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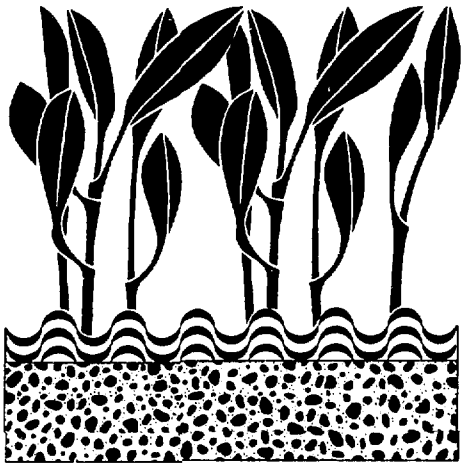

Soils Newsletter

JOINT FAO/IAEA DIVISION
OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE
AND FAO/IAEA AGRICULTURAL LABORATORY, SEIBERSDORF
INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA

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TO OUR READERS

On behalf of my colleagues in the Soil Fertility, Irrigation and Crop Production Section, and the Soil Science Unit, IAEA Seibersdorf Laboratory, let me first extend to you our best wishes for a happy, prosperous and rewarding 1995.

In 1994, we celebrated the 30th Anniversary of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. The year has been a busy one with many developments and achievements.

Four Research Co-ordination Meetings (RCM) were held. The third RCM on "Enhancing soil fertility and crop production by better management of *rhizobium*", hosted by Prof. W.J. Broughton, Head of the Molecular Biology Laboratory at the University of Geneva, took place in Geneva from 15 to 19 August 1994. The first FAO/IAEA RCM on "The use of nuclear techniques for optimizing fertilizer applications under irrigated wheat to increase the efficient use of fertilizers and consequently reduce environmental pollution", as well as the final FAO/IAEA/SIDA RCM on "The use of isotope studies on increasing and stabilizing plant productivity in low phosphate and semi-arid and sub-humid soils of the tropics and sub-tropics", were held in Vienna, Austria. The final FAO/IAEA/UNDP RCM on "The use of isotopes in studies to improve yield and N_2 fixation of grain legumes", was hosted by Dr. O.P. Rupela at the International Crop Research Institute for the Semi-Arid Tropics, in Hyderabad, India from 7 to 11 November 1994. Some excerpts from these RCMs can be found in this issue of the Soils Newsletter.

We organized two symposia this year. These were dedicated to the 30th Anniversary of the Joint FAO/IAEA Division. One was on "The Use of Nuclear and Related Techniques in Soil/Plant Studies, with Special Emphasis on Environmental Preservation for sustainable Agriculture", organised as part of the XV World Congress of Soil Science, held in Acapulco, Mexico from 10-16 July and the other on "Nuclear and Related Techniques in Soil/Plant Studies for Sustainable Agriculture and Preservation" held in Vienna, Austria from 17-21 October. The Acapulco Symposium was considered a great success by the International Society of Soil Science and by the XV World Congress of Soil Science. The Symposium reviewed the contribution of isotope methods to increasing soil fertility and crop production, and the contributions from various FAO/IAEA Co-ordinated Research Programmes and IAEA Technical Co-operation Projects to developments of nuclear technique in soil science studies.

The Vienna Symposium which was attended by over one hundred participants from forty three countries, included 48 oral presentations and 31 posters. This Symposium reviewed the recent developments in analytical methods, fertilizer use and management studies, biological nitrogen fixation in sustainable cropping systems, soil organic matter and nutrient cycling, water use efficiency studies, plant physiological aspects in crop production, environmental pollution and preservation , soil conservation, soil erosion and desertification. The symposium provided "food for thought" for the challenges mankind is faced with in attempts to solve the growing food demands of the ever increasing population. The proceedings of the Symposium will be published in April 1995.

I would like to mention here that I was very fortunate and am proud that I had the chance to be involved in the activities of the Joint FAO/IAEA Division for 30 years since its inception in 1964. Firstly, my involvement was as a Chief Scientific Investigator in different Co-ordinated Research Programmes, then as a field expert in soil fertility and fertilizer studies and finally as a staff member since 1991. For this reason I enjoyed very much my participation in all of the activities dedicated to this anniversary.

The following meetings were also held in 1994: Forth workshop of the IAEA Technical Co-operation Regional Africa Project on Biological Nitrogen Fixation (RAF/5/010) from 14-25 March in Nairobi, Kenya; Final Middle East and Europe Co-ordination meeting on Biological Nitrogen Fixation, Nutrient and Water Balance Studies (RER/5/004) from 5-9 December, Teheran, Iran and the Consultants Meeting on "Radiation Processing of Sewage Sludge and its Use to Increase Crop Yields and to Preserve the Environment, from 5-9 December in Vienna, Austria.

Two Training Courses on the "Use of Isotopes in Soil Fertility and Plant Nutrition Studies" held in Nairobi, Kenya from 6 June to 8 July, and the "Advanced Fellowship Group Training in Soil/Plant Research", held from 26 September to 21 October 1994 in Vienna, Austria, provided participants with knowledge on the use of isotopes and radiation technique for sustaining soil fertility and crop yields through efficient management of fertilizer nutrients, water and biological processes.

Taking into consideration that the productive capacity must be conserved for future generation and that the dangers of a reduction in soil fertility and production capacity due to the use of deficient technology is growing day by day, we have taken steps to initiate the following new Co-ordinated Research Programmes for the coming years: "The use of irradiated sewage sludge to enhance soil fertility and crop production and to protect the environment" (1995-1999); "The use of isotopes in soil organic matter turnover and management for promoting sustainable agriculture at optimum yield levels" (1995-1999); "The assessment of soil erosion as a basis for soil conservation by using ^{137}C and other techniques" (1995-1999); "The use of nuclear techniques to increase the yield of root and tuber crops through soil, plant and fertilizer management" (1996-2000); "The use of isotope techniques to quantify the production of greenhouse gases and minimize their emission" (1996-1999); "The use of nuclear techniques to assess nutrient uptake and water use efficiency under rainfed agriculture"; "The use of nuclear techniques to improve crop production in acid soils". The implementation of these programmes would however depend upon availability of financial resources.

Preparations are now underway for the publication of the results received under the CRP on "The use of nuclear techniques to improve crop production on salt-affected soils, results of the CRP on "Increasing and stabilizing plant productivity in low phosphate and semi-arid and sub-humid soils of the tropics and sub-tropics, results of the Phase I of the regional Africa project on biological nitrogen fixation, as well as the proceedings of an FAO/IAEA regional seminar for Asia and the Pacific on "nuclear methods in soil-plant aspects of sustainable agriculture.

At the beginning of 1995 there are a few major staff changes that will be taking place in the Joint FAO/IAEA Division Director's Office. Firstly, after 12 years of efficient and dedicated service, Dr. Björn Sigurbjörnsson will leave Vienna at the beginning of 1995. He is expected to take up the position of Secretary General of the Ministry of Agriculture in Iceland. Also in January 1995, our colleague from the Soil Section, Dr. M.P. Salema will start his new duty as Deputy Director of the Joint FAO/IAEA Division. We wish them both all the best and every success in their future endeavors

I would like to take this opportunity to express my thanks and gratitude to all my colleagues from Headquarters and the Seibersdorf Laboratory for their valuable contributions in the year 1994.

This issue of the Soils Newsletter was compiled and edited by Saliya Kumarasinghe and myself with contributions from our colleagues from Soil Section at the Headquarters and Soils Unit at the Seibersdorf Laboratory. The manuscript was typed by Ms. Daniela Panzenböck.

Thank you for your continued collaboration and I wish you once again a Merry Christmas and a Happy New Year.

Christian Hera
Head, Soil Fertility, Irrigation and
Crop Production Section

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3. **Staff Changes**

One of the major changes in staff was at the Soil Unit in Seibersdorf. Aldo Sebastianelli, who joined the Lab on 1 November 1974 as a Junior Laboratory Technician, left on 30 September

1994 after 20 years of yeoman service. During this period he was promoted to the level of a Senior Laboratory Technician where he became in charge of radioisotope assays in Soil/Plant Research. He also participated, actively and willingly, in every activity where the soils unit was involved. He was one of the "live-wires" in the organization of practical work for the Soil/Plant training courses in Seibersdorf. In the R & D area, Aldo was instrumental in the development of the all-metal vacuum line for ^{15}N sample preparation which is now widely used in many Member States of the Agency. In November, Aldo went into early retirement and is now planning to go back to his home country, Argentina, where he is looking forward to a quieter life, away from radio and stable isotopes. We wish Aldo and his family all the very best for the future.

Louise Taylor, who joined as the Section Secretary in June 1994, resigned in August to take up a position in UNIDO. We wish Louise every success in her future endeavors.

Daniela Panzenböck, an Austrian who grew up in South Africa, joined the Section on 15 November 1994 as the new Secretary, a position left vacant by Louise. All our staff extend a warm welcome to Daniela and wish her all the best.

CO-ORDINATED RESEARCH PROGRAMMES

1. **FAO/IAEA/SIDA CRP on The Use of Isotope Studies on Increasing and Stabilizing Plant Productivity in Low Phosphate and Semi-arid and Sub-humid Soils of the Tropics and Sub-tropics (D1-50.02)**

   (Project Officer: K. Saliya Kumarasinghe)

   This CRP, funded by the Swedish International Development Authority (SIDA) was successfully completed with the last RCM held in Vienna from 10-14 October 1994. The contract holders (8): Messrs. M. Abdou (Egypt), D.O. Nyamai (Kenya), M. Bazza (Morocco), B.A. Ogunbodede (Nigeria), D. Amara (Sierra Leone), K.H. Elamin (Sudan), M. Mechergui (Tunisia) and Ms. Bui Thi Hong Thanh (Viet Nam) and the five agreement holders: Messrs. W. Horst (Germany), N. Sanginga (Nigeria), A. Haystead (New Zealand), P. Högberg and E.Haak (Sweden) participated in this RCM. Excerpts from presented reports are given elsewhere in this Newsletter.

2. **FAO/IAEA/UNDP CRP on The Use of Isotopes in Studies to Improve Yield and N_2 Fixation in Grain Legumes with the Aim of Increasing Food Production and Saving N-Fertilizer in the Tropics and Sub-tropics of Asia (D1-40.04)**

   (Project Officer: Seth K.A. Danso)

   This CRP is closing in December this year, and the final Research Co-ordination Meeting has already been held (7-11 November at ICRISAT, Hyderabad, India). A tripartite mission consisting of Drs M.D. Tantera (Indonesia), D.F. Herridge (Australia), K.A. Malik (Pakistan) and J.V.D.K. Kumar-Rao (India) and the project officer, S.K.A. Danso, visited the Philippines, Malaysia, Thailand, Bangladesh and India in order to review achievements of the CRP; they also participated in the RCM. They are to submit a report to UNDP. The Final RCM was attended by the following 14 participants: M.A. Sattar and M.A.O. Shaik (Bangladesh), Thea Ah Kow and K.A. Rahim (Malaysia), G.A. Dayatilake and S.Subasinghe (Sri-Lanka), M.X. Hon, and Tran D. Long (Viet Nam), Li Haixan, Wang Zhidong and Li Xiomin (China), M. Boonkerd (Thailand), D.F. Herridge (Australia) and O.P. Rupela (India). In all cases, the reports showed a very wide genetic variability in N_2 fixation among the following legume genotypes: soybean, cowpea, mungbean, chickpea and groundnuts. Some participants have reached various stages of

crosses between high yielding and high N_2 fixing lines, as an avenue for incorporating both high yield and high N_2 fixation into single cultivars.

3. **FAO/IAEA/OPEC CRP on The Use of Nuclear Techniques in the Management of Nitrogen Fixing Trees for Enhancing Soil Fertility and Soil Conservation (D1-40.05)**

(Project Officer: Seth K.A. Danso)

The CRP is still active, as presented in the previous Soils Newsletter, and will now proceed to 7 years instead of 5 years. This is to make it reasonable for our participants to obtain worthwhile data, considering the perennial nature of trees. Participants are continuing with their studies on the use of ^{15}N techniques to measure N_2 fixation in various trees, and efforts are being made to overcome problems with the selection of reference trees. Genotypic variation among trees for N_2 fixation has been demonstrated and remains one of the achievements of this project. Studies on the management of nitrogen fixing trees for greater nitrogen fixation and the role of nitrogen fixing trees as sources of organic matter for soil fertility improvement are running alongside. The postponed RCM will now be held at the end of May 1995.

4. **FAO/IAEA CRP on The Use of Nuclear and Related Techniques in Assessment of Irrigation Schedules of Field Crops to Increase Effective Use of Water in Irrigation Projects (D1-20.05).**

(Project Officer: Pierre Moutonnet)

This programme included sixteen participants: C. Angueira (Argentina), P.L. Libardi (Brazil), M. Qi (People's Republic of China), B.G. Pene (Côte d'Ivoire), M. Calvache (Ecuador), M. El-Haris (Egypt), G. Vachaud (France), T. Kovacs (Hungary), M. Tayaa (Morocco), M. Iqbal and R. Waheed (Pakistan), I. Graciun (Romania), F. Moreno (Spain), M.S. Anaç and Ç. Kirda (Turkey) and J. Hopmans (USA).

The final RCM will be held in Rabat, Morocco, 24-28 April, 1995. The results of this CRP is planned to be published as an IAEA TECDOC.

5. **FAO/IAEA CRP on The use of Nuclear Techniques for Optimizing Fertilizer Applications under Irrigated Wheat to Increase the Efficient Use of Fertilizers and Consequently Reduce Environmental Pollution (D1-40.07)**

(Project Officer: Pierre Moutonnet)

This programme includes seventeen participants; five agreements holders: R. Rennie (Canada), G. Vachaud (France), R.A. Fisher (Mexico), W. Baethgen and J. Schepers (USA) and twelve contract holders: M.I. Khalil (Bangladesh), A.E. Boaretto (Brazil), I. Vidal-Parra(Chile), X. Wen (People's Republic of China), M.A.S. Abdel Monen (Egypt), M.S. Sachdev (India), X. Uvalle-Bueno (Mexico), J.M. Sanchez-Yañez (Mexico), M. Bazza (Morocco), G. Cioban (Romania), A. Arslan (Syria), Ç. Kirda (Turkey). The Consultants Meeting, held in Vienna from 29 November to 2 December 1993, established the goals and objectives of this new CRP, initiated in co-operation with CIMMYT, Mexico, and IFDC, USA. The first RCM was held in Vienna from 3 to 6 October 1994. So far, ^{15}N labelled fertilizers as well as several technical documents to be used as a guideline during the 1994-1995 winter wheat cropping campaign, have been sent to the contractors. Excerpts from the first RCM are given elsewhere in this Newsletter.

6. **FAO/IAEA CRP on Enhancing Soil Fertility and Crop Production by better Management of *Rhizobium* (D1-40.06)**

(Project Officer: Gudni Hardarson)

The third FAO/IAEA Research Coordination Meeting on "Enhancing Soil Fertility and Crop Production by Better Management of *Rhizobium*", was held at the Laboratoire de Biologie Moléculaire des Plantes Supérieures (LBMPS) at the University of Geneva, from 15 - 19 August, 1994. Professor W. J. Broughton, an agreement holder in the above programme, was the local organizer of the meeting. Excerpts of presented reports are given elsewhere in this Newsletter.

7. **FAO/IAEA/French Government CRP on The Use of Nuclear and Related Techniques for Evaluating the Agronomic Effectiveness of Phosphate Fertilizers, in particular, Rock Phosphates (D1-50.03).**

(Project Officer: Felipe Zapata)

This CRP is starting its second year of operation. Funding of the programme is done under Regular Budget to secure continuity of implementation. At present there are 18 scientists collaborating in this CRP, i.e. 12 contractors and 6 research agreement holders. The second RCM is planned to be held from 24 to 28 April 1995, in Montpellier, France. The IAEA Publication Committee approved that the proceedings of the Consultants Meeting be published as an outside publication, as recommended by the consultants. All manuscripts have been sent to Fertilizer Research for peer-review and publication.

8. **FAO/IAEA CRP on The Use of Irradiated Sewage Sludge to Increase Soil Fertility and Crop Yields, and to preserve the Environment.**

(Project Officer: Saliya Kumarasinghe)

This is a joint programme of the Joint FAO/IAEA Division of Nuclear Techniques in Food Agriculture, and the Division of Physical and Chemical Sciences of the IAEA, to be initiated in 1995. In support of this CRP, a Consultants Meeting was held from 5-9 December at the Vienna International Centre. This was attended by Consultants from Austria, Germany, India, Indonesia, Japan and the United Kingdom. About five agreement holders and ten contract holders are expected to be selected and the first RCM is planned to be held in mid 1995.

FAO/IAEA TRAINING COURSES

Planned

1. **The FAO/IAEA Interregional Training Course on the Use of Isotope and Radiation Techniques in Studies of Soil/Plant Relationship with Emphasis on Better Nutrient Utilization to Improve Crop Production.**
   29 May - 7 July 1995, Seibersdorf, Austria

Technical Officer: Christian Hera; Course Director: Felipe Zapata

The objective of this six-week training course is to give scientists, from developing countries, a sound working knowledge of the use of isotope and radiation techniques in soil/plant relationship studies. The course also aims at training local personnel to develop capabilities to carry out isotope-aided experiments as part of national programmes for increasing sustainable agricultural

productivity. The course will cover the relevant isotope and nuclear techniques in soil fertility and plant nutrition studies. Broad coverage of techniques will be given through lectures, laboratory sessions, greenhouse/field experiments, films and discussion groups. Emphasis will be placed on application of these techniques to solve practical problems from their own experience. Please note that the language of the course is English. A written certificate of English proficiency is required.

The circular announcement and prospectus will be dispatched to the National Atomic Energy Authority or Government Office responsible for nuclear matters in the respective Member States during the last quarter of 1994. For additional information, please contact the Technical Officer, Christian Hera, Head, Soil Fertility, Irrigation and Crop Production Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria.

Nominations should be submitted in duplicate on the standard IAEA nomination forms for training courses. Completed forms should be endorsed and forwarded through the official channels established (the Ministry of Foreign Affairs, the National Atomic Energy Authority, the Office of the UNDP, FAO or the Ministry of Agriculture); they must be received by the International Atomic Energy Agency, P.O. Box 100, A-1400 Vienna, Austria, not later than 1st March 1995. Nominations received after that date or applications sent direct by individuals or by private institutions cannot be considered.

2. **FAO/IAEA Regional Training Course on the Use of Isotopes and Radiation Techniques in Studies of Soil/Plant Relationships with Emphasis on Crop Production on Acid Soils. 6 March - 7 April 1995, Bangkok, Thailand.**

Technical Officer: Saliya Kumarasinghe; Course Director: Patoom Snitwongse

The objective of the course is to train scientists in Asia and the Pacific region on the use of relevant isotope and radiation techniques in research, to find ways by which soil fertility, nutrient uptake and water use efficiency of crops could be increased, particularly on acid soils. The course will provide both theoretical and practical knowledge on the use of radioactive and stable isotopes as well as radiation techniques in research on fertilizer and water use efficiency, plant nutrition, biological nitrogen fixation, carbon metabolism, soil chemistry and soil physics. Broad coverage of the techniques will be given through lectures, laboratory sessions, field/greenhouse experiments, calculation exercises, films and discussion groups. The language of the course is English.

The announcement and prospectus have been dispatched to the National Atomic Energy Authority or Government Office responsible for nuclear matters in the respective Member States in September 1994 and the closing date for applications was 30 November. The selected participants will be informed towards the end of January. For additional enquiries, please contact the Technical Officer, Saliya Kumarasinghe, Soil Fertility, Irrigation and Crop Production Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria.

Completed

1. **FAO/IAEA Regional Training Course on the Use of Nuclear Techniques in Soil Fertility and Plant Nutrition Studies (RAF/5/030). 6 June - 8 July 1994, Nairobi, Kenya,**

Technical Officer: Seth K.A. Danso; Course Director: F. Muchena

The course was organized by IAEA and FAO in co-operation with the Government of Kenya through the Kenya Agricultural Research Institute in Nairobi, Kenya. Twenty-one participants from 12 Member States of IAEA and FAO participated. The training course was a great success.

2. **FAO/IAEA Advanced Fellowship Group Training on Modern Nuclear Techniques in Soil-Plant Research for Sustainable Agriculture Development and Environmental Conservation.**
26 September - 21 October 1994, IAEA Laboratories, Seibersdorf, Austria

Technical Officer: Christian Hera; Course Director: Felipe Zapata

The main objective of this group training was to update the knowledge of local counterparts of IAEA Technical Co-operation projects and/or former participants of FAO/IAEA Training Courses on the ongoing and future project areas as well as specific aspects of the use of nuclear techniques in soil/plant relationship studies. Eleven participants took part in this training. In addition to IAEA staff, invited specialists participated as key-lecturers providing overview lectures, assisting in the design of isotope-aided experiments and interpretation of isotopic data, and in leading group discussions on selected topics of soil-plant research.

MEETINGS / WORKSHOPS

1. **The third FAO/IAEA Research Coordination Meeting of the FAO/IAEA CRP on Enhancing Soil Fertility and Crop Production by Better Management of *Rhizobium* (D1.40.06)**

(Scientific Secretary: Gudni Hardarson)

The third FAO/IAEA Research Coordination Meeting on "Enhancing Soil Fertility and Crop Production by Better Management of *Rhizobium*", was held at Laboratoire de Biologie Moléculaire des Plantes Supérieures (LBMPS) at the University of Geneva between 15 - 19 August, 1994. Professor W. J. Broughton, an agreement holder in the above programme, was the local organizer of the meeting. The meeting was attended by 30 participants; 8 contractors, 5 agreement holders. Twelve of the participants were from the University of Geneva, 1 from FAO Rome and 4 from FAO/IAEA. The programme included two and half days of presentations given by all participants on research performed during the past year, three half days of practical exercises, planning of future research and a workshop on the ^{15}N methodology. Many of the participants have already gathered several important results which can be published at the end of the present programme. The location of the meeting gave the participants the unique opportunity to get to know the research performed at the LBMPS and to learn practical aspect of molecular biology e.g. GUS-fusion, plasmid isolation, DNA sequencing and PCR of DNA and whole rhizobia. The laboratory in Geneva is very well recognized for its work on molecular biology of the legume/*Rhizobium* symbiosis.

Excerpts from presented reports:

i. *Do Nod-factor Levels Play a Role in Host-Specificity?*
B. Relic, C. Staehelin, R. Fellay, S. Jabbouri, T. Boller, and W.J. Broughton
LBMPS, Geneva, and University of Basel, Switzerland

Rhizobial Nod-factors are N-acylated oligomers of N-acetyl-D-glucosamine carrying a variety of substituents. At pico- to nano-molar concentrations, they provoke deformation and curling of nodulation competent root-hairs. Higher concentrations (10^{-7} to 10^{-6} M) elicit pre-infection structures, allow Nod$^-$ rhizobia to enter legume roots and to fix nitrogen within nodules. Evidence is accumulating that nodulation capacity is controlled both by specific Nod-factors as well as by Nod-factor concentrations. As an example, root-hairs of *Macroptilium atropurpureum* show identical "shepherd's crook" type curling when challenged with optimal (= 10^{-9} M) concentrations

of Nod-factors of *Bradyrhizobium japonicum* and *Rhizobium sp.* NGR234 (which are Nod$^+$) or with Nod-factors of *R. leguminosarum* and *R. meliloti* (both bacteria are incapable of nodulating *Macroptilium).* Similarly, conjugation of the *nodD1* gene of NGR234 into *R. meliloti* allows nodulation of *M. atropurpureum.* We have used alkanisation of the tomato cell growth medium as a quantitative bioassay for Nod-factors. Upon induction with flavonoids, wild-type *Rhizobium sp.* NGR234 produces abundant Nod-factors, which may partly explain its extremely broad host-range. Nod-factors are produced throughout the log-phase of growth, while mutations in *nodSU* drastically reduce Nod-factor synthesis. Good correlations exist between the levels of Nod-factors and the ability to nodulate specific legumes.

ii. Molecular responses of rhizobia in root-hairs.

A. Krause, and W. Broughton
LBMPS, Geneva, Switzerland

Developmental changes in the synthesis of root-hair proteins were followed to monitor whether *Rhizobium sp.* NGR234 is modulating the expression. Comparison of the 2-D electrophoresis patterns of proteins isolated from *Vigna* root-hairs inoculated with wild-type or Nod$^-$ mutants of NGR234 revealed 12 symbiosis-specific proteins. Synthesis of three of these symbiosis-specific proteins was repressed 4 days after inoculation, while synthesis of the remaining nine proteins was induced by *Rhizobium* 1, 2, or 4 days following treatment. Three of these were specifically and transiently expressed in root-hairs during the deformation process. Five proteins were first observed 24 h after inoculation. Two days after inoculation, three additional proteins became apparent, while another was visible on day 4. All 12 symbiosis-specific proteins seemed to be associated with root-hair deformation and nodule development.

To isolate genes involved in the early stages of nodule development, a cDNA library was constructed in kNM1149 of poly(A)$^+$ RNA isolated from root-hairs of *Vigna unguiculata* inoculated with *Rhizobium sp.* NGR234. This library was screened in four different ways to isolate putative symbiotic genes. A. Differential screening with probes synthesized from poly(A) RNA isolated from root-hairs treated with *Rhizobium sp.* NGR234, or with the Nod - mutant *Rhizobium sp.* NGRDD*nodABC.* No positive clones were obtained. B. A second differential screen was performed using probes synthesized from poly(A)$^+$ RNA of root-hairs or of roots. C. Nested PCR. D. Finally, by screening with heterologous probes. The isolated cDNA clones can be grouped into 1. clones coding for cell wall proteins like extensins, prolin-rich protein and glycin-rich protein. 2. clones homologous to defense genes coding for chitinases or chalcone synthase. 3. final clones coding for proteins with different function, like involved in the cell cycle or in lipid transfer.

Concentrating on the chalcone synthase gene, molecular analysis were started. The expression pattern of this multi copy gene was followed in all parts of the plant, showing that there were transcripts visible only in leaves and in symbiotically relevant tissue, like root-hairs and roots after inoculation as well as nodules. Accumulation of transcripts was inducible in root-hairs and roots through inoculation with *Rhizobium* sp. NGR234. The level of transcripts increased with the days after inoculation reaching a maximum in nodules. A transient expression was observed after treatment with a *nodABC* mutant in root-hairs, but not in roots. As potential regulator of gene expression in root-hairs the Nod-factor was shown as an important one, suggesting that induction of chs-gene expression can be correlated to root-hair deformation.

iii. Molecular ecology, diversity and host specificity of Rhizobium isolates that nodulate African species of Acacia

K. Haukka and P. Young
University of York, United States of America

In recent years the importance of finding efficient nitrogen-fixing, root and stem nodulating bacteria for use in tropical countries has promoted research on rhizobial species that infect

leguminous trees. A recent study carried out in connection with an afforestation program in Sudan showed convincingly that inoculation of the tree seedlings with efficient *Rhizobium* bacteria helped the roots to reach the depth of ground water faster thus improving survival of the plants in the harsh conditions. In our laboratory we aim to define clearly the taxonomy and genetic diversity of these rhizobia that nodulate African acacias, especially Sudanese *Acacia senegal* species, and the basis of their host specificity, using the methods of molecular genetics. The methods include 16S rDNA sequencing, PCR based restriction fragment length polymorphism, pulsed-field gel electrophoresis, and metabolic product analysis. The study should aid the development of African agroforestry as well as provide valuable fundamental information on the evolution of the legume-*Rhizobium* symbiosis.

iv. Genes of Vigna unguiculata regulated by attachment of rhizobia?
M. Shayya
LBMPS, Geneva, Switzerland

VuA, 1.5 kb, a cDNA clone extracted from root cortical cells of *Vigna unguiculata* cultivar Purple hull inoculated with *Rhizobium fredii* USDA 257 and shown to be nodulated very early after inoculation, was analyzed. VuA was hybridized with a cDNA library from mRNA of root hairs of *Vigna unguiculata* cultivar Red caloona inoculated with *Rhizobium* NGR 234. Out of seven cDNAs that hybridized with Vua, two cDNAs, VuAl and VuA3, 1.338 kb and 1.488 kb, respectively, were chosen to be studied because of their largest size and because they do not cross hybridize. Vual and Vua3 were sequenced and VuAl had a high proline content and had a conserved repitive motive of the amino acid residues Proline-Proline-Valine-Glutamine-Lysine and of Proline-Proline-Valine-Tyrosine-Lysine. The cDNA Vual was strongly homologous to a cell wall rich protein of *Glycine max* while Vua3 did not show any homology to any plant gene in the database.

v. Nod-factors of wild-type and overproducing NGR234 strains.
S. Jabbouri, C. Staehelin, R. Fellay, and W. J., Broughton
LBMPS, Geneva, Switzerland

The first characterization of the Nod factor was from *R. meliloti.* The extremely low production of these factors by the wild-type strain was enough for biological activity, but not for complete structural determination. Introduction of extra copies of regulatory *nodD* genes permits an increase in production of Nod-factors and the structural determination. Using over-producing strains, others workers have described minor products which supposedly play a role in host specificity. The relative abundance of the different Nod-factors in wildtype NGR234 is unknown. Certain *nod* genes involved directly in the biosynthesis of Nod-factor are not regulated by nodD genes, for example the nodZgene in *B. japonicum.* The initial characterization of NGR234 - Nod-factors was from an overproducing strain obtained by the introduction of extra copies of the *nodD1* gene. We have observed that wild type NGR234 produces abundant Nod-factors. The difference in Nod-factor production between wild type and overproducing NGR 234 strains, as well as the structural difference in Nod-factor produced by these two strains are small.

vi. Nod-factors of Rhizobium are a Key to the Legume Door
B. Relic and W.J. Broughton
LBMPS, Geneva, Switzerland

Symbiotic interaction between rhizobia and legumes are largely controlled by reciprocal signal exchange. Legume roots excrete flavonoids which induce rhizobial nodulation genes to synthesize and excrete lipo-oligosaccharide Nod-factors. In turn, Nod-factors provoke deformation of the root-hairs and nodule primordium formation. Normally, rhizobia enter roots through infection

threads in markedly curled root hairs. If Nod-factors are responsible for symbiosis-specific root hair deformation, they could also be the signal for entry of rhizobia into legume roots. We tested this hypothesis by adding NodNGR-factors to signal production deficient mutants of the broad host-range *Rhizobium sp.* NGR234 and *Bradyrhizobium japonicum* strain USDA110 at inoculation. 10^{-7} M to 10^{-6} M NodNGR-factors permitted these NodABC$^-$ mutants to penetrate, nodulate and to fix nitrogen on *Vigna unguiculata* and *Glycine max*, respectively. Furthermore, NodNGR-factors allowed *R. fredii* strain USDA257 to enter and fix nitrogen on *Calopogonium caeruleum*, a non-host. Detailed cytological investigations of *V. unguiculata* showed that the NodABC$^-$ mutant, in the presence of NodNGR-factors, entered roots in the same way as the wild type bacterium. Since infection threads were also present in the resulting nodules, we conclude that Nod-factors are the signals which allow rhizobia to penetrate legume roots.

vii. Root hair deformation and curling in Macroptillium induced by a compound produced by E coli.

D. Quesada, S. Jabbouri, and W.J. Broughton
LBMPS, Geneva, Switzerland

E. coli produces a compound which has a biological activity on *Macroptillium* root hairs (hair deformation and hair curling). We tried, to identify which type of molecule causes this activity, and if this compound is similar to nod factors, or not. This compound was extracted on a XAD column using methanol as eluant. The methanolic phase was fractioned on a preparative reverse-phase C18 column HPLC. The active fraction, on *Macroptillium*, was further purified on a propylamin direct phase column HPLC. Its UV absorption spectrum showed two peaks at 234 and 276 nm. These absorptions and the chemical displacements in 13C NMR suggest the presence of aromatic ring or conjugated bonds and/or carbonyl group. The TLC indicates that this compound does not contain any saccharidic part.

viii. Chitinases of Vigna unguiculata root hairs

V.T.T. Lan, W.J. Broughton, and A. Krause
LBMPS, Geneva, Switzerland

Plant chitinases, which have been studied extensively in plant pathogen interactions, are able to hydrolyze internal p-1,4 linkages of chains of N-acetylglucosamine. This compound is found in chitin, a major component of most fungal cell walls, as well as in Nod factors, a signal molecule secreted by rhizobia, which induces root-hair deformation, root-hair curling and nodule formation. Using biochemical and molecular techniques, we tried to identify the role of chitinases in the interaction between the broad host-range *Rhizobium* sp. NGR234 and the widely compatible legume *Vigna unguiculata*. Interestingly, the specific chitinase activity is much lower in *Vigna* roots and root-hairs than in *Phaseolus* roots. No changes in activity could be observed after inoculation with *Rhizobium*. On chitinase activity gels, the protein pattern was identical in roots and root-hairs with and without treatment with *Rhizobium*, but an additional chitinase was visible in nodules. Using nested PCR with degenerated primers, cDNA clones were isolated from a root-hair cDNA library, which after sequencing were shown to code for chitinase class I, class III and class IV. Sequence analysis showed that the clones from *Vigna* were only 60% to 80% homologous to *Phaseolus* chitinase genes. Southern blots showed that the genes coding for the different chitinases were single genes. Northern blot analysis of all three cDNA clones underlined the above mentioned results. No activation in transcription of any chitinase genes was detected from roots or root-hairs after inoculation by *Rhizobium*. Further expression studies showed that one transcript is observed in root-hairs treated with ethylene.

ix. *Identification of new symbiotic loci in Rhizobium sp NGR234.*
M. Hanin, R. Fellay and W. J. Broughton
LBMPS, Geneva, Switzerland

Usually, in different rhizobia, new symbiotic loci were isolated by transposon mutagenesis and/or complementation. In *Rhizobium* sp NGR234, wide-spread dispersal of the symbiotic loci as well as the large number of the host plants, reduces greatly the efficiency of these techniques. As an alternate strategy to identify new symbiotic genes in this strain, we combine two techniques: competitive RNA hybridization and shot-gun sequencing. Competitive RNA hybridization technique reveals flavonoid inducible transcripts carried on the fragments from the canonical ordered cosmid library of the symbiotic plasmid. Shot-gun sequencing of these fragments and search for homologies in protein and DNA data bases, allow us to identify genes as well as their putative functions.

x. *Use of Rhizobium as plant growth promoting rhizobacteria for non-legumes*
R. Chabot, N. Goussard, C.J. Beauchamp, and H. Antoun
Laval University, Quebec, Canada

Many evidences in the literature indicate that rhizobia can perform many of the beneficial characteristics found in other plant growth promoting rhizobacteria (PGPR). We have determined the production of siderophores, indole acetic acid (IAA), HCN and the solubilization of inorganic phosphate by 266 different strains of rhizobia and bradyrhizobia. The majority of the strains (83%) produced siderophores, while 58% produced IAA, and 54% were P-solubilizers. Only 8 strains (3%) produced HCN.

In a greenhouse assay, we have observed that inoculation of radish seeds with some strains of rhizobia or bradyrhizobia increased plant dry matter yield. Yield increase varied from 50% to 103% higher than the yield of the uninoculated control. Some strains of the legume nodule bacteria, behaved as deleterious micro-organisms with radish. In fact reductions in dry matter yields ranging from 19 to 44% were observed with some strains.

Field assays were performed with P-solubilizing strains of *R. leguminosarum* bv. *phaseoli* (strains P31 and P121) and R. *leguminosarum* bv. *viciae* (strain Tal 1402). Among all bacteria tested, these 3 strains were the most effective inorganic P solubilizers. In a soil rich in P, strain P121 significantly increased the dry matter yield of lettuce and the P-content of the shoots. In soils poor in P, strain P31 increased the dry matter yield of lettuce when half the recommended level of P fertilization was applied. Under the same conditions, strains P31 and P121 significantly increased P content of maize shoots.

Our results indicate that some strains of rhizobia and bradyrhizobia can be used as PGPR with non-legumes.

xi. *Selected studies on Rhizobium and Azospirillum ecology in the Philippines.*
J.C. Mamaril
National Institute of Biotechnology, Laguna, Philippines

Field experiments were conducted to study the populations of indigenous and introduced strains of bradyrhizobia in soil and rhizosphere in a soybean-rice cropping sequence. Antibiotic resistant mutants of *B. japonicum* strains USDA 110 were used in the two soybean crops.

Indigenous bradyrhizobia population in the soil increased 100-fold from planting to harvest of the first soybean crop while the introduced inoculant, USDA 110 SpcRif recovered from the soil was 100 times lower. Counts of bradyrhizobia in the rhizospheres were 200 times higher than in the soil for both indigenous and introduced strains. Bradyrhizobia number in flooded soil declined during rice growth but remained higher in the rice rhizosphere.

During the growth of the second soybean crop, the first crop inoculant fluctuated from 100-10,000/g dry soil in the presence or absence of the second crop inoculant, USDA 110 StrRif, whose number equilibrated around 1,000/g dry soil. In the rhizosphere both inoculants maintain a population of 10^6/g dry root in the presence or absence of competition.

The first crop inoculant formed 64% of the nodules of the initial soybean crop and 25% of the nodules of the second soybean crop. With reinoculation, the first crop inoculant occupied only 9% of the nodules while the second crop inoculant dominated nodule occupancy by 46%. Results show that introduced bradyrhizobia survived flooding of soil and persisted up to the next soybean crop after rice but failed to dominate nodule occupancy. Inoculation of every soybean crop after rice is therefore necessary.

xiii. Using Molecular Biology to Detect Rhizobia in Agro-Ecosystem

N. Teaumroong and N. Boonkerd
Nakhon-Ratchasima, Thailand

For preliminary study, 23 strains of *Bradyrhizobium japonicum* were identified. Using serological method one could classify them into 4 serogroups and 4 uncross related strains.

By using restriction pattern of chromosomal DNA utilizing restriction enzymes Eco RI, Hind III, Pst I and Bam HI one could classify the 23 *B. japonicum* strains into 5 groups and 4 individual pattern of USDA 7, USDA 94, USDA 142 and TAL 432 which was similar to serological technique, ELISA. Utilizing nif HDK hybridization after Eco RI and Pst I digestion it was possible to classify those strains into 4 and 5 groups, respectively. The individual ungrouped strains by ELISA and restriction pattern could be grouped into hybridization pattern (RFLP) group I for strains USDA 142 and TAL 432 and into group 5 for strains USDA 76 and USDA 94. By using *nod* ABC and D hybridization after Bam HI digestion of those strains, except USDA 35, USDA 184 and TAL 377, 8 groups of rhizobia were formed.

In evaluating the relationship of Southern blot hybridization of *nif* structural gene and common *nod* genes RFLP and the effectiveness in nitrogen fixation of *B. japonicum* strains, we found no relationship. There were no statistical differences in nitrogenase activities among strains. Most strains could nodulate in high number, 20 - 40 nodules on S.J. 5 soybean cultivar. Nodule formation produced two different patterns, tap root and lateral root nodulation. We also found no relationship between nitrogenase activity and *nif* HDK grouping; and between nodulation and *nod* ABC, D probing indicating the effectiveness in nitrogen fixation and nodulation were not dependent on *nif* and common *nod* gene only.

The future plans will focus on the methodologies such as DNA-extraction from soil, MPN-DNA hybridization and PCR technique by using molecular probe to detect rhizobia in ecosystems. These will provide the possibility to gather information on their survival and competition.

xiv. Symbiotic nitrogen fixation contribution and limitations in Phaseolus bean in Romania

A. Popescu
Fundulea, Romania

Phaseolus bean usually achieves grain yields less than genotypic potential of varieties under Romanian field condition due to various limiting factors. For a better understanding of the contribution of the symbiosis to the yield formation, a long term (1977-1993) evaluation of inoculation effect of 19 selected *R. leguminosarum bv. phaseoli* strains on *Phaseolus* bean cultivated in several locations with distinct pedoclimatic conditions has been made. The grain yields were significantly influenced by all selected strains, years, locations and by the interaction between years and locations in all experimental cycles and only partially by the interactions

between strains and years or locations. Mean grain yields and variation limits recorded along the rhizobial strain evaluation in locations with different soil pH values showed similar patterns of yield level increases from the acidic to neutral pH values. Linear regression between mean grain yields and average temperatures recorded during flowering and pod-filling stages in the experimental locations demonstrates the limiting effect of this factor on yield formation. The curves expressing the accumulated effect of the mean temperatures during flowering and pod-filling stages and the total amounts of rainfalls along the vegetation period on grain yields obtained in non-inoculated and inoculated treatments follow the yearly characteristics of favorability for *Phaseolus* bean crop in every location.

The influence exerted on plants by the interaction between bacterial strains and progressive increased nitrogen fertilization rates, studied along three years in two locations with similar climatic conditions, but different soil characteristics, indicate that in some conditions, higher and more stable yields could be obtained by associating the selected strains with low nitrogen fertilization levels.

An evaluation of N_2-fixed amounts in three *Phaseolus* bean varieties, inoculated with two bacterial strains and cultivated on two N-fertilization levels suggested differences between plant genotypes in supporting nitrogen fixation.

xv. Design and application of rRNA-targeted specific hybridization probes.

W. Ludwig and R. Amann
München, Germany

Comprehensive analyses of ribosomal ribonucleic acids (rRNA) have proven these molecules to be not only reliable phylogenetic markers but also excellent targets for specific hybridization probes. The structures of rRNAs contain evolutionary highly and less conserved regions which provide phylogenetic information on higher and lower levels of relatedness, respectively. Concurrently, the differently conserved primary structure elements often contain diagnostic stretches which represent useful target sites for group and species specific hybridization probes.

A variety of hybridization and detection techniques are available to perform rapid analyses of pure and mixed cultures, enrichments of microorganisms and even complex environmental samples. Probing of purified nucleic acids applying conventional fluid and solid phase procedures allows qualitative and quantitative analyses with respect to the presence of species or groups of organisms and the fraction of their rRNA genes, respectively. The sensitivity of qualitative analyses can be substantially improved by polymerase chain reaction (PCR) based amplification of the target nucleic acids (rDNA). The so called reverse dot blot probing approach allows the use of a large number of immobilised probes of different specificities in combination with *in vitro* amplified target rDNAs. Colony-forming units can be determined for the particular specificity groups applying *in situ* colony hybridization techniques. Currently, the most attractive probing approach is whole cell hybridization. Individual fixed cells are specifically stained by intracellular probe hybridization and their abundances and spatial distributions can be microscopically monitored *in situ* even within complex samples.

The latter technique usually depends on the use of fluorescent probes. Sensitivity and specificity of the approach are often hampered by interfering autofluorescence of sample components especially while probing complex environmental samples such as soil and plant materials. Alternative nonfluorescence-based detection systems have been developed and currently are further improved. Computer-aided image analysis and the application of confocal laser scanning microscopy were introduced as valuable tools improving the efficiency of whole cell hybridizations with specific probes.

Group and species specific rRNA targeted probes are under development or testing for nitrogen fixing, plant associated rhizobia, bradyrhizobia, cyanobacteria, and their phylogenetic neighbors as well as for other organisms reported to be important members of rhizosphere populations. Given the complexity of the habitat, emphasis is given to the development of group specific probes.

xvi. Application of subtraction hybridization for the isolation of species- or strain-specific Rhizobium DNA sequences.

J.E. Cooper and A.J. Bjourson
The Queen's University of Belfast, United Kingdom

A previously reported DNA subtraction hybridization and amplification procedure (Soils Newsletter 16 (1) p9, July 1993) has been used in three different applications for the isolation of unique nucleic acid sequences from rhizobia: 1) isolation of a genomic DNA probe which hybridizes specifically to one *R. leguminosarum* bv *trifolii* strain from a group of eight strains of this biovar; (2) development of a *R. leguminosarum bv phaseoli/R.tropici* group-specific DNA probe which distinguishes these two organisms from other *Rhizobium/Bradyrhizobium* biovars and species and which does not cross hybridize with DNA from other common soil bacteria (in collaboration with Professor D. Werner and Dr W. Streit, Philipps University, Marburg); (3) isolation of new, symbiotically active loci from the broad host range *Rhizobium sp.* NGR234 (in collaboration with Professor W.J. Broughton, Dr X. Perret and Dr R. Fellay, LBMPS, Geneva,).

Current research is directed towards adaptation of the subtraction hybridization system for the purpose of isolating *Rhizobium*-induced plant mRNA (after conversion to cDNA) from localized regions of root tissue during the very early phases of symbiotic interaction.

xvii. Strategies to improve the symbiosis of bean rhizobia.

E. Martinez-Romero
Cuernavaca, Mexico.

Phaseolus vulgaris bean forms symbiotic nitrogen fixing nodules with different *Rhizobium* species: *R. etli*, *R. tropici* and *R. leguminosarum* bv. phaseoli. Different strain constructions have been made and by this way we have identified genes that improve symbiotic abilities: e.g. *nod*PQ genes that participate in the addition of sulfate to Nod-metabolites, and a *citrate synthase* gene that could provide citrate as an iron chelator.

xviii. A class of root-hair specific extensins involved in Rhizobium/Legume interactions.

I. Arsenijevic-Maksimovic, W.J. Broughton, and A. Krause
LBMPS, Geneva, Switzerland

Root-hairs of leguminous plants play an important role in recognition between plants and microorganisms. Presumably, changes in regulation of root-hair specific genes are an early and essential symbiotic step. In order to identify *Vigna unguiculata* root-hair specific transcripts, differential screening of a root-hair cDNA library was performed. Of 30 possibly unique clones, 20 strongly cross-hybridized with one another. Five of the latter clones were sequenced. All possessed the repetitive motif KSP4SPSP4Y3 which is characteristic of extensins, a class of structural cell-wall proteins. Another cDNA clone, isolated by screening the same bank with the heterologous probe - *ext*12-7, displayed the almost identical motif KSP4SPSP4YVY. A large gene family encodes extensin-like proteins in *Vigna*. Transcripts homologous to cDNAs *ext*3, *ext*12-7 and *ext*26 were only detected in root-hairs. *Rhizobium sp.* NGR234 caused a decrease in their mRNA levels as early as 1 day after inoculation. mRNA levels of extensin-like transcripts were reduced to 10% (ext3 and ext26) or to 30% (ext12-7) following inoculation with wild type NGR234, and to 50% (ext3 and ext26) or to 75% (ext12-7) after inoculation with the NGR234 *nodABC* mutant, suggesting that all three extensin-like genes play a symbiotic role. Application of NGR234 Nod factors, significantly reduced *ext*12-7 transcript levels, but had no effect on the amounts of mRNA's homologous to ext3 and ext26. Kinetin increased the levels of all three

transcripts, while BAP affected each transcript differently. Similar and variable results, were obtained with GA_3 and NAA. Thus, none of the potential regulators of gene expression in root-hairs fully mimicked the effect of the wild-type bacterium. The genomic locus of ext26 has been isolated and is being analyzed.

xix. Inducible nodulation genes of Rhizobium sp. NGR234

R. Fellay
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In a systematic approach to identify genes involved in the early steps of the legume-*Rhizobium* symbiosis, we studied transcription patterns of symbiotic plasmid-borne loci. A competitive hybridization procedure was used to identify DNA restriction fragments carrying genes, whose expression is enhanced by plant root exudates or by purified flavonoids. The fragments with induced genes were then located on the physical map of the 500 kb pNGR234a. New inducible loci as well as previously described genes were identified and their time course of induction determined. After initial induction, transcription of loci such as *nodABC* and the host specificity genes *nodSU,* decreased to undetectable levels 24 h after incubation with purified flavonoids. In contrast, expression of other loci is detectable only after several hours of induction. Surprisingly, many genes remained transcribed in the *nodD1*⁻ mutant suggesting the presence of other flavonoids dependent activators in NGR234. Hsnl region, involved in host specificity, was shown to carry several induced transcripts independently regulated. Sequencing analysis revealed several open reading frames whose products, based on sequence similarity, may be involved in the L-Fucose metabolism and its adjunction to the Nod factors.

xx. The agronomic significance of native populations of R. leguminosarum bv phaseoli in Mexico.

J. J. Peña-Cabriales
Irapuato, Mexico.

Response to inoculation of common bean (*Phaseolus vulgaris*) in Mexico has not been consistently successful. This failure seems to be associated, among other factors, to the high competitive ability of the indigenous rhizobial populations which occur in high numbers and are also very diverse in the soils of the most important bean producing area of Mexico, i.e. the states of Zacatecas and Durango.

Field experiments were conducted with the aim of increasing the number of nodules produced by inoculant strains. Rhizobial strains resistant to high concentrations of antibiotics were used. The results showed that by inoculating the soil as well as the seed the proportion of nodules produced by the introduced strains was enhanced. However, this proportion was never higher than 10% of the total number of nodules produced. Differences among the plant genotypes tested were also observed.

Studies to evaluate the role of supplemental inoculation, as well as other means to increase nodulation by inoculant strains are in progress.

xxi. Ribosomal RNA analysis to detect and quantify Frankia and other micro-organisms

A.D.L. Akkermans
Wageningen, The Netherlands

Current ecological studies on N_2-fixing organisms are often hampered by the lack of reliable methods to detect and to quantify microbial populations in soil. This is particular relevant for microorganisms that are difficult to isolate and/or to quantify, such as the N_2-fixing, nodule-forming actinomycete, *Frankia.*

One approach to study the ecology of these organisms is to analyze and to quantify group-specific 16SrRNA sequences. We have demonstrated the significance of this approach by analyzing the 16SrRNA sequences of Frankia and other Gram+ microorganisms (Segmented Filamentous Bacteria, Clavibacter, Microthrix) that are currently studied in our research group.

A description was given of the development and use of oligonucleotide probes that recognizes 16SrRNA sequences of *Frankia* in root nodules of *Coriaria nepalensis* and *Datisca cannabina* in Pakistan. Although we were unable to isolate the endophytes of these nodules, we have characterized these organisms on the basis of 16SrRNA sequences. *D. cannabina* could be infected by inoculating with crushed nodules of either *D. cannabina* or *C. nepalensis*. By sequencing the PCR amplifies of the 16SrDNA from the nodules we have demonstrated that *D. cannabina* can be nodulated by both the endophytes of both species.

The biodiversity of *Frankia* in nodules collected in different parts of Pakistan has been described on the basis of sequences of PCR-amplifies of the 16S rRNA gene.

A new approach to quantify the different groups in the microbial community on the basis of 16SrRNA sequence diversity has been described. A method to collect 16SrRNA from a soil system has been proposed. Quantification of the major components (10^6cells/g) was possible by quantifying hybridization signals with a Phosphor Imager. For lower numbers the reverse transcriptase PCR approach was used. This method allowed us to decrease the detection level 100 times. Initial results were reported on the use of the PCR System 5000 (Perkin Elmer) for quantifying small numbers of PCR-fragments with an electrochemoluminescent marker mixtures of low numbers of *E. coli* and *Pseudomonas aeruginosa* 16SrRNA molecules.

xxii. Application of molecular biology techniques for studying competition between rhizobial strains.

H. Moawad A. Al
Cairo, Egypt

The diversity of large collection of the rhizobia nodulating soybean, beans and lentil was investigated using intrinsic antibiotic resistance, serological markers and PCR. The results indicated wide diversity of symbiotic efficiency among indigenous rhizobial populations of lentil and bean nodulating strains. In some regions it was clear that the symbiotically less efficient strains were dominating. In general, large percentage of indigenous rhizobial populations was not fixing as high nitrogen as compared with N fertilized control plants. This shows the need to increase the balance of highly effective strains within the pool of rhizobial population through the use of high quality inoculant strains that can survive and compete with other less desired rhizobia for the target legume.

The use of PCR in studying competition for nodulation among soybean bradyrhizobia gave promising results in understanding the intraserogroup competition. Using the same technique showed the diversity among the soybean, common bean and lentil rhizobia based on the PCR fingerprinting. This will allow better understanding of competitive interaction between rhizobial strains for nodulation of legume hosts.

xxiii. Using molecular markers to determine evolutionary and ecological host-Rhizobium interactions in common bean

S. M. Tsai
Piracicaba, Brazil

Several biochemical, physiological and molecular alterations may have occurred throughout the common bean domestication period, so that a functional symbiosis between bean-*Rhizobium* was possible to occur at present. Environmental changes may have influenced the *Rhizobium*

establishment in the host rhizosphere, and studies with the bean host suggest the increased adaptability of its *Rhizobium* under tropical conditions, such as Brazil. Studies developed with *R. tropici* in Brazil have shown a high predominance of *R. tropici* over *R. etli*, the other *Rhizobium* which nodulates specifically bean plants. Furthermore, *R. etli* "prefers" the Mesoamerican group of bean, whereas *R. tropici* the Andean bean plants. Other fact, involving the nodule proteins - nodulins, important for the development, structure, maintenance and general metabolism of the nodule also contribute to reinforce the possibility that two distinct co-evolution events have occurred in the Middle America and in the Andes for the bean-*Rhizobium* symbiosis. We have indications from RFLP (Restriction Fragment Length Polymorphism) data that leghemoglobin, considered a late nodulin highly conserved among cultivated legumes, was shown to be highly polymorphic only when wild and domesticated materials are compared.

Data from elite N_2-fixing cultivars also suggest that *Rhizobium* can develop an efficient symbiosis with his bean host, through increased activity of nodulins. High levels of nitrogenase activity and allantoin were also present in those cvs. The yield capability and biological nitrogen fixation (BNF) were positively correlated at semicontrolled field conditions, under low level of N-mineral, and using ^{15}N-enriched soil N. Puebla-152 and Mexico-309 were capable of yielding as high as 3200 kg/ha and 2700 kg/ha, respectively, with the highest amounts of 42 kg N/ha and 38 kg N/ha from BNF.

xxiv. Hyper-saline protection of diazotrophs from saline soils of Pakistan
J. A. Qureshi
Faisalabad, Pakistan

Klebsiella salinarium was previously isolated from the rhizosphere of Kallar grass growing in a hypersaline environment. Western blot data indicate that this diazotrophic organism has a *proU*-like transport system for glycine betaine, the most effective exogenous osmoprotectant for this and many other microorganisms. Other osmoprotectants such as glutamate, glycine, and proline also enhanced growth and nitrogen fixation activity when *K. salinarium* was grown under hyperosmotic conditions. *K. salinarium* contains a plasmid pNIAB-1, of about 50kb that confers salt-tolerance on *E. coli*, HYO47, a salt sensitive strain that is deficient in the uptake of osmoprotectants. HYO47/pNIAB-I was rescued from salt inhibition when minimal medium was supplemented with glycine betaine, suggesting that pNIAB-1 carries genes specifying a transport system. NMR analysis supported the above observation, showing that HYO47/pNIAB-1 could accumulate high levels of glycine betaine and proline when they were supplied in the medium. Plasmid pJK21, which contains a 1.9kb fragment of pNAB-1, confers the same salt-tolerance as the entire plasmid. Western blot data indicates that the plasmid-encoded glycine betaine system is not related to proU. Southern blots confirmed the absence of homology to proU or proP, which suggests that a novel glycine betaine transport system is encoded on pJR21. Two proteins of Mr 10,000 and 35,000 were strongly expressed from pJK21 in an E. coli S-30 coupled transcription/translation system.

2. The First FAO/IAEA Research Co-ordination Meeting on "The Use of Nuclear Techniques for Optimizing Fertilizer Applications Under Irrigated Wheat to Increase the Efficient Use of Nitrogen Fertilizers and Consequently Reduce Environmental Pollution". 3-6 October 1994, Vienna, Austria.

Scientific Secretary: Pierre Moutonnet

This is the first RCM organized under this CRP. Fourteen scientists from 12 countries, including contractors and agreement holders, participated in this meeting. Results of the first phase of this programme were presented and follow-up experiments planned.

Excerpts from presented reports:

Ç. Kirda, R. Kanber, M.R. Derici and A. Yazar
University of Çukurova
Adana, Turkey

In this project two irrigation treatments will be superimposed on four nitrogen treatments taking advantage of the continuous gradient characteristics of the line source irrigation system. The experimental area is situated in Southern Turkey (subtropics). Soils in the area contain significant amounts of smectite type clay minerals (Vertisols). The elevation of the experimental site is 40 m and its latitude and longitude are 36° 30' North and 37° 40' East, respectively. The project will commence in October 1994.

A.E. Boaretto, T. Muraoka, P.C.O. Trivelin and J.C. Chitolina
University of Sao Paulo, CENA
Piracicaba SP - Brazil

Brazil almost reached self-sufficiency in wheat production in 1987. However, since then the cultivated area and production has been decreasing from 6 million tonnes in 1987 to about 2 million tonnes in 1993. Two factors have contributed to this: reduction in the cultivated area and in the productivity. With respect to water management, wheat has been grown in Brazil without irrigation, mainly in Parana state, where climatic conditions are favourable. Sprinkler irrigation is used in the centre west region. Recent studies show a good response to nitrogen fertilization, but this response depends on the previous crop. When a green manure crop is cultivated before, there is no response to nitrogen, as against a good response when rice is grown as a prior crop. Nitrogen is applied at planting (20 kg/ha of N along with phosphorus and potassium). In irrigated treatment, nitrogen ranged from 0 - 120 kg/ha, applied in one single application for lower doses and split twice with higher doses. The potential yield is approximately 5 tons.

The objective of our project is to study the effect of seeding and N fertilization on the recovery of N by wheat crop, under irrigated and non-irrigated conditions, using ^{15}N labelled fertilizers.

M.I. Khalil
Soil Science Division, Bangladesh Institute of Nuclear Agriculture
Mymensingh 2000, Bangladesh

In Bangladesh, wheat ranks the second staple, in terms of both area and production and about 85% of the total wheat area follows rice crops. Rice-Wheat or Rice-Rice-Wheat is the major cropping system in Bangladesh. Climate is dominated by tropical monsoon and about 85% of the total rainfall occurs during autumn season (June-September) and a small portion in wheat growing season (November-March). The soils of Bangladesh are mostly occupied by floodplain (80%), hill (11%) and terrace (8%) and they are generally poor in nitrogen, organic matter and cation exchange capacity. Cultivation of wheat has been expanded with varying irrigation regimes and fertilizers application. The increasing levels of irrigation and nitrogenous fertilizers being used, might pollute the soil and water resources.

The present research programme is initiated to make an attempt to reduce environmental pollution through judicial use of irrigation water and balanced application of nitrogenous fertilizer and also for increased yield potential of wheat and sustained productivity. Moreover, field experiments will be conducted to generate information both qualitative and quantitative regarding application of nitrogenous fertilizer and irrigation water with emphasis on the enrichment of nitrate-nitrogen in soil and water resources. The generated information will be used for simulation of CERES-wheat model. The project works are in progress.

D. Abdel Monem
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Cairo, Egypt

The experiment is conducted in the experimental station of the Faculty of Agriculture, Cairo University at Giza at latitude 30° 02', longitude 31° 13' and altitude 18 m. The area available for the experiment is 6000 m^2. The rotation practiced in the site is wheat-corn. Although the experimental farm has its own management with respect to rotation, irrigation and different other agronomic practices, in our selected site specific treatments will be followed according to general recommendations. As located in the rain-less area of the Great Desert of North Africa, the climate of Egypt is extremely arid, with hot summers and mild to cool winters. Although the northern part of the country is affected by the Mediterranean climate, where rainfall could be as high as 200 mm, it decreases to reach 25 mm at Giza.

Soil of the site was classified as Typic Torrifluvents, representing large areas of the Nile Valley. Soil profile was established at the site. Water content at saturation is 52%, 36% at field capacity, 18% at wilting point. Bulk density ranges between 1.15 and 1.40 g/cm^3, and porosity ranges between 0.57 and 0.47. Surface irrigation from the Nile Valley is the main irrigation system in the Delta; meters will be fixed on the distribution canal to measure the exact amount of irrigation water. Water samples were collected for analysis; it was found pH=8.2, E.C.=0.32 m.mhos/cm, total salt content=210 ppm and (NO_3-N)=0.81 mg/l.

M.S. Sachdev
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A field experiment has been laid-out in the Main Block-8C of the Institute farm to evaluate the fate of applied nitrogen fertilizer (urea) to wheat crop under irrigated conditions using ^{15}N techniques and neutron moisture meter. As per the recommendations of the Consultants meeting, the field experiment has been established and a preceding crop of maize was planted in the monsoon season (July-September 1994) with a uniform fertilizer N application (120 kg/N/ha, applied in three equal splits) and same amount of irrigation. The normal recommended doses of P and K, and plant protection measures are being followed. The site of the field experiment is at latitude 28.4°, longitude 77.1° and altitude 218 m above mean sea level. The soil of the experimental field has been classified as Coarse Loamy non-acid hypothermic family of typic Ustochrepts and belongs to Mehrauli Series.

Urea fertilizer will be applied in two splits with four rates from 0 to 180 Kg N/ha. One-third at planting and two-third at maximum tillering stage. In each of the N treated plots ^{15}N micro-plots of size 1.0 m x 1.0m (1 m^2) will be established by partitioning with corrugate plastic sheets to a depth of 40 cm. Two plots of size 6m x 4m have been made side by side for each N treatment with three replications. One plot will be used for soil and plant sampling and the other containing micro-plot will be used for grain, biomass and N yield and ^{15}N determinations. In each of the yield plots neutron moisture meter access tubes will be installed before wheat planting to a depth of 2.0m for periodically recording the soil water content. In each of the micro-plots two soil solution samplers will be installed at 90 cm and 120 cm depths. In each N treated yield plots, a set of tensiometers will be installed at 30, 60, 90, 120, 150 and 180 cm depths to record the water gradient during the entire wheat growth period.

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In China, the annual output of nitrogen of fertilizers is 45 million tonnes, among which about 50% is ammonium bicarbonate. About 35 million tonnes of nitrogen fertilizers are applied to wheat production. The nitrogen uptake efficiency of ammonium bicarbonate is around 25% because ammonium bicarbonate is a nitrogen fertilizer which volatilizes easily. Therefore it is necessary to study the effect of fertilizer placement in relation to irrigation on yield and nitrogen balance, which could bring about a large economic and social benefits for the country. The objectives of this experiment are as follows: (i) To study the effect of fertilization and irrigation on the nitrogen uptake efficiency and wheat production under local management; (ii) To explore the movement of nitrate and its contribution to environmental pollution; (iii) To set up a package for optimizing fertilizer application. The results from field trials with ^{15}N labelling in 1994 showed that winter wheat took nitrogen continuously from fertilizer during the period of vegetation. When soil was irrigated immediately after application of urea, the nitrogen uptake efficiency at mature stage was 48.8%, higher by 9.4% and nitrogen loss decreased by 28.5% than the corresponding ones without immediate irrigation after application. The nitrogen uptake efficiency was only 22% when ammonium bicarbonate spread on the soil surface without immediate irrigation after application, while it was 29.3% with immediate irrigation after application. Nitrogen loss decreased by 14.0% .

J.M. Sanchez-Yañez
University of Guanajuato
Irapuato, Mexico

The propose of this first step was to determine the wheat's response under irrigation to the inoculation with some nitrogen fixing bacteria (NFB) in soil with high organic matter content (OM). A glasshouse experiment was conducted using Leornard's jar containing soil poor in nitrogen. Wheat seeds were inoculated with *Azospirillium lipoferum* (*A.l.*), *A. brasilense*, (*A.b.*) a mix of A.l.-A.b. (1:1) in non sterile soil and *Azotobacter beijerinckii* (Az.b.), under sterile condition, using in all cases ca. 3.0×10^3 bact/seed. The responses were evaluated on the two growth stages of wheat (tillering and early stem extension). The results suggest that in general NFB stimulated both stages in comparison to the controls with and without N. They also influenced the growth of the plant more than the OM alone. The bacteria may increase the dry matter of wheat by producing growth promoting substances and improving the mineral uptake. These results will be confirmed on the field experiments using N^{15}.

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Agricultural and Zootechnical Research Station
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In Romania there is a vast network of research stations under the coordination of the Research Institute for Cereals and Industrial Crops (Fundulea) and long term stationary fields have been set up, where investigations are being carried out on the effect of chemical and organic fertilizers on yields of main crops. The results obtained in experimental fields have shown that the effectiveness of N utilization in wheat is most variable and generally low, often ranging between 25 and 33% owing to the N losses within the system through leaching, NH_3 volatilization and denitrification. The most common method is sprinkler irrigation which is used on 2,700,000 ha. Since wheat is cropped on large areas in Romania, great stress is laid on increasing the economic efficiency of

this crop. As on irrigated wheat large amounts of N fertilizers are applied, the producers are facing problems in connection with their application such as: lodging, foliar diseases, loss through leaching.

These long-term studies using nuclear techniques (^{15}N and neutron gauge respectively) offer the possibility of achieving rigorous and valuable scientific results that will contribute to solving the problems mentioned above.

R.J. Rennie
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H.J. Janzen, E.M. Bremer, K. Volkmar
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Plant growth-promoting rhizobacteria (PGPR's) associated with Canadian wheat have been shown to increase yield due to associative asymbiotic N_2 fixation and also due to enhancement of nutrient uptake. However, amounts of N_2 fixed are low (approximately 10% of plant N yield) and are not sufficient to have a dramatic agronomic effect. The ability of PGPR's to enhance plant growth by means other than N_2 fixation has received considerable attention. Kucey used ^{32}P-labelling techniques to show that inoculation with *Azospirillum brasiliense* altered the rooting pattern of wheat but that inoculation with *Bacillus* C-11-25 did not. He concluded that the yield benefit of *A. brasiliense* inoculation increased nutrient uptake while that of *Bacillus* C-11-25 increased N_2 fixation. Our scientific hypothesis is simple: if PGPR's could stimulate larger, healthier wheat roots, the plant would literally "see"more moisture and nutrients. If these two factors are rate-limiting, inoculating with PGPR's may increase seed yield.

The first part of the paper described the effects of inoculating Katepwa cultivar in an attempt to stimulate greater uptake of soil and fertilizer nutrients. The second part described preliminary ^{15}N research in collaboration with Agriculture Canada (Lethbridge) to document alteration of roots and/or increased fertilizer use efficiency (FUE) due to inoculation with the same PGPR strain.

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The reason CIMMYT is keen to maintain their collaboration with IAEA is because they believe that there is scope to increase the efficiency of use of the 5+ M t of N fertilizer applied to irrigated wheat in developing countries, and that this project is an excellent vehicle by which new technologies can be tested and extended to NARS. The CIMMYT Economics Program carried out a production practices survey in April 1994 in the Yaqui Valley returning to the 100 fields surveyed in 1990-91 season by the wheat program. Eighty five of the field were planted to wheat and the mean N application was 251 kg N/ha, an increase of 13% over 1990-91. With a mean yield of 5.46 t/ha, fertilizer N recovery in grain remains well below 50% as pointed out last year from other data. Although farmers appear to be splitting the N application more, sixty-four percent of the N was applied before sowing. The most popular form used was urea, followed by aqua ammonia, then anhydrous ammonia.

Several experiments in 1993-94 looked at N timing, comparing all at seeding, with all at first node stage (DC31), and sometimes with part at seeding and part at DC31. In N responsive situations (15-20 kg grain/kg N for initial 100 kg N/ha at sowing), in 3 out of 4 experiments the yield response to intermediate N levels was on average 13% greater with all N at DC31 (around 50 DAS) than with all at seeding. In the 4th experiment there were variable results but variable leaf rust infection could have been a factor. In 2 of the experiments 1/3 sowing, 2/3 at DC31 was compared to all at DC31, and the yield response was 20% greater still. These results corroborate those of earlier years in Mexico: DC31 N is more efficient than sowing N, but a little N (one third only) at sowing plus the rest at DC31 is most efficient.

J.S. Schepers
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Understanding and measuring the dynamics of nitrogen (N) in soil is a challenge for plant and soil scientists alike. It may seem that measuring the quantity of plant available N in soil should be a trivial matter, but it can also be a never-ending task. This is because N transformations in soil involve a wide variety of microorganisms, which are influenced by climate changes and soil properties. For these reasons, it may be more practical to monitor the net effect of soil N dynamics by measuring crop N status than extensively analysing soil. Being able to characterize the nitrogen status of a crop has the advantage over soil testing procedures because roots integrate a much large volume of soil than is typically sampled and analyzed.

Chlorophyll meters were originally developed by the Minolta Corporation to quantify the N status of rice and identify the need for N fertilizer. Recently, this technology has been applied to many other crops. The unique characteristic of the chlorophyll meter approach compared to other tissue testing techniques is that the meter is only responsive to N deficient situations. While this feature may be a limitation for some applications, it can be an asset in terms of monitoring N availability under situations where the crop is responsive to N fertilizer. Chlorophyll meters have been used extensively at the Nebraska Management Systems Evaluation Area (MSEA) project to monitor soil N dynamics and to schedule fertigation of corn. The meters have been shown to detect an approaching N deficiency several weeks before visual symptoms are present. They are also able to rank the relative N status of plots receiving different rates of N fertilizer. For corn, the meters are reliable from about the 8-leaf stage until near maturity.

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Universidad de Concepcion
Chillan, Chile

A substantial proportion of the wheat crop in Chile is grown under conditions of the Mega-environment 1 (ME1) which accounts for about 40% of the wheat production in the developing world. In the South-central region of Chile few farmers are achieving the productive potential (6 to 10 t/ha) of the new varieties. The biggest production constrains in this region is soil fertility, particularly nitrogen. Specific objectives for the first year include: a) to measure the contribution of the applied and native nitrogen taken up by two wheat varieties under three irrigation levels [excessive, optimal and sub-optimal], and b) to generate an experimental data set of soil, crop and climate within ME1 conditions for validating Ceres-Wheat simulation model. The field experiment will be conducted on a typic dystrandept in south central Chile. A line-source sprinkler irrigation system will be used to establish three water levels and all treatments will be replicated four times. Stripped at right angles to the line-source will be a factorial of four fertilizers N levels [0, 75, 150, and 225 kg N/ha] and two wheat cultivars. Within each fertilized treatment ^{15}N microplots of 1 m^2 will be installed and ^{15}N labelled urea (1% a. e.) will be applied in the same doses as in the main plot.

3. **Final Research Co-ordination Meeting of the FAO/IAEA/SIDA Co-ordinated Research Programme on The Use of Isotope Studies on Increasing and Stabilizing Plant Productivity in Low Phosphate and Semi-arid and Sub-humid Soils of the Tropics and Sub-tropics** (D1-RC-415.4)
10 - 14 October, 1994, Vienna, Austria

Scientific Secretary: Saliya Kumaransinghe

This final meeting brought together all the contractors and agreement holders of the CRP from Egypt, Germany, Kenya, Morocco, New Zealand, Nigeria, Sierra Leone, Sudan, Sweden, Tunisia and Viet Nam during which the final reports of this five year programme were presented. The reports highlighted the existence of substantial genotypic differences in phosphate and water use efficiency in food crops as well as in tree species. Some morphological as well as physiological parameters responsible for these differences have also been studied.

Excerpts from presented reports:

i. Carbon-13 discrimination as criterion for identifying high water use efficiency wheat genotypes under water deficit conditions

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During four consecutive years, 20 durum wheat (*Triticum durum* Desf) and bread wheat (*Triticum aestrivum* L.) genotypes were grown under rain-fed conditions and supplementary irrigation with the objective of assessing the possibility of using ^{13}C discrimination (Δ) as a criterion to screen for wheat genotypes that produce high yields and have a better water use efficiency under water deficit conditions. In all four growing seasons, both treatments were subjected to some water stress which was higher under rain-fed conditions and varied according to the intensity and time of rainfall. During the first growing season, and despite the small differences between the two treatments in terms of the amounts of water used, the grain and straw yields as well as Δ were significantly higher in treatments which received an irrigation at installation than in the one without irrigation. There was substantial genotypic variation in Δ. When both treatments were considered, the total aboveground dry matter yield and grain yield were positively correlated with Δ although the correlation coefficient of grain yield versus Δ was not high ($< 0.45^{**}$). Moreover, the Δ value was also correlated positively with water use efficiency. This is in contrast to greenhouse experiments with wheat where plant water use efficiency and Δ were negatively correlated. The data suggest that high Δ values can be used as a criterion for selecting genotypes of wheat that have a relatively higher grain yield potential and high water use efficiency under water deficit conditions. Eleven genotypes were identified using this technique. During the second growing season, the grain yield and Δ were similar to that of the previous season. Their linear relationship was loose, but significant ($r = 0.34^{**}$); this became more evident when straw ($r = 0.41^{**}$) or the total dry matter (0.49^{***}) was used. From this second season study, 13 genotypes were selected as potentially suitable candidates for water deficit conditions on the basis of their high yield and Δ values. All the genotypes selected during the first season were also among this second selection. During the last 2 years of the 4 year experiment, grain yield data were used to validate the selection performed during the first two seasons. However, a very high inconsistency was noted between the genotype performances in terms of Δ during the first two seasons and the yield during the last two seasons. Of the

genotypes tested, only three were among the best in terms of grain yield during the last two seasons. Three genotypes produced non-competitive yields thereafter. Four genotypes only produced a good yield in one of the last 2 seasons. Three genotypes that were not selected also produced high yields during at least one of the last two seasons. The data suggest that while a high Δ value may be used as a criterion for selection of genotypes of wheat with potential for high yield and high water use efficiency in wheat under field conditions, caution must be exercised in the selection process as the size of the canopy and the changes in environmental factors mainly soil water content, can result in changes in Δ and the yield of a cultivar. However, Δ of a genotype can also provide valuable information with respect to plant parameters responsible for the control of Δ and this information can be usefully employed in breeding programmes aimed at developing wheat cultivars high in yield and high in water use efficiency, and suitable for cultivation in arid and semi-arid regions of the tropics and sub-tropics.

ii. Performance of Acacia tortilis, Prosopis juliflora and Casuarina equisetifolia provenances in soils low in available phosphorus

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Acacia tortilis, *Prosopis juliflora* and *Casuarina equisetifolia* provenances were screened to determine their potential for adaptability under P limiting conditions as a strategy to exploit genotypic differences interms of utilization and uptake efficiencies. The experiment was conducted in the greenhouse at the Kenya Forestry Research Institute using soils taken from the field which are critically low in available P. The experimental treatments comprised of P application at 0 and 60 Kg P_2O_5/ha for 11 provenances of *Acacia*, 6 *Prosopis* and 4 *Casuarina* spp. Trait for adaptability to P deficiency was determined by measuring the growth performance, P uptake and utilization efficiencies at zero and moderate application of P. The results indicated considerable differences in the growth performance and phosphorus use efficiency (PUE). *Acacia* provenances showed the highest PUE compared with *Prosopis* and *Casuarina* spp. although this was not reflected in the total dry matter yield. However, it was observed that P application resulted in an increase in shoot dry matter, height, root collar diameter and root dry matter in the case of *Casuarina*. Similarly, the highest total P uptake was obtained in *Casuarina* and *Prosopis* spp. The results further indicated that P application probably contributed to the reduction in root dry matter and root: shoot ratios of *Acacia* and *Prosopis* but not *Casuarina* spp.

iii. Use of the ^{13}C discrimination technique for identifying durum wheat cultivars efficient in uptake and use of water

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The ^{13}C isotope discrimination method and the water balance model using a neutron moisture probe were used in this field study to rank durum wheat genotypes for water use efficiency. The results focus on the first two years of a five-year study. Eighteen durum wheat cultivars were used in the first experiment. The $^{13}C/^{12}C$ ratio was measured to examine the

correlation between this ratio and the water use efficiency. Total water consumption was calculated and the grain and straw yields and other parameters were also recorded. The results show differences between cultivars with respect to water use efficiency, ^{13}C discrimination and grain yield. From this experiment, four cultivars were selected for a detailed study in the second year. The data from this study show that there is a positive correlation between grain water use efficiency and Δ. Thus, it may be possible to use Δ as a tool for screening out water use efficient cultivars in semi-arid regions.

iv. Genetic variability in phosphorus use efficiency in Acacia senegal (L) Willd Provenances

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Two experiments were conducted with *Acacia senegal* provenances collected from within the natural gum belt to screen for high phosphorus use efficiency (PUE) and water use efficiency (WUE). A preliminary screening was done in phase 1 of this programme, from which 4 provenances and 6 provenances were further subjected to screening for PUE and WUE respectively. The study revealed that provenance 11 (from Elobeid) is significantly different from the others in total biomass production. However the application of P did not influence the biomass production. Provenance 11 had the highest PUE and was intermediate in root length density (RLD). The WUE study revealed that provenance 7, 3 and 11 were the best amongst the provenances tested, in biomass production, water use efficiency as well as the RLD. These results conclude that provenance 11, which is collected from the centre of the gum belt is the most promising one to be used to restock the vast degraded hashab habitats.

v. Evaluation of hedgerow trees in alley cropping for phosphorus use efficiency and N_2 fixation in low P soils in moist savanna in Nigeria.

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S.K.A. Danso, F. Zapata
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G.D. Bowen
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Low soils P and N are common in moist savanna zone establishments and growing hedgerow trees in alley cropping systems might require addition of N and P fertilizers. This is not possible for small scale farmers who have limited access to fertilizers and therefore depends only on no input cropping systems. Exploiting genetic differences in P use efficiency and selecting hedgerow trees with high N_2 fixing capacity can improve tree establishment and growth on N and P poor soils and preserve soil from degradation.

Field experiments carried out at Fashola (moist savanna) have shown that large differences in growth and P use occurred between N_2 fixing trees such as *Gliricidia sepium* and no N_2 fixing trees such as *Senna siamea* and *Senna spectabilis*. Provenances or isoline differences in P also occurred within species and varied depending on level of P application and period of growth. Differences between species and provenances to P uptake and growth were largely related to differences in physiological P-use efficiency (PPUE), root length and VAM infection rate, especially at low P.

In general, nodulation was improved by P application, but varied amongst provenances. For example, *G. Sepium* fixed about 61% of its N from atmospheric N_2 in the pot experiment and 40% in the field. The percentage of N fixed was effected by the low rate of P application, i.e., 20 kg P ha^{-1} and, at high rates of P, no further increase in % N_2 fixed occurred. Differences in P and N accumulation and use was also influenced by management practices such as pruning. Distribution of total P followed the same trend as that of dry matter yield, while no significant correlations were found between partitioninning of dry matter and total N. Uncut and cut *G. sepium* derived 35% and 54% respectively of their N from atmospheric N_2. About 54% of fixed N_2 was partitioned to shoots and roots and this was not attributed to the size of these organs in relation to others.

vi Phosphorus use efficiency of cowpeas in Sierra Leone

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Of the cowpea genotypes tested IT86d-1010 and IT 86-719 had combined features high dry matter and grain yields with high total and grain phosphorus use efficiency. These genotypes also showed higher root dry weight, root length and root fitness than the inefficient genotypes with which they were compared, under low and high P. The capacity of these genotypes to use P efficiently could be attributed to their rooting characteristics. There is a high and positive correlation between yield and nitrogen fixation parameters and phosphorus use efficiency. Phosphorus efficiency is therefore an important estimate of yield and nitrogen fixation of these genotypes. Multilocational testing has shown that the genotypes selected cannot do well in areas with low rainfall in Sierra Leone.

vii Genotypic differences in acquisition and utilization of phosphorus in wheat

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In an attempt to evaluate whether breeding and selection for high yielding capacity did change the P requirement of modern wheat cultivars, the response of two wheat cultivars to different levels of P supply was investigated. A traditional cultivar ('Peragis') and a modern cultivar ('Cosir') were cultivated in a soil low in available P and high in $CaCO_3$ in 120cm high PVC tubes. Shoot growth, root growth, P uptake, P translocation and P distribution, within the shoot at different developmental stages were compared.

The grain yield of the modern cultivar 'Cosir' was high at limiting and non-limiting P supply and, therefore, this cultivar can be considered as more P-efficient than the traditional cultivar. Grain yield reduction at low P supply was mainly due to an inhibition of tillering and thus lower number of ears per plant, whereas the number of grains per ear were hardly affected. Reduced tillering at low P supply could not be related to P concentrations in the shoot meristematic tissues which were generally much higher than in other plant tissues and kept at an elevated level even at limiting P supply. Also root branching was reduced at limiting P supply in 'Cosir' but not in 'Peragis' which, generally, had lower numbers of laterals at the beginning of tillering.

From the results, it can be concluded that the main factors contributing to the higher P efficiency of the modern cultivar 'Cosir' are; (i) efficient use of assimilates for root-growth which enhance P acquisition: enhanced root branching and thus smaller mean root diameter and longer root hairs; (ii) efficient P uptake system; (iii) efficient remobilization of P from vegetative plant organs to the grains, and most important; (iv) lower P requirement for grain yield formation because of lower ear number per plant but higher grain number per ear.

viii *Can the ^{15}N dilution technique be used to study N_2 fixation in tropical tree symbioses as affected by water deficit?*

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Three methods were used to study N_2 fixation and effects of water deficit on N_2 fixation C_2H_2 reduction assay (ARA), ^{15} N dilution technique and accumulated N content. In addition, ^{15}N dilution was calculated both in a traditional way and in a modified way, which takes into consideration N and ^{15}N content for the plants before the experiment started. The three methods were applied on the following *Rhizobium*-symbioses: *Acacia albida* Del *(Faidherbia albida* (Del) A. Chev.) and *Leucaena leucocephala* (Lam) de With., and the *Frankia*-symbiosis *Casuarina equisetifolis* L. The plants were about 4 months old when they were harvested.

Nitrogen derived from N_2 fixation in control plants of *Acacia albida* was 54.2 mg as measured with ARA, while it was 28.5 mg as measured with the ^{15}N dilution technique, compared to 30.7 mg calculated as accumulated N. In comparison, *L. leucocephala* fixed 41.6 mg N (ARA), 53.5 mg N (^{15}N dilution technique) and 56.3 mg N (accumulated N). The *Frankia*-symbiosis had fixed 27.4 mg N as measured by ARA, 8.1 mg N as measured by ^{15}N dilution technique and 12.3 mg N as accumulated N. There were no differences between the estimates based on traditional and modified ways of calculating ^{15}N dilution.

The immediate effect of water deficit treatment on N_2 fixation was continuously measured in all species with ARA, which started to decrease approximately 10 days after the initiation of the treatment, and declined to less than 5% of the initial level after 21-28 days.

The decrease in the amount of N derived from N_2 fixation was studied in *L. leucocephala* during the period of treatment. There was a 26% decrease in amount of N derived from N_2 fixation as a result of water deficit (as measured with ARA), while the decrease was 23% when measured with both the ^{15}N dilution method and as accumulated N.

Since there was no difference in measured values of N_2 fixation between the different methods, we conclude that the ^{15}N dilution technique can be conveniently used to study effects of water deficits on N_2 fixation.

Note: This work has been published in the Journal of Experimental Botany, Vol. 44, No.269, pp. 1749-1755, December 1993.

ix. *Genotypic differences in phosphorus use efficiency of wheat*

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The modern wheat cultivar "Cosir" can be considered as more efficient in the utilization of the P supply from the soil than the traditional cultivar "Peragis". Three factors contribute to the high P efficiency of "Cosir"; (i) efficient P uptake system; (ii) efficient P uptake through smaller root diameter and longer root hairs and (iii) more efficient internal use of P for grain yield formation. However, the most important characteristic confering P efficiency to "Cosir", appeared to be the yield structure. The cultivar "Peragis" appeared to be more sensitive to low P supply because the number of ears/area which is reduced most sensitively at limiting P supply is more important than for "Cosir" with its much higher grain number per ear.

In a nutrient solution experiment, the higher P uptake efficiency of cultivar "Cosir" and its lower sensitivity of tiller formation at liming P supply could be confirmed. P is preferentially transported into the meristematic tissue in the basal stem section and P concentration in this tissue is much less affected by P supply than in other plant organs. Reduced tiller formation can not be explained on the basis of reduced P concentrations in the meristematic tissue.

Cultivar "Cosir" produced much higher numbers of first order laterals than "Peragis". In agreement with lower tiller formation, the number of laterals was clearly reduced at low P supply in "Cosir". However, lateral root formation could not, consistently, explain the differences in tiller formation between all P supplies and especially between the cultivars.

In a pot experiment with low P soil, cultivar "Cosir" responded in grain yield more to increasing P supply than a "uniculm" cultivar from Australia. This confirms the high sensitivity of the yield component number of ears per plant to low P supply.

x. *Genotypic differences in phosphate nutrition of wetland rice (Oryza sativa L.) on grey soil of south Viet Nam.*

Le Dac Lieu, Luong Thu Tra, Mai Thanh Son, Nguyen Dang Nghia, and Do Tung Binh.

Centre of Nuclear Techniques of Hochiminh City, Hochiminh City, Viet Nam.

This study was conducted from 1992 to 1994, to examine genotypic differences in phosphate uptake and use in wetland rice.

In the first experiment, twenty four genotypes of rice (*Oriza sativa. L*) were screened for phosphate use efficiency (PUE) under field conditions at low P (O P) and high P (90kg P_2O_5/ha) in a grey soil of South Vietnam during a dry season from July to November, 1992. Significant genotypic differences were found in grain yield, ranging from 0.245 to 0.511 and 0.271 to 0.534. Total P ranged from 1.27 to 1.83 and 1.35 to 1.83, PUE ranged from 0.181 to 0.322. The results show that there are significant genotypic differences at both P levels. Grain yield was highly correlated with PUE ($r=0.85$) but not with total P uptake ($r=0.53$). Based on PUE, the genotypes were separated into three groups: low, high and medium PUE types.

Four genotypes which differ in PUE were selected from the first experiment for the second experiment, which involved a detailed investigation of the superiority in phosphate use efficiency. Plants were grown in greenhouse conditions for four weeks. P was supplied at 0, 30, 90 ppm. Rice genotypes with a high P efficiency grew well at all P levels. But inefficient genotypes grew well only at high P. Dry weight of shoots was not effected by P levels, but dry weight of roots increased with increasing P in soil. Root/shoot ratio also showed significant differences. The data shows that the root dry weight, and the R/S ratio can be considered as important parameters in P uptake and use.

xi *Tracer methods to quantify nutrinet uptake from plough layer, subsoil and fertilizer implications to sustainable nutrient management.*

E. Haak

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Two soil injection methods were presented. The first method consisted of homogeneously labelling the whole plough layer with carrier free tracers. This was done in two treatments. One in a *reference treatment* without connection with the subsoil and the other in an *experimental*

treatment where the subsoil is freely available for root penetration. The second method, which is now under development, consisted of using labelled fertilizers instead of carrier free tracers.

By application of the A-value concept it is possible to quantify the plant uptake of nutrients from plough layer and sub-soil. Based on this tracer field experimentation, a fertilizer application strategy has been developed for phosphorous.

4. Final Research Co-ordination Meeting of the FAO/IAEA/UNDP CRP on "The Use of Isotopes in Studies to Improve yield and N_2 Fixation in Grain Legumes with the Aim of Increasing Food Production and Saving N-Fertilizer in the Tropics and Sub-tropics of Asia.
7 - 11 November 1994, Hyberabad, India

Scientific Secretary: Seth Danso

Excerpts from presented reports presented at this meeting will be published in the next Soils Newsletter.

TECHNICAL ASSISTANCE CO-OPERATION PROGRAMMES

The Soil Fertility, Irrigation and Crop Production Section is currently responsible for 61 Technical Co-operation Projects. In the July 1994 issue, we gave summaries of projects in Asia and the Pacific Region. In this issue, we highlight the activities of TC Projects in the Middle East and Europe.

Afghanistan

Use of ^{15}N techniques in improving crop production (AFG/5/002)

University of Kabul, Department of Soils and Irrigation, Faculty of Agriculture

Counterpart: **A. G. Ayubi**

This project was initiated in 1993 with the objective of strengthening research and development activates in the field of ^{15}N techniques to increase the yield of major crops through improved fertilizer practices. Arrangements have been made to supply ^{15}N labelled fertilizer However, a three week technical assistance mission, had been postponed due to other difficulties.

Iran

Nuclear techniques in wheat production (IRA/5/008)

Atomic Energy Organization of Iran, Nuclear Research Centre, Agricultural Department, Teheran.

Counterpart: **M. Faramarz**

This is a multi-disciplinary project initiated in 1987 involving Plant Breeding and Genetics, and Soil Fertility, Irrigation and Crop Production Sections. The objectives are to initiate radiation and isotopes aided studies for increasing wheat production through the development of superior varieties and the introduction of more efficient fertilizer and irrigation water management practices. These activities were related to mutation breeding, including, more recently, the use of

double haploid techniques for wheat improvement. Local staff were trained on the use ^{15}N techniques in assessment of biological nitrogen fixation of grain legumes. Expert missions, fellowship training, scientific visits and laboratory equipment were also provided.

Middle East - Regional Project

Nitrogen fixation nutrients and water balance studies (RER/5/004)

1) Ministry of Agriculture and Natural Resources, Agricultural Research Institute, Nicosia, Cyprus

   Counterparts: **I. Papadopoulos, I. Papastylianou**

2) Atomic Energy Organization of Iran, Karadj Nuclear Research Centre, Karadj, Iran

   Counterparts: **N. Sagtieb, M.S. Alemzadeh**

3) Ministry of Agriculture and Fisheries, Ras al Khaimah, United Arab Emirates.

   Counterpart: **M.H. Al Sharnsi**

4) University of Jordan, Faculty of Agriculture, Amman, Jordan

   Counterparts: **B. H. S. Khattari**

5) King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia

   Counterpart: **A.A. Al Jaloud**

6) Syrian Atomic Energy Commission, Damascus, Syria

   Counterpart: **K.H. Khalifa, and A.F. Asfari**

7) Turkish Atomic Energy Authority, Saraykoy Research Centre, Nuclear Agricultural Research Centre, Ankara, Turkey

   Counterpart: **M.B. Halitligul**

This regional project was initiated in 1991 to promote regional co-operation in the use of biological nitrogen fixation (BNF) and increased efficiency in fertilizer management to help improve the production of cereals and legumes, particularly when legumes are included in crop rotations. ^{15}N technique was used to assess N contribution of leguminous crops to residual soil N and to the subsequent cereal crops. Additionally, the participants of the programme used neutron soil moisture gauges to compare different cropping systems from the point of view of crop water consumption and soil water conservation. Several training courses and workshops were organized from 1991 to 1994. Expert missions, fellowships and equipment were provided. The final co-ordination meeting was held in Teheran (Iran) from 3 to 7 December 1994. According to the recommendations made by the participating Member States of this programme, a fellowship activity will be organized for the biennium, 1995-1996. The emphasis will be shifted from BNF to fertigation, a process which is most appropriate to the participating countries.

Romania

Use of beneficial bacteria for increased crop production (ROM/5/006

Research Institute for plant protection, Department of Biochemistry and Bacteriology, Bucharest, Romania

Counterparts: **V. Severin, I. Zurini**

This project aims at developing the uses of beneficial bacteria for crop production in Romania through nuclear and related methods. It was initiated in 1993 to introduce modern biotechnology and nuclear technologies as part of an integrated programme for plant nutrition and protection. The IAEA assistance included; provision of expert services, equipment, scientific visits and fellowships. In the long term, isotopic techniques in biochemistry and bacteriology will contribute to the development of sustainable agriculture in Romania.

Turkey

Soil fertility and plant nutrition (TUR 5/016)

Turkish Atomic Energy Agency, Ankara Nuclear Agriculture Research Centre, Saray Koey, Ankara, Turkey

Counterpart: **M.B. Halitligil**

The main objectives are to consolidate research on soil/plant/water relationships of staple crops, and also to determine the efficiency of the fertilizer/water use of genotypes and N-fertilizer management practices. The Government of Turkey requested technical assistance from the IAEA to increase their research capabilities so as to improve fertilizer practices in the country. ^{15}N labelled fertilizers were provided as well as expert assistance, fellowships and equipment. Emphasis is now being placed on the analysis and transfer of results of immediate practical relevance to agricultural production.

SYMPOSIA and SEMINARS

The FAO/IAEA International Symposium on Nuclear and Related Techniques in Soil/Plant Studies on Sustainable Agriculture and Environmental Preservation, 17 - 21 October 1994, Vienna, Austria

Scientific Secretary: Christian Hera

Co-Scientific Secretary: Saliya Kumarnsinghe

This symposium, which was attended by nearly one hundred participants from forty-three countries, was successfully completed in October. There were forty-eight oral presentations and thirty- one posters which were divided into eight sessions. The sessions included:

i) Recent developments in analytical methods and equipment. In this, there were three presentations on high productivity stable isotope analysis, a GCQMS aided incubation systems for trace gas studies and a system for $^{15}N/^{13}C$ determination in one sample.

ii) Fertilizer use and management studies. This session included eight papers which highlighted the methods that may be used for efficient management of nutrients using isotope techniques, FAO soil fertility and integrated plant nutrient management programmes, and application of ^{15}N and ^{32}P in fertilizer studies.

iii) Biological nitrogen fixation in sustainable cropping systems. Biological nitrogen fixation-present and future, and the role of biological nitrogen fixation in sustainable agricultural production were the keynote addresses in this session. In addition, papers were also presented on management of legume N_2 fixation in cereal studies and studies of rhizobial ecology using marker genes.

iv) Soil organic matter studies and nutrient cycling. The presentations in this session highlighted the recent studies on organic matter turnover with special reference to N and C.

v) Water use and management studies. Papers on the use of neutron probe in soil/plant water studies, and the Tensionic technique for assessment of soil solution inorganic-nitrogen were among those that were presented. A paper on Carbon-13 discrimination as a criterion for identifying high water-use efficiency wheat genotypes under water-deficit conditions presented interesting new findings using this technique under field conditions.

vi) Plant physiological aspects in crop production. Analysis of carbon fluxes in crop-environment interactions and the use of radio- and stable isotopes was an invited paper. In addition, there were several papers that dealt with nutrient uptake and partitioning as well as nitrogen nutrition aspects in wheat: legume cropping systems involving lupins.

vii) Environmental pollution and preservation. These papers brought to the attention of the participants, the major environmental problems caused by irrational agricultural practices. The problems of trace organic chemicals were also discussed.

viii) Soil conservation, soil erosion and dertification. The invited paper in this session highlighted the use of fallout radionucleides in soil erosion investigations. Other nuclear methods in soil erosion and siltation were discussed in subsequent papers.

FROM OUR READERS

XXIVth Annual Meeting of ESNA, 12 - 16 September 1994, Varna, Bulgaria

Report of Working Group Chairman, Dr. Martin Gerzabek
WG 3 - Soil - Plant - Relationships

The working group on soil-plant relationships held four topical sessions in which 39 papers were presented by scientists from 17 countries.

Session 1, Nitrogen in the Soil Plant System, was opened by E.HAUNOLD (Austria) presenting critical comments on the present and future nitrogen use with respect to the growing world population and it's possible impact on the ozone layer. Most of the papers focussed on the rational use of nitrogen fertilizers. KHALIFA (Syria) showed that a combined NH_4 and NO_3 application is favourable for maize growth and N-uptake under saline conditions. AKIN (Turkey) emphasized the importance of nitrogen fertilization of wheat varieties for increasing water use efficiency in semi-arid regions. INAL (Turkey) investigated the impact of N-applications on the sucrose content of sugar beet. He found out that sucrose content stays stable, up to an application

level of 300kg N/ha, on soils relatively poor in total nitrogen. Water use efficiency can be increased by a combination of pumice stone and N-treatments, as it was shown by KARAMAN (Turkey). PAVLOV (Bulgaria) elaborated on the control of nitrate uptake in natural meadows by applying different nitrogen forms and by alternate fertilization. Especially for grassland, excess of nitrogen supply by liquid waste from animal farms, may be a sever problem. MEGUSAR (Slovenia) reported about a new biotechnological method to reduce NO_3-N through bioconversion to molecular nitrogen to less than 100ppm N without dilution. The appropriate use of N- and P-fertilizers on two Romanian soils was tested by SERDINESCU. He showed that the combination of P-K- and N- applications resulted in the application methods and showed that foliar applied nitrogen contributed 7.7% to the total nitrogen in grapevine. In the poster session further results on differences in nitrogen dynamics between N-forms, crop varieties and the behaviour of soil types were presented. Much attention was paid to the presentation of MAKARIEV (Bulgaria) about graft incompatibility in fruit trees. The use of ^{15}N can decrease the time for investigating graft incompatibility from 12 to 2 years.

Session II, Plant Nutrition, was opened with the presentation of a paper from BROHI (Turkey) on, the use of poultry manure to wheat crops. It was clearly shown that even extremely high treatments (100 t/ha) had no detrimental effect on plants. However, a positive influence on yields was only obtained up to 15 t/ha. KIRKBY (England) presented a lecture in which he showed that the major driving force on root nutrient uptake is the shoot demand and not the root uptake ability. Several papers were presented on phosphorus behaviour in soil and plant uptake. AYDENIZ (Turkey) discussed the phosphorus-lime relationships. OSZTOICS from Hungary gave an overview about P-sorption and the short term behaviour in soil. Special attention was given to the two slow processes of field diffusion and intraparticle diffusion and the fast sorption reaction. Phosphorus double-labelling is suitable to evaluate the contribution of different phosphorus pools to P extracted by various standard methods, as was proven by the experiments of NIKOLOV (Bulgaria). In combination with field experimental data, the proposed method could help to better define the plant available P-pool. TONEV (Bulgaria) elaborated on the N-P-K nutrition of sunflowers and stressed the distinct influence of the precipitation levels on the predictability of nutrient uptake. The presentation of ASFARI (Syria) led to the conclusion that wheat seed irradiation can have a similar positive growth effect like fertilizer additions.

In Session III, on Plant Stress and Environment, BUJTAS (Hungary) reported about the influence of soil properties on herbicide toxicity to maize plants. She pointed out that soil texture seems to especially alter the toxic effects. FIGLIOLIA (Italy) presented results on the influence of soil organic matter on heavy metal toxicities. The detrimental impact of excess Zn- and Cu-concentrations on oat plants was significantly lower in an organic rich soil, despite the fact that soil extractant hardly showed any response. The last presentation in this session focussed on the practicability and accuracy of soil sampling for agricultural and environmental purposes. ILIEV (Bulgaria) described the function of a new type of motor-driven portable soil sampler and elucidated the obtained high precision.

The largest session of this annual meeting of WG 3 was the ***Joint IUR/ESNA-Session on Radioecology (Session IV)***. ERIKSSON (Sweden) presented results from lysimeter experiments, already running for nine years. Generally Pu showed a higher leaching rate than plant uptake. Annual neptunium-leaching reached nearly 1% of total Np present. KIRCHNER (FRG) and FAWARIS (Sweden) elaborated on the mechanisms of caesium uptake into pasture grasses. The latter author identified soil organic matter and potassium nutrition to be the major influencing factors causing great variability between different soils. KIRCHNER showed that the time variations of Cs-concentrations in pastures are mainly due to the moisture content of the soil and the number of cuttings. ROSEN (Sweden) reported that 0.9% of total deposited ^{137}Cs can be transferred to plants under the condition of a mountain farm in Sweden. The Cs concentraion in single plant species decreased in this order: herbs > grasses > woody plants > trees and shrubs.

A group of papers focussed on appropriate countermeasures. From the presentation of LÖNSJÖ'S (Sweden), we learned that the combination of ploughing under and potassium application is most effective to reduce Cs-transfer on grasslands with soils poor in potassium. These findings were generally confirmed by the data shown by JONES (England) on the effect of different amendments to upland grasslands in the United Kingdom. Besides potassium, especially the clay mineral clinoptilolite, seems to be quite effective in decreasing the Cs concentration in vegetation and its effectiveness increased with time. ONCZIK (Hungary) successfully used zeolite and Cabentonite to decrease Cs-uptake into rye. At the same time, crop yields and physiological activities could be enhanced. SKARLOU (Greece) tested the influence of soil parameters on Cs and Sr uptake into a number of agricultural crops. A negative correlation between pH, CEC and clay content and concentration ratios could be clearly demonstrated. GERZABEK (Austria) discussed the impact of soil mass loading on transfer values and showed that soil adhesion could contribute up to 23% to Cs contamination of cereal straw. In the case of broad bean, rain splash may account for 68% of the total soil adhesion. TOMPKINS (England) presented an alternative to the traditional transfer factor concept, based on data observed in lysimeter experiments. In this model, radionuclide transport - even upward movement - and root density distribution are taken account. In the lecture on a homeostatic - partly dynamic model for ^{137}Cs in trees, FRISSEL (Netherlands) explained the main process involved, starting from the assumption that the $^{137}Cs/K$ ratio in soil determines the Cs/K ratio in the various parts of a tree. He analysed the sensitivity of discrimination factors between Cs and K; the problem of luxurious consumption of K and stressed the lack of data on cycling of K in the system. McGEE (Ireland) showed that an appropriate plant species like *Calluna vulgaris* can be used as an indicator for the Cs-contamination of other species and that such estimates result in a considerably lower coefficient of variation than estimations based on soil contamination data. In the poster by NAVARCIK (Slovak Republic) a microbiological process with mild extractant was proposed.

In the final discussion of the WG 3, two topics were defined, which should be, preferably, addressed in the presentations next year. For the nitrogen session papers on nitrogen mineralization potential of soils and the subsequent plant uptake are encouraged. For the radioecological session papers focussed on radionuclide fluxes, special emphasis on recycling of radionuclides in natural environments should preferably be submitted. The task is to provide modellers with the data needed and to discuss presently unexplained fluxes and variations.

Soils Newsletter

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