	1.93
245 10^9	2.4	11	3.0	1.70
245 NR$^-$ 10^6	3.07	8	3.0	1.6
245 NR$^-$ 10^7	3.0	8	2.9	1.6
245 NR$^-$ 10^8	1.2	12	2.7	1.4
245 NR$^-$ 10^9	2.1	10	3.0	1.7
246 10^6	2.0	9	2.6	1.4
246 10^7	3.7	9	3.0	1.8
246 10^8	2.3	14	3.0	1.7
248 10^9	2.1	11	2.8	1.4

Three plates per treatment, all roots were measured

a) Exp. I: Inoculation of wheat in petri dishes, 64 h growth at 30 C.

1. Plates were inoculated with 1 ml suspension of bacteria per plate, 5 seeds per plate.

2. Appearance of root hairs was estimated on a scale of 1 to 5 where 1 = no root hairs and 5 = long dense mass of root hairs. The roots were observed with a binocular on a dark background.

3. Length of roots was measured with a ruler.

Observations to experiment 1, Wheat inoculation Table 1

1. There was low percent germination but it is aparently improved by inoculation with 10^7 - 10^8 bacteria.
2. Germination was not uniform therefore root length does not give any information..
3. The average index of root hairs show enhancement with all strains tested at 10^7 - 10^8 but diminishing at 10^9 .

Table II: Experiment II. Inoculation of a) wheat and b) sorghum in petri dishes 72 h growth at 30 C. 1 ml suspension of inoculum per plate 5 seeds per plate, 9 replications.

Wheat	No. germinated seeds/plate Average	No. roots per seed Average	Index of root hairs	Average length of roots (cm)
CONTROL	5	5.01	2.23	1.58
245 10^6	5	5.42	1.97	1.69
245 10^7	5	5.00	2.12	1.82
245 10^8	5	4.37	2.55	1.58
245 10^9	4	4.77	1.90	1.56
Cd 10^6	5	4.68	1.99	1.48
Cd 10^7	5	4.58	2.03	1.54
Cd 10^8	5	4.61	2.09	1.86
Cd 10^9	4	3.44	1.38	1.33
Sorghum				
CONTROL	4.3	1.00	1.32	2.28
245 10^6	3.1	1.00	1.28	1.70
245 10^7	3.22	1.00	1.51	1.66
245 10^8	2.66	1.00	1.43	1.27
245 10^9	2.77	1.00	1.40	1.02
Cd 10^6	3.11	1.00	1.40	1.94
Cd 10^7	2.1	1.00	1.40	2.50
Cd 10^8	2.0	1.00	1.50	2.80
Cd 10^9	1.2	1.00	1.30	1.1

Comments on Experiment II. Seeds were pregerminated overnight

Wheat

Incubation was too long, there were already 5 roots per seed some with developed root hairs and some without and that mixed up estimation of root hair development. An increase is clearly seen at 245 10^{8} , at 10^{9} there is a diminishing effect. Cd 10^{9} clearly diminished root length. The main problem was degeneration of root tips by some disease that altered results.

Sorghum

Seed germination was bad. Root length was inhibited by 10^{9} 245 and Cd. Index of root hairs increased at 245 10^{7} and Cd 10^{8} but differences compared to control and other inoculum levels were not large. Also in this case roots were diseased probably due to a fault in seed sterilization.

Experiment III. Inoculation of wheat and sorghum with *A. brasilense* in petri dishes incubated for 48 h at 30 C 6 replicates per treatment, 5 seeds per plate

	Index of root hairs	Average length roots (cm)	No. roots/seed
Wheat (~ 60% germination)			
CONTROL	1.37	2.12	3
245 10^6	1.64	1.99	3
245 10^7	2.38	2.22	3
245 10^8	1.79	1.55	3
245 10^9	2.27	1.61	3
Cd 10^6	1.77	1.73	3
Cd 10^7	2.03	1.96	3
Cd 10^8	1.61	1.44	3
Cd 10^9	1.55	1.65	3
Sorghum (50%) germination			
CONTROL	1.35	1.64	1
245 10^6	2.13	1.22	1
245 10^7	2.55	2.49	1
245 10^8	2.98	1.29	1
245 10^9	1.50	0.55	1
Cd 10^6	1.73	1.11	1
Cd 10^7	1.82	1.36	1
Cd 10^8	2.43	0.86	1
Cd 10^9	2.32	0.59	1

Comments on experiment III, Table 3

1. The system was kept contamination-free and disease by preparing seeds, inoculum, petri dishes etc. while taking care to avoid contamination. However, % germination was low because seeds were pregerminated with too much water.

2. 245 10^7 clearly favoured root hair formation in wheat, while 10^5 - 10^9 inhibited root length. Cd 10^4 also favored root hair formation but not to the extent of 245. 10^9 245 and Cd caused the formation of very thin root hairs.

3. 245 10^7 - 10^8 clearly favoured root hair formation in sorghum and inhibited root length at 10^9. The same trend was observed with Cd, 10^5 increased root hair index but index values are somehow lower than with 245.

Summary of Petri dish experiments

Similar trends as reported in the literature were observed in the experiments. Inoculation with optimal inoculum concentrations of 245 or Cd increased root hair formation, while higher concentrations 10^9 decreased root length and root hairs were thinner and less developed. A gradual effect with an optimum of 10^7 - 10^8 was observed from inoculum size. All strains tested showed similar effects but strain 245 gave relatively the most consistent and clearer effects.

Table 5: Greenhouse experiment (10 replicates)

	Root surface area ml NaOH 0.5M	Dry weight shoots g	No. cells/root system (2 replicates)	Dry weight of roots g	No. leaves & adventitious roots	Length of leaves 1	2	3
Cd CONTROL	5.36	0.073	1.0×10^4	0.028	4 adv.2-3cm	16.45	11.6	-
Cd 10^5	5.59	0.106	2.5×10^5	0.077	4 adv.2-3cm	19.13	9.82	3.5
Cd 10^7	5.55	0.090	1.6×10^5	0.065	3 adv.1-2cm	19.16	11.00	-
Cd 10^9	5.73	0.098	5.0×10^5	0.067	4 adv.2-3cm	21.54	12.35	3.37
245 CONTROL	6.65	0.04	3.0×10^4	0.083	3 adv.1-2cm	20.55	13.66	3.9
245 10^8	5.47	0.08	2.5×10^5	0.091	4 adv.1-2cm	19.41	10.64	4.6
245 10^7	7.12	0.09	6.0×10^5	0.086	4 adv.7-9cm	21.20	12.20	5.5
245 10^9	5.15	0.097	7.5×10^6	0.057	4 adv.5-6cm	18.83	10.58	3.5
NR CONTROL	6.26	0.079	-	0.043	4 adv.2-3cm	20.16	9.26	4.33
NR⁻ 10^5	6.47	0.093	0.4×10^5	0.061	4 adv.3-4cm	19.45	10.64	5.2
NR⁻ 10^7	5.96	0.095	5.2×10^5	0.106	4 adv.	19.16	11.00	5.46
NR⁻ 10^9	5.08	0.090	2.5×10^6	0.058	4 adv.1-2cm	18.83	10.58	4.0
246 CONTROL	5.84	0.093	-	0.041	3 adv.1-3cm	14.53	10.58	5.0
246 10^5	5.00	0.118	1.4×10^5	0.079	3 adv.3-4cm	20.29	11.66	4.0
246 10^7	4.70	0.091	0.9×10^6	0.070	4 adv.4-5cm	19.79	11.04	4.77
246 10^9	5.84	0.093	4.2×10^6	0.041	3 adv.5-6cm	19.42	11.58	6.0

Comments to results on the greenhouse experiment

1. 245 at 10^3 increased surface area above controls, and adventitious roots were observed to be more developed.

2. All strains at 10^5 and 10^7 increased leaf dry weight and root dry weight above controls. All strains decreased root dry weight at 10^9 . In general effects of 245 at 10^7 were clearer than effects of the other strains tested.

3. Number of bacteria per root system reflect root surface area and root dry weight. The more roots the more bacteria were found. All inoculated roots 10^5 - 10^7 were colonized by Azospirillum brasilense at ~ 10^5 per root system. There were not enough replications for a clearer conclusion. Control 245 was contaminated with Azospirillum at 1 x 10^4 . This was most probably due to contamination in the greenhouse since no attempts were made to avoid cross contamination. However, 245 increased root development and plant growth above controls. This indicates that even if Azospirillum is present in Brazilian soils, inoculation with large numbers of cells can improve plant growth.

Conclusions

Partial answers were given to the question raised:

1) There is a clear effect of inoculum size on root development by all strains tested. 10^7 - 10^8 cells seem to be optimal while 10^9 inhibited root growth.

2) Strain 245 gave the more favorable effects at 10^7 cells, compared with the other strains tested. This is in agreement with results obtained in inoculated wheat in Brazilian fields.

Strain Cd of Azospirillum brasilense used in Israel ATCC 29729, has given in Israel effects comparable to those observed by 245 in Brazil. It is possible that strain Cd used in Brazil underwent changes or it is a different strain.

The experiments carried out will be repeated in Israel and Argentina to compare Cd-Brazil, Cd-Israel and to observe the performance of 245 under Israeli conditions.

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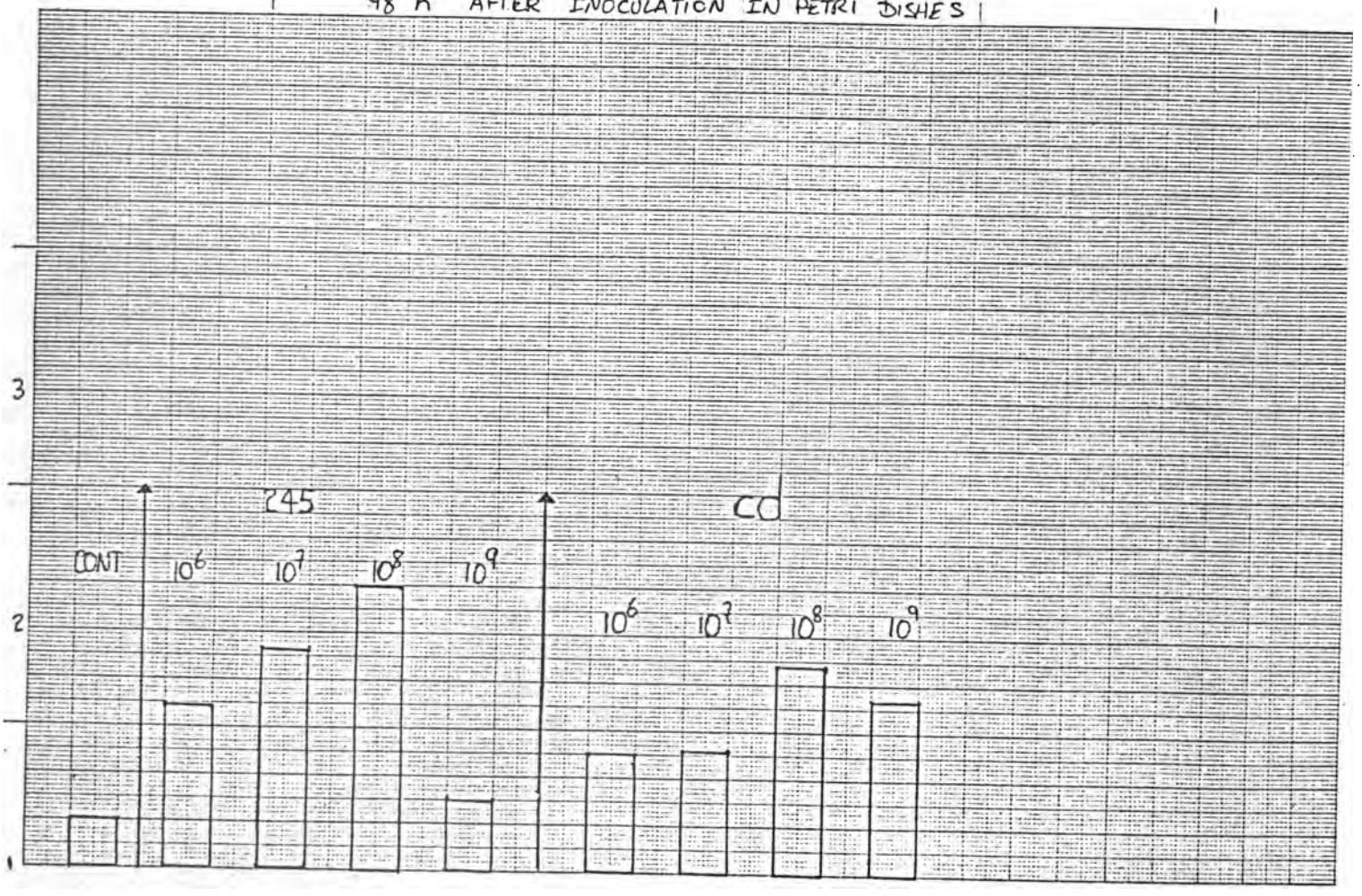
Carley, H.E. and Watson, R.D. (1976). A new gravimetric method for estimating root surface area. Soil Science 102,189-291.

AVERAGE OF 30 PLANTS

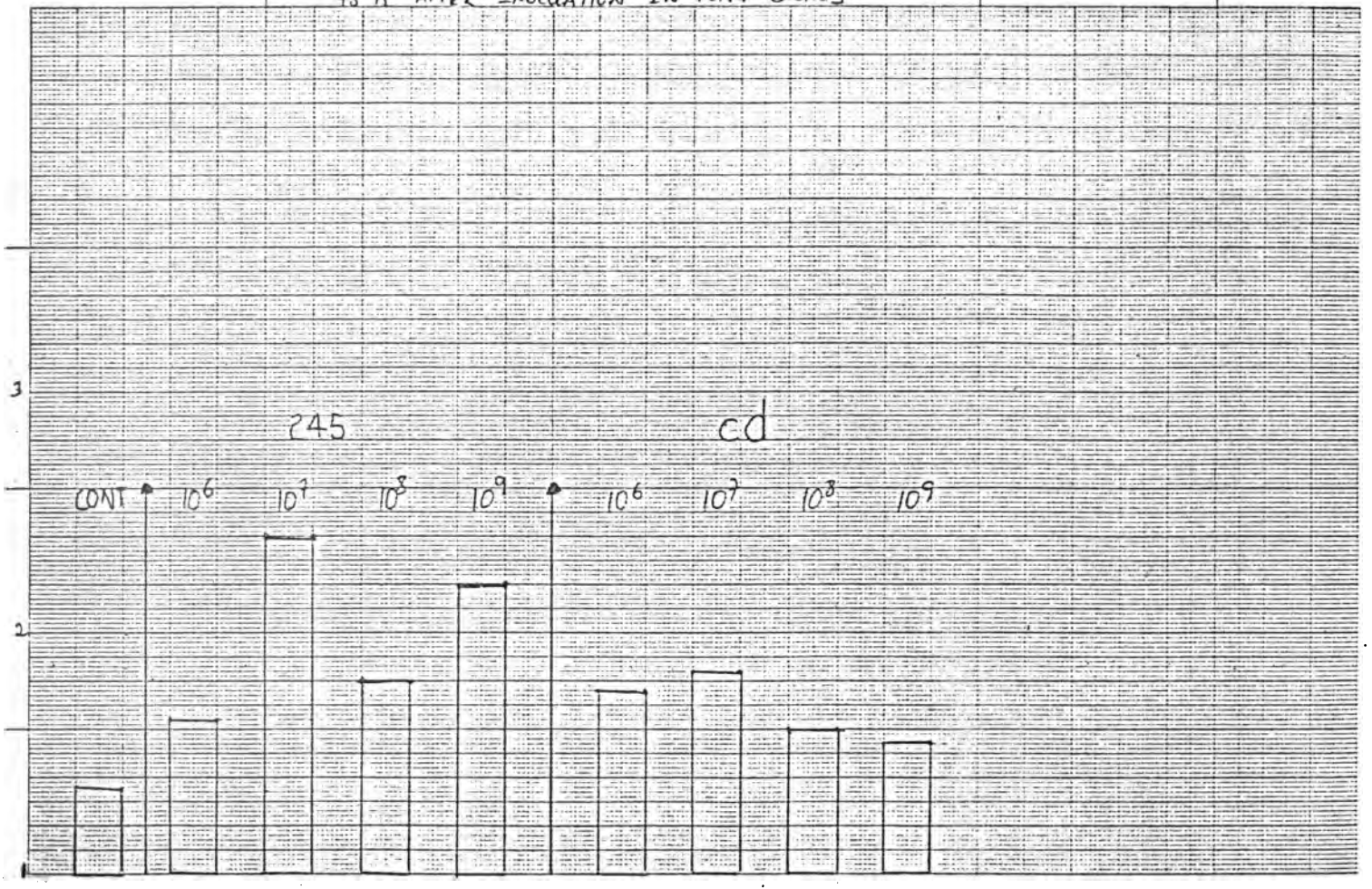
EXPERIMENT III. SORGHUM

48 h AFTER INOCULATION IN PETRI DISHES



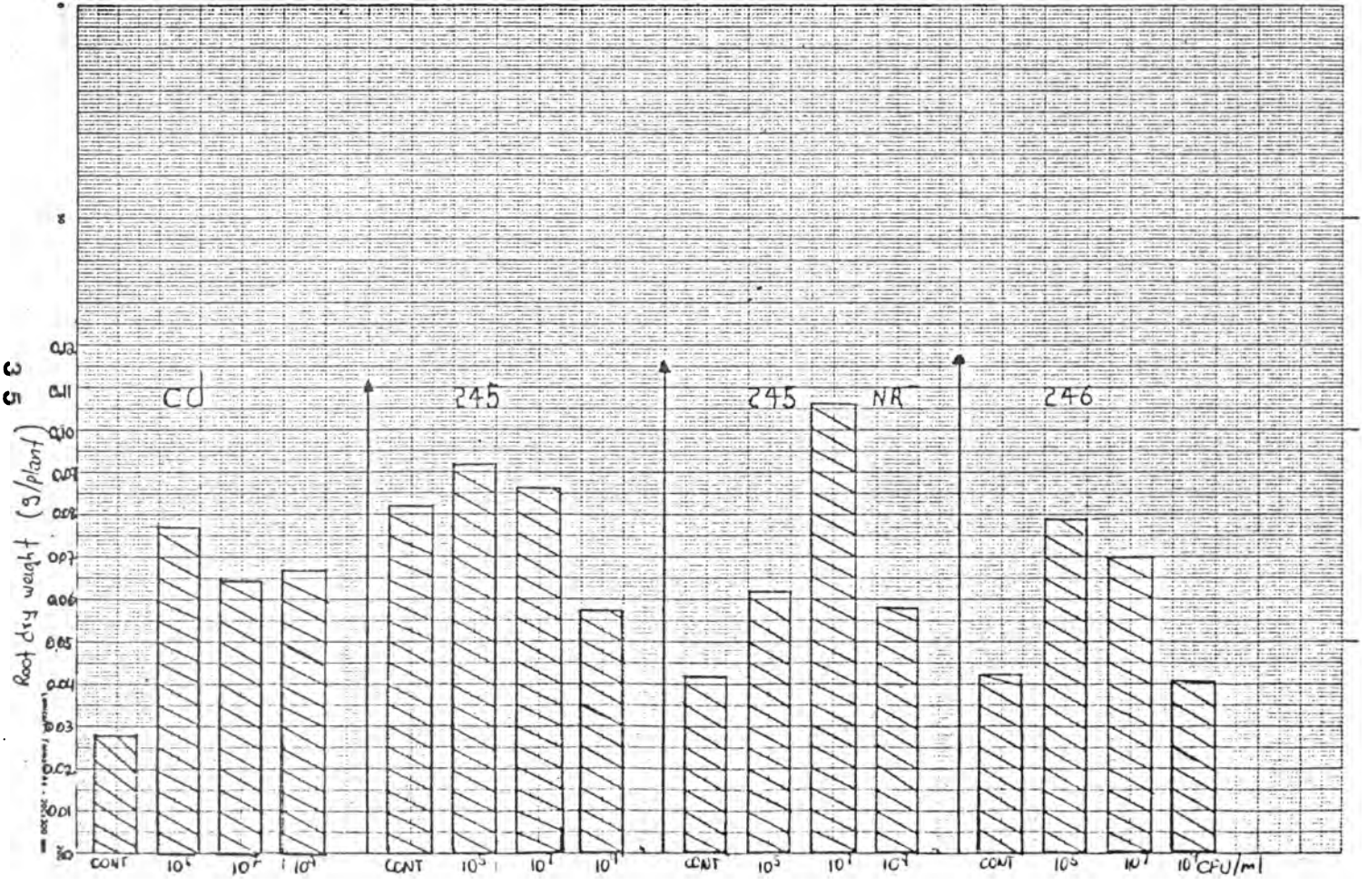
EXPERIMENT III WHEAT

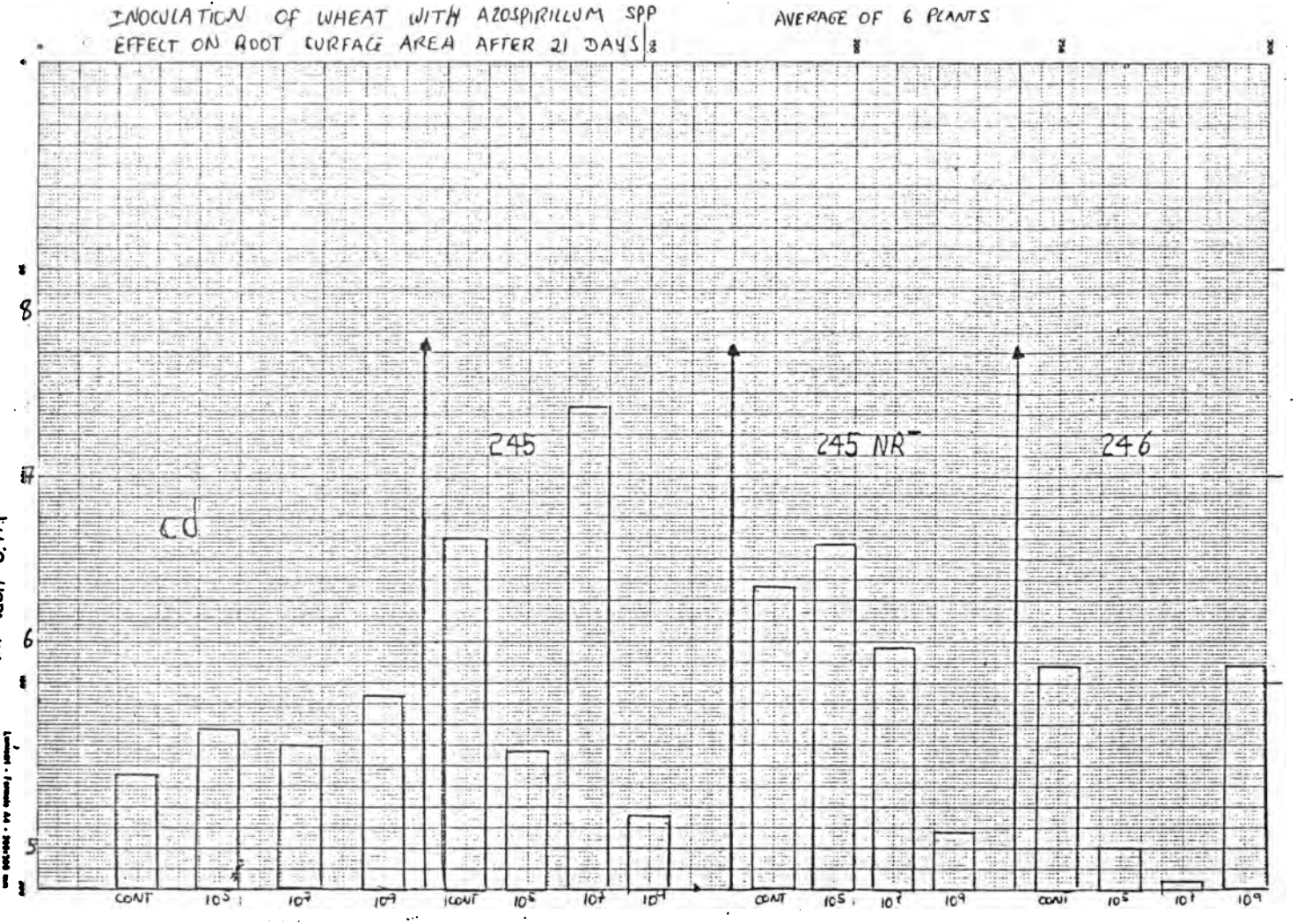
48 h AFTER INOCULATION IN PETRI DISHES



34

INOCULATION OF WHEAT WITH AZOSPIRILLUM SPP. AVERAGE OF 10 PLANTS
EFFECTS ON PLANT GROWTH AFTER 21 DAYS (ROOT DRY WEIGHT)




INOCULATION OF WHEAT WITH AZOSPIRILLUM SPP
EFFECT ON ROOT SURFACE AREA AFTER 21 DAYS
AVERAGE OF 6 PLANTS
ml NaOH 0.1M
8
7
6
5
CO
245
245 NR⁻
246
CONT
10⁵
10⁷
10⁹

Programa II. Geração e Transferência de Tecnologia

O Programa de Geração e Transferência de Tecnologia é a resposta do IICA a dois aspectos fundamentais: (i) o reconhecimento, por parte dos países e da comunidade técnico-financeira internacional, da importância da tecnologia para o desenvolvimento produtivo do setor agropecuário; (ii) a convicção generalizada de que, para aproveitar plenamente o potencial da ciência e da tecnologia, é necessário que existam infra-estruturas institucionais capazes de desenvolver as respostas tecnológicas adequadas às condições específicas de cada país, bem como um lineamento de políticas que promova e possibilite que tais infra-estruturas sejam incorporadas aos processos produtivos.

Nesse contexto, o Programa II visa a promover e apoiar as ações dos Estados membros destinadas a aprimorar a configuração de suas políticas tecnológicas, fortalecer a organização e administração de seus sistemas de geração e transferência de tecnologia e facilitar a transferência tecnológica internacional. Desse modo será possível fazer melhor aproveitamento de todos os recursos disponíveis e uma contribuição mais eficiente e efetiva para a solução dos problemas tecnológicos da produção agropecuária, num âmbito de igualdade na distribuição dos benefícios e de conservação dos recursos naturais.

INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA

O Instituto Interamericano de Cooperação para a Agricultura (IICA) é o organismo especializado em agricultura do Sistema Interamericano. Suas origens datam de 7 outubro de 1942, quando o Conselho Diretor da União Pan-Americana aprovou a criação do Instituto Interamericano de Ciências Agrícolas.

Fundado como uma instituição de pesquisa agronômica e de ensino, de pós-graduação para os trópicos, o IICA, respondendo às mudanças e novas necessidades do Hemisfério, converteu-se progressivamente em um organismo de cooperação técnica e fortalecimento institucional no campo da agropecuária. Essas transformações foram reconhecidas oficialmente com a ratificação, em 8 de dezembro de 1980, de uma nova convenção, que estabeleceu como fins do IICA estimular, promover e apoiar os laços de cooperação entre seus 31 Estados membros para a obtenção do desenvolvimento agrícola e do bem-estar rural.

Com um mandato amplo e flexível e com uma estrutura que permite a participação direta dos Estados membros na Junta Interamericana de Agricultura e em seu Comitê Executivo, o IICA conta com ampla presença geográfica em todos os países membros para responder a suas necessidades de cooperação técnica.

As contribuições dos Estados membros e as relações que o IICA mantém com 12 Países Observadores, e com vários organismos internacionais, lhe permitem canalizar importantes recursos humanos e financeiros em prol do desenvolvimento agrícola do Hemisfério.

O Plano de Médio Prazo 1987-1991, documento normativo que assinala as prioridades do Instituto, enfatiza ações voltadas para a reativação do setor agropecuário como elemento central do crescimento econômico. Em vista disso, o Instituto atribui especial importância ao apoio e promoção de ações tendentes à modernização tecnológica do campo e ao fortalecimento dos processos de integração regional e sub-regional.

Para alcançar tais objetivos o IICA concentra suas atividades em cinco áreas fundamentais, a saber: Análise e Planejamento da Política Agrária; Geração e Transferência de Tecnologia; Organização e Administração para o Desenvolvimento Rural; Comercialização e Agroindústria, e Saúde Animal e Sanidade Vegetal.

Essas áreas de ação expressam, simultaneamente, as necessidades e prioridades determinadas pelos próprios Estados membros e o âmbito de trabalho em que o IICA concentra seus esforços e sua capacidade técnica, tanto sob o ponto de vista de seus recursos humanos e financeiros, como de sua relação com outros organismos internacionais.

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Responsáveis pela reprodução: Jadir José dos Santos e Murillo Sodré da Silva.

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Autor
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Título fixacao de nitrogenio das
nao-leguminosas
Fecha Devolución
Nombre del solicitante

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