IICA

Consultant Final Report
IICA/EMBRAPA-PROCENSUL II

IONIC EQUILIBRIUM IN NITROGEN FIXATION SYSTEMS

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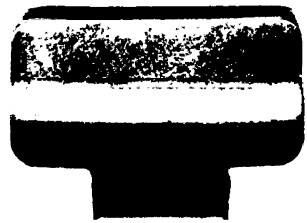

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John Albert Raven

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 Agropecuária 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

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

CONSULTANT FINAL REPORT

1. Consultant's full name: John Albert Raven

2. Specialist in: Ionic Equilibrium in Nitrogen Fixation Systems

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

4. EMBRAPA Program for which consultancy is providdd:

PROGRAM : PROCENSUL II

SUB-PROGRAMA : PESQUISA DE SOLOS

IICA Project Activity Code: 2.SB.3.06	Administrative Code: R 4884 BIB
Title of Activity of IICA Project corresponding to this consultancy	Cooperation with EMBRAPA on research on soil manage... and soil analysis.

CONSULTANT CONTRACT PERIOD	DUTY LOCATION (Center)
July 14th to 21st., 1988	UAPNBPS/EMBRAPA
CONTRACT EXTENTION PERIOD (If any)	DUTY LOCATION (Center)

5. Financial support: PROCENSUL II

6.5 ACTIVITIES IN SUPPORT OF RESEARCH STRATEGY AND PLANNING

Research subject matter	Research program to which subject matter is concerned

On the subject of acid excretion by N_2-fixing plants in relation to the availability of soil-derived resources, discussions were held with Jorge Jacob-Neto and Avilio Antonio Franco in relation to quantification of acidification and its effects on the availability of P, Fe, Mo and Zn (see also 6.2 and 8).

6.6 ACTIVITIES IN SUPPORT OF OTHER CENTERS AND UNIVERSITIES IMPROVING THE RESEARCH CENTERS LINKS WITH ABROAD

Subject matter on which links were recommended	Persons, centers and universities recommended for contact

NIL.

6.7 PUBLICATIONS AND REPORTS UNDERTAKEN WITH THE CONSULTAI'S PARTICIPATION

Author(s)*	Title of publication or Report and other bibliografic identification
Raven, J.A.	Possible interactions between symbiotic nitrogen fixation and electric currents circulating around and within root hairs. Anais da Academia Brasileira de Ciências, in press. (abstract).

* Personal, institutional, etc.

6.8 SUPPORT PROVIDED TO EMBRAPA RESEARCHERS IN THESIS AND DISSERTATION WORK

Name of the student	Thesis subject matter and sinthesis of advice

NIL.

6.2 SUPPORT TO RESEARCH UNDERTAKEN BY OTHER EMBRAPA RESEARCHERS

Research activities developed	Results achieved

Discussions were held with Avilio Antonio Franco and Eli Lino de Jesus on the use of "green manure" in providing N (via N_2 fixation) and P (via acid-induced solution of calcium phosphate). These discussions centred on the methodology of measuring pH changes in the rhizosphere in soil in relation to the objective of solubilizing phosphate from calcium phosphate by acid-excreting N_2-fixing legumes. Quantitation of the pH change in parts of the rhizosphere is possible, using pH-indicator dyes and, for preference, small pH-sensitive (e.g. antimany) electrodes of the type under development at EMBRAPA. However, use of soil measurements of pH to quantify total H^+ excretion by a crop is not currently feasible (see 6.5 and 8 below).

6.3 TRAINING ACTIVITIES DEVELOPED BY THE CONSULTANT

Date	Training subject matter	Type of event*	Number of beneficiaries	
			From EMBRAPA	From other institutions
15 July/88	Possible role of circulating electric currents flowing around and within root hairs in symbiotic N_2 fixation	Conference of Brazilian Academy of Sciences	15	20
18 July/88	General aspects of acid-base balance of plants.	Short Course of Biological N_2 Fixation	5	25
18 July/88	Ionic, acid-base and redox aspects of plant-rhizosphere interactions.	"	"	"
19 July/88	Acid-base balance of plants and plant parts during N_2 fixation.	"	"	"
19 July/88	Resource (photons, watter, Fe, Mo) use efficiency and N_2 fixation.	"	"	"

* Short courses, seminars, conferences, etc.

6.4 IN-SERVICE TRAINING PROVIDED BY THE CONSULTANT

In-service training subject matter	Names of counterparts

NIL.

TABLE 1. Results of analyses of media and plants before and after the 5 days culture period.

Treatment Source of nitrogen	gfw/gdw at start	gfw/gdw at end.	pH of culture solution (air-equilibrated) at start.	pH of culture solution (air-equilibrated) at end.	gfw of plants at start.	gfw of plants at end.	ash alkalihity of plants, µmol/gfw at start.	ash alkalihity of plants, µmol/gfw at end.	total N of plants, µmol/gfw at start.	total N of plants, µmol/gfw at end.
	16.13%	14.68%		5.11%	1	2.540				5,9
N_2	14.29%	16.54%	6.88	4.92%	1	2.650	10	20	2,9	6,4
	15.38	14.34		5.30	1	2.653	14	22		5,7
	16.13%	11.17%		3.77%	1	1.427				4,28
NH_4^+	14.29%	11.20%	5.31	3.82%	1	1.396	10	11	2,9	4,16
	15.38	9.97		3.80	1	1.386	14	13		4,00
	16.13%	14.76%		6.93%	1	2.094				5,0
NO_3^-	14.29%	15.22%	6.78	6.84%	1	2.372	10	24	2,9	6,2
	15.38	14.20		6.85	1	2.457	14	24		6,1

TABLE 2. Differences in extracellular H^+, plant ash alkalinity, total plant N, and plant fresh weight, over the 5 day culture period.

A Treatment		B Increase in fw.over 5 days/g (initial=1g)	C Increase in H^+ in medium per f.g.w. gain (µmol/gfw)	D Increase in plant ash alkalinity (µmol/gfw)	E Predicted increase in organic N (µmol/gfw)	F Measured increase in total N (µmol/gfw)
N_2 as only N source	(1)	1.540	+ 15.58	+ 25.2	N.P.	3,0
	(2)	1.650	+ 14.55	+ 28.1	N.P.	3,5
	(3)	1.653	+ 14.52	N.D.	N.P.	2,8
NH_4^+ present	(1)	0.427	+ 67.45	+ 15.3	52.15(a)	1,38
	(2)	0.396	+ 48.48	+ 8.47	40.01(a)	1,26
	(3)	0.386	+ 74.61	N.D.	N.D.	1,10
NO_3^- present	(1)	1.094	- 3.20	+ 34.97	38.17(b)	2,1
	(2)	1.372	- 0.73	+ 32.75	33.48(b)	3,3
	(3)	1.457	- 1.72	N.D.	N.D.	3,2

N.D. = no data. N.P. = Not Possible to compute - N source (N_2) uncharged.
(a) = computed from column (c) minus column (D).
(b) = computed from column (D) minus column (C).

6. ACTIVITIES UNDERTAKEN BY THE CONSULTANT AND RESULTS

6.1 RESEARCH DONE UNDER DIRECT RESPONSIBILITY OF THE CONSULTANT

Research activities developed	Results Achieved

The research initiated by the consultant, and undertaken by the consultant in collaboration with Eli Line de Jesus, Patricia Póvoa de Mattos and Dr. Avílio Antonio Franco (all of EMBRAPA) was concerned with the acid-base-balance of the heterosporous watter fern Azolla supplied with various N sources. The objective of the research was to complement the large body of data on acid-base balance of symbiosis of higher plants with Rhizobium, Bradyrhizobium or Frankia growing with NH_4^+, N_2 or NO_3^- as N source with data on the Azolla/Anabaena symbiosis. Essentially no data on acid-base balance are available for higher plant/cyanobacteria symbioses (e.g. Azolla/Anabaena; Gunnera/Nostoc; Cycadales/Nostoc).

In addition to the different N_2-fixing symbiont, the Azolla/Anabaena symbiosis is of interest as a Free-Floating aquatic N_2-fixing plant. The N_2-fixing land plants with Rhizobium/Bradyrhizobium or Frankia as symbionts frequently excrete more H^+ for each N assimilated from N_2 than is predicted from the H^+/N ratio during growth on NH_4^+, i.e. the plant makes more organic acid per N assimilated when growing on N_2 than when growing on NH_4^+. The additional H^+ excreted may be important in mobilizing phosphate (from insoluble calcium phosphate) or iron imobilization of Fe^{3+} to Fe^{2+} using reductant and acid); N_2 fixation may have P and Fe requirements additional to those found during growth on combined N. These nutrient-mobilizing properties are much less readily expressed by roots in water than by roots in soil. Teleologically, there is less "need" for extra H^+ excretion by a floating water plant. If such extra H^+ excretion is found in Azolla then some other possible significance to the plant must be sought.

The starting material was an Azolla culture (infected with Anabaena) growing in water above soil. Three culture conditions were employed, with triplicatiation of each treatment. A standard N-free Azolla medium was supplemented with either 10 ml m^{-3} NH_4Cl ("NH_4^+"), or 10 mol m^{-3} $NaNO_3$ ("NO_3^-") or 10 ml m^{-3} NaCl ("N_2"). The pH of the media at the start of the experiment was measured at intervals throughout the 5 days of culture in a glasshouse under natural illumination. At the end of the growth period the Azolla plants were removed from the culture medium and their fresh weight was determined to see what increase had occurred over the 1g fresh weight in each culture at the start of the exporiment.

A portion (weighed) of the Azolla was dried for dry weight and, subsequently, total N determination. The remainder of the Azolla was ashed in a muffle furnace at about 650°C for subsequent determination of ash alkalinity. For comparison, similar measurements were made on 1g fresh weight samples taken at the beginning of the growth period. The media remaining after the Azolla was removed was re-equilibrated with air (to remove any accumulated respiratory CO_2) and titrated back to the original pH with NaOH solution ("NH_4^+" and "N_2" treatment) or HCl ("NO_3^-" treatment).

6. ACTIVITIES UNDERTAKEN BY THE CONSULTANT AND RESULTS

6.1 RESEARCH DONE UNDER DIRECT RESPONSIBILITY OF THE CONSULTANT

Research activities developed	Results Achieved

The data obtained are summarised in tables 1 and 2. It will be seen that, as is almost invariably found in other symbioses, the NH_4^+ - assimilating plant excretes more H^+ per g.f.w. increase than does the N_2-fixing plant, while the NO_3^- - assimilation plant excretes OH^-. Another finding in accord with measurements on other symbiosis is that growth on NH_4^+ yields the lowest ash alkalinity (approximately equal to organic anions: see below) on a fresh weight basis, and NO_3 as N source yields the highest. The finding that the ash alkalinity of N_2-fixing plants exceeds that of the NH_4^+ - assimilating plants suggests that "extra" H^+ is excreted by N_2-fixing plants despite the absence of a rhizosphere (in the soil sense) to acidify.

The discrepancies between the "predicted" and the "measured" N content have a number of possible sources. One is that the computation of column "E" in Table 2 (as well as the conclusions drawn in the previous paragraph) implicitly assumes that N_2 does not contribute to N increase in the media containing NH_4^+ or NO_3^-; this could explain a greater than predicted increased in total plant N. Another source of error relates to the significance of "ash alkalinity". Ashing removes any NH_4^+ and NO_3^- present in the plant as well as organic material. Significant NH_4^+ levels in the original material means that ash alkalinity under-estimates organic anion content. Conversely, significant NO_3^- levels in the plant means that ash alkalinity over - estimates organic anion content. Furthermore, ash alkalinity does not just represent organic anion whose H^+ counterpart is available for neutralizing OH^- generated in NO_3^- assimilation or as a source of H^+ for excretion during NH_4^+ or N_2 assimilation. Some of the H^+ equivalent to "ash alkalinity" is used to neutralize OH^- generated in SO_4^{2-} assimilation. The absence of organic S, NH_4^+ and NO_3^- measurements means that the ash alkalinity measurements are less useful than would otherwise be the case in drawing conclusions as to acid-base regulation.

It is clear that more work is needed on Azolla and on soil-based cyanobacterial symbioses. However, despite constraints of time due to the consultants other engagements, significant results were obtained which point the way to more detailed studies.

8. CONSULTANT'S SUGGESTIONS AND TECHNICAL OR INSTITUTIONAL RECOMMENDATIONS FOR THE IMPROVEMENT OF THE RESEARCH SERVICE

The results of discussions in 6.2 and 6.5 above on soil acidification by plants were that both soil and plant measurements were helful in determining the <u>total</u> H^+ flux from plant to soil <u>provided the dominant N source</u> for plant growth is known. The plant measurements are ash alkalinity, NH_4^+ , NO_3^- and (when possible) organic S. This permits computation of <u>total</u> H^+ efflux during growth on N_2 comparison with <u>spatial</u> data on efflux from soil measurements (6.2 and 6.5).

9. AGREEMENTS OR COMMITTMENTS ESTABLISHED WITH EMBRAPA RESEARCHERS IN-SERVICE OF THE FUTURE DEVELOPMENT OF RESEARCH IN THE CONSULTANT'S FIELD OF SPECIALIZATION

An informal agreement was reached to exchange results and ideas between the consultants research group and interested parties in EMBRAPA.

10. CONSULTANT'S COMMENTS ON CIRCUMSTANCES WHICH AFFECTED THE CONSULTANCY WORK

The only constraints were those imposed by the consultants other committments which only allowed him to spend 8 days in Seropédica. EMBRAPA staff were extremely helpful in expediting the teaching and laboratory parts of the consultancy.

Date: 21 July 1988

Signature J. A. Raven

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 ás condições específicas de cada país, bem como um lineamento de politicas 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 própios 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.

Esta publicação foi reproduzida na Gráfica do Escritório do IICA no Brasil, em Brasília, em janeiro de 1989, numa tiragem de 100 exemplares.

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

Interessados em receber mais exemplares deste o de outros Relatórios de Consultores poderão solicitá-los a:

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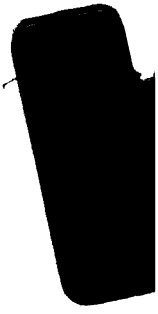

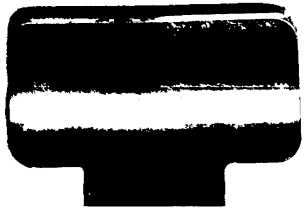

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