The dynamics of rooting patterns in relation to nutrients and water in soils:

Development, standardisation and documentation of methodologies

First Progress Report of the EEC-Concerted Action AIR3-CT93-0994

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ab-dlo

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The DLO Research Institute for Agrobiology and Soil Fertility (AB-DLO) is part of the Dutch Agricultural Research Department (DLO-NL) of the Ministry of Agriculture, Nature Management and Fisheries.
The institute was founded on 1 November 1993 by the amalgamation of the Centre for Agrobiological Research (CABO-DLO) in Wageningen and the institute for Soil Fertility Research (IB-DLO) in Haren.

The DLO organization generates new knowledge and develops and maintains the expertise needed for implementing government policies, for improving the agro-industry, for the planning and management of rural areas and for protecting the environment.

AB-DLO, with locations in Wageningen and Haren, will carry out research into plant physiology, soil science and agro-ecology with the aim of improving the quality of soils and agricultural produce and of furthering sustainable plant production systems.

Key areas of expertise in AB-DLO are: plant physiology, soil biology, soil chemistry and soil physics, nutrient management, crop and weed ecology, grassland research and agrosystems research.

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Table of Contents

1. Introduction

The objectives of Concerted Action AIR3-CT93-0994 are to establish a representative network of research centres where plant root studies are done in relation to nutrient and water dynamics of the plant/crop/soil system. During the Concerted Action an update of old root research methodologies is to be made and a description of new methods in used nowadays will be included. If appropriate an attempt to standardise specific methods will be made. Updates, descriptions and standardisation protocols will lead to a document (a Handbook). The concerted Action aims further at an improved exchange of research results and methodological aspects between the participants by initiating and maintaining electronic discussion or newsgroup on the Internet.

Among the participants to this Concerted Action "Users" and "Core members" are distinguished. "Core members" will have a special task in designing and writing the so-called Handbook, during the writing process "users" will be asked to collect and to provide relevant research data.

Three Workshops are to be hold, addressing the following points:

Workshop I: Participation by the User-group. In this workshop the state of the art of several methodologies shall be identified and an information exchange shall take place. At this point already a discussion on the outline of the "Handbook", a methodological paper describing methods in root research will take place

Workshop 2: Participation of the "Core-group". Finalising the preparation of the "handbook"

Workshop 3: Final results and presentation of the Handbook

The current progress report describes the first workshop which was hold in June 1994 in Wageningen and contains also the results of an inventory on current root research methods.

In order to identify the current "state of the art" of root research each of the participating research groups (Appendix I) was invited on the first day of the workshop to inform the other participants about their research project(s), emphasising the methodological aspects or methodological difficulties (See also Chapter 6 with short descriptions of the research items and relevant references of the contributing researchgroups).

On the second day of the workshop (Appendix II for the program) specific subjects were treated in more detail in the form of sessions. Each session was presided by an expert in the field who was asked to give an introductory paper in order to i) give a broad outline of the techniques now available and the degree of standardisation (or lack of standardisation) ii) identify existing problems which impair further development iii) identify and initiate relevant coöperative activities in the next years. Chapter 3 will give a brief description of the outcome of these sessions. During the last day of the workshop four different working groups discussed the following topics which were considered as relevant for future root research: the use of minirhizotrons, image analysis, sampling methodology and root activity (Chapter 4).

2. Inventory on root research methods

2.1. Introduction

To start the information exchange between the participants of this Concerted Action an inventory (Appendix III) was sent to each research group. In this inventory, all participants have described i) the major research questions in the projects, ii) the methodology they use and iii) the root parameters they assess in their research.

2.2. The results

Appendix IV gives a survey of the research questions treated by the participants, it becomes clear that all groups together cover in their research a wide range of agricultural systems (including forestry), plant species and physiological processes. It can be concluded that the participating groups create an excellent platform to carry out the tasks described in this Concerted Action.

On the basis of Appendix V the subjects/techniques were chosen which were treated in the sessions on the second day of the workshop. Considering the response to question # 9 of the inventory it can be concluded that the assessment of root functioning, including the criteria for declaring a root dead or alive, is a topic of high interest for most of the root researchers today. Also model development, the use of minirhizotrons and image analysis were considered as important for current root research.

To quantify root length dynamics in the field the greater part of the researchers use minirhizotrons, use the profile wall method or carry out auger sampling (Appendix VI). Especially the use of minirhizotrons usually is accompanied by methodological difficulties and was therefore treated in a separate session.

Appendix VII shows (based on question # 14 of the inventory) the most important root parameters assessed in current root research. It follows from the appendix that Image Analysis is used increasingly to assess root length and root diameter. Also for this relatively new technology it was concluded during the workshop that an update and description of the method was necessary, considering the various systems and programs which are used by the participants (Appendix VIII).

Appendix IX finally makes clear that most studies on functioning of roots deal with the uptake of the major nutrients (NPK) and water. There is, however, a broad spectrum of external factors which are studied to investigate the effect on root functioning.

3. Report of the sessions

3.1. Session 1: Sampling methodology

Chairman: dr. G. Bengough
In his introduction, Glen Bengough pointed out that there is still much room for improvement in the assessment of root parameters. More attention should be paid to the positioning of samples as root densities decrease non-linearly with the distance from the plant. Van Noordwijk and colleagues provide sampling scheme's to account for this.

The storage and processing of soil cores can be a subsequent source of errors leading to an underestimate of root densities. Especially fine roots (with a great contribution to root density per unit weight) can easily be lost. Losses appear to be greater for samples that have been stored too long at too high a temperature and for samples containing stones. Root extraction can be improved if samples are incubated with dispersion fluids. Incomplete separation of roots and debris (including dead roots), however, may overestimate root intersections (with a grid) and thus root density.

The participants agreed that a certain standardisation for sampling strategy is needed.

Statistics should always play an important role both in designing sampling scheme's and in interpreting data both in terms of means and their variability. It was suggested to pay more attention to geo-statistics (Michigan State Univ) as individual sample values can not be considered independent.

It was suggested that models which predict the rooting pattern, like the one developed by Pages and colleagues (France), may be very helpful in defining optimum sampling scheme's. Participants concluded that a standardised ring research program would be valuable in order to have various methods compared by exchanging samples among several labs.

3.2. Session 2: Minirhizotron techniques

Chairman: dr. A.L. Smit
With Minirhizotrons, root quantification is directed at root countings per unit area and can be compared with trench wall methods. As such the method is affected with errors too, although minirhizotrons have become a major tool in root research in recent years.

Bert Smit reviewed the pro's and con's of minirhizotrons. There seems to be a lot of variation concerning installation procedures. As an example the angle with the vertical of the minirhizotrons was mentioned: it varies between horizontal to vertical. Recently an article appeared which mentioned 54° as optimal. Furthermore, installation may be problematic unless partial or complete refilling takes place. Inflatable minirhizotrons seem to deserve more attention as they overcome some of the problems frequently found at the interface of soil and minirhizotron wall. Even in the case of inflatable minirhizotrons, however, the number of roots rather than their length should be used for further processing since preferential growth along the wall may occur.

It was generally recognised that minirhizotrons are excellently suited for C-allocation and turnover studies though it may be difficult to discriminate between living and dead roots. UltraViolet (UV)-fluorescence seems to tell us more about the presence of phenolic substances rather than about the senescence of roots. Besides, it was suggested that live and dead may be inappropriate terms as even dead xylem vessels may still facilitate water transport and are hence to be considered active. Probably, UV-light can be used to distinguish species in mixed stands.

As indicated, observations from both auger samples and minirhizotrons may be affected with errors. Consequently, it is not surprising that linking these two techniques seldom produces Meluish & Lang's L= 2 x N. Even without errors the conversion factor may deviate from the value of 2 due to a non-random orientation of roots resulting from the crop growth stage, the relative contribution of various branching orders and temperature effects. With few exceptions, participants have often had difficulties in getting comparable root density values from auger samples and minirhizotron observations.

It was (as in session I) suggested that models, like the one developed by Pages and colleagues, may be very helpful exploring possible deviations from expected conversion factor values, finding the optimal angle of installation etcetera. Participants concluded that a standardised ring research program would be valuable in order to have various methods compared.

Correct root density assessments require a considerable effort. Consequently, researchers should decide a priori whether other parameters (e.g. number of root tips, rooting depth) would not suffice to answer their questions and, if not, at what accuracy root density should be determined. Crude methods (like visual calibration) may sometimes be perfectly suitable and save money and time. If, for instance, one is interested in nitrate uptake it is not so relevant to know whether root densities is exactly 7.5 or something in between 5 and 10 cm per cm^3. It would be worthwhile to develop and standardise visual methods for higher root length densities

3.3. Session 3: Assessment of physiological functioning of roots

Chairman: dr. E. George

After a keynote presentation by Eckhard George it was concluded that root researchers need a reliable criterion for distinguishing functional and non-functional roots. The physiological functioning of roots however is complex and consists of different independent processes. Roots produce and transfer substances which are then further used in the shoot (e.g. plant growth regulators). Roots also play a role in the avoidance of uptake of toxic elements like aluminium. The root function which was identified as the most important in root research was the uptake and transport towards the shoot of water and mineral nutrients. Again however these are two largely independent processes therefore needing different research methods.

The method to be used for measuring the root functioning depends on the research questions. One should consider whether actual or potential uptake has to be measured, whether a limitation by plant or by soil factors has to be expected, whether uptake should be expressed relative to root length, surface or volume. In addition the important role of rhizosphere processes and micorrhizae in the uptake of some nutrients should not be overlooked.

These preliminary considerations should result in the definition of the root activity of interest, and the scale and precision with which one wants it to be measured. A large overview of available techniques was given and discussed. These include labelling, staining, collection and analysis of root exudates or root samples. Implication of these techniques in a field situation however is often hazardous or not feasible.

Therefore in field research, traditionally, distinction is made only between live and dead roots. This is mostly done on a visual basis. This technique is rather arbitrary and can not be used for all plant species. An alternative is then to measure root fluorescence. However, ample experience of this method and evidence of its accuracy is still lacking.

It was remarked that "live" and "dead" roots are not clear terms as such. The root stele can still be functional in water transport while the outer root cells have already died off.

Final remarks were also made with respect to the importance of root activity in heterogeneous soils. In this type of soils also a large heterogenity in root activity is generated. As a consequence there can in these situations no scientific value be given at average figures of root activity. Heterogenity in the field can be mimicked at the lab by split-root experiments.

Experiments on a lab scale however are not always possible (e.g. trees).

3.4. Session 4: Image analysis in root research

Chairman: dr. W. Richner
Most root length-determination methods are based on manual or visual analysis of root samples. They are constrained by one or more of following factors: time of analysis, cost, limited resolution. An alternative method using computer-aided Image Analysis has been developed in recent years. This new tool is characterized by hard and software, the usage for washed root samples and minirhizotron images, and the different parameter it provides.

Root Image Analysis of washed roots
There is a low degree of standardization of hardware and software. Mostly, root length, area and mean diameter are provided. Few systems additionally provide distribution of root length vs. root diameter, number of root tips, information on root morphology, and architecture. There is no unique system that provides all these information. A general lack of these systems is the inability to discard debris from root measurements according to morphological traits.

Hardware
The standard equipment are CCD cameras (756 x 581 pixels). Relative inexpensive flatbed scanners (400 to 600 dpi) are gaining more importance. 3-D scanners as described by Smit and Groenwold (1992), are characterized by high resolution of 4000 x 3600 pixels. Used are all types of computers. Most widespread are IBM-compatible systems.

There is a large variety of products of digitizing boards depending on hard and software (standard products, e.g. Truevision targa cards).
The storage media for images varies from video tapes, hard disks, optical rewritable disks to DAT (digital audio tape) drives. The storage of large amounts of images on video tapes or computer hard disks is unsatisfactory and optical disks or DAT drives might become an alternative.

Software

The software is based on two main principles: the digital line-intercept methods (based on the Line-Intercept theories of Newman (1966) and Tennant (1975)) and edge detection (based on identifying the parallel edges of each root segment and separating the root from the background; most often followed by skeletonization algorithms resulting in center lines of the segmented root images). The first method is widely accepted as a standard method, is relatively fast and requires inexpensive equipment. Drawbacks are the sensitivity to non-random distribution of the roots and the requirement of clean root samples (without debris). Examples for the digital line-intercept method are Delta-T's Mk II (Harris and Campbell, 1991) and Delta-T "Scan" (Kirchhof, 1992).

The edge detection method is independent on random distribution of roots in a sample. In addition to root length and area, information on root morphology (e.g., branching patterns, root architecture, fractal dimension) are provided. Compared to the line-intercept method the edge detection method requires more processing time. Examples for that method are Smucker's system (unpublished), Fitter et al., 1991, Pan and Bolton, 1991, Berntson, 1992, and Fitter and Stickland, 1992.

Root Images Analysis of Minirhizotron Images

To analyze longevity and turnover of single roots of minirhizotron images there are two methods in use. The first method is tracing of roots on transparent sheets which requires no additional hardware but is very time consuming (e.g., Cheng et al., 1990). The second one is using computer programs, combined with manual or automated tracing roots. This method is less laborious, length and diameter measurements can be combined, identification and development stage codes may be assigned to the root, root tracing and identification codes may be saved and recalled and overlaid to follow the same roots at subsequent dates. Drawbacks of using this kind of computer programs are the interactively tracing of roots and the need of expensive hardware for digitizing of images. Examples are C-Map/Roots software (Hendrick and Pregitzer, 1993) and a color composite technique (Heeraman et al, 1993), which includes automated root tracing.

To analyze automatically morphological root parameters on minirhizotron images edge detection and filtering techniques are combined. Examples are Smucker et al. (1987), Casarin et al. (1991), Nater et al. (1992).

Conclusions

There is a wide interest to overcome the time consuming and laborious root sample processing by using computer-aided image analysis. Further demands on image analysis techniques have been developed by increasing usage of Nuclear Magnetic Resonance and X-Ray Tomography technique in root research.

Currently good products at reasonable prices are commercially available such as Delta-T "Scan" for washed root samples (root length, area, diameter classes) or will be soon available such as a program by A. Smucker, Michigan State University, for minirhizotron images (available in 1995, analyses a wide range of root parameters, requires an UNIX-based system). So far, no program is available for an automated analyzing of roots on minirhizotron images.
However, there is a need of collaboration with specialists from other fields, especially pattern recognition and image analysis to improve algorithms for better discrimination between washed roots and debris and for automated image analysis of minirhizotron images.

A collection of information about available image processing hardware, software and algorithms in a database that would be kept up to date is suggested to convey this information to interested scientists beyond this concerted action in cooperation with the american research groups of root image analysis. Furthermore, a documentation of the state-of-the-art is required in a handbook chapter.

A co-operation development or improvement of techniques, e.g., algorithms for the separation of debris from washed roots, would be desirable.

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3.5. Session 5: Model development

Chairman: dr. P. de Willigen
In this session a short survey was given on different types of models and the problems involved. Peter de Willigen distinguished between models in which uptake is governed by uptake kinetics (Barber, Nielsen) and models in which uptake is determined for the greater part by soil physical conditions and in which the plant is regulating uptake by a feed back principle (De Willigen & Van Noordwijk). Root length densities of 0.2 to 0.5 cm/cm^3 are sufficient for depletion of soil for nitrate. Root clustering or partical contact of the roots with the soil may change substantitally the required amounts of roots for depletion.

Lester Simmonds distinguished between lumped parameter models (SWATRE type) and "Single Root" models (anolog to the electrical resistance model). According to Simmonds the required amount of roots for water uptake (3 mm day) would be 5 km/m^2. With an exponential distribution of roots over the profile at 10 km/m2 (mostly in top layers) it means that at low water content soon "active" soil layers will contain < 5 km $root/cm^2$.

Senthold Asseng described a different kind of model in which the question was raised whether root assimilates were transported to soil layers according to economic principles (where is investment in roots economical?).

The discussion revealed that modelling root growth based on dry matter is extremely difficult because of the fact that the specific root length is very variable. In fact simulation must be done on a fresh weight basis which is almost impossible.

3.6. Session 6: New methods in root research

Chairman: dr. S.C. van de Geijn
During this session an inventory among the participants was made to establish which new methods have to be considered in this concerted action. Below the first list (not in order of priority):

- Develoment of statistical procedures to further refine "old methods" such as the pinboard method (Updating old methods with new statistical methods)
- Heat flow rate technique to measure transpiration *in situ*
- Root exudates (C-content)
- Turgor probes, root tips in soils
- The use of stable isotopes in root research
- How to assess root architecture from washed samples
- Functioning of roots in relation to NMR technique
- The use of ^{15}N in ammonium/nitrate uptake resarch
- Image analysis in root architechture and mychorrhiza
- Electical capacity measurements
- The use of minirhizotrons in relation to research about the mechanical influence of the soil to root development
- The use of Se in stead of S to see where new protein is built in to the roots
- Techniques to study P-uptake in relation to VAM-mychoriza
- the use of models for sampling strategy

The "Core Group" will decide on the second Workshop whether and which of these techniques will be described in the Handbook

4. Report of the Working groups

4.1. Minirhizotrons

Rapporteur: K. Groenwold

Installation

It was concluded that installation procedures should be better described or standardised with emphasis on the following items:

- Angle of installation
- Contact with soil (disturbed and non-disturbed soils)
- The material of the minirhizotrons: glass, lexane, open frame (flexible minirhizotrons)

Observations

With minirhizotrons the following observations are possible:

Observation	Question/Lack of standardisation
- # of root intersections/cm^2	What is the definition of a root intersection?
- length of roots/cm^2 minirhizotron surface	
- length of roots/cm^3	after assuming a certain "depth of view"?
- root turnover	what is the definition of root turn over? Criteria for dead or alive
- rooting depth	what is the definition of rooting depth
- horizontal distribution of roots in row crops	How to measure
- morphology of rooting (branching)	How to measure

Interpretation of minirhizotrondata

Goal

modelling
rooting depth
root turnover
root functioning?

Conversion to root length density

For uptake studies the lower rooting intensities are more interesting. However, calibration with auger sampling often means that the higher values are dominating the conversion factor.

If rootorientation is not at random, observing only the upper side of the minirhizotrons might lead to spurious results. But are additional observations at the lateral sides unbiased?

How important is the angle of installation of minirhizotrons in this respect? Is there a change in time of the conversion factor (due to changed orientation of root growth, or are roots seen longer on minirhizotron tubes in comparison to auger sampling).

Problems
Voids/spaces interfere with rooting depth, rooting intensity etc.

This problem is very difficult to tackle. How to determine the viability of the roots?

Who has experience with UV/ fluorescence of roots. Is there a relationship with age/functioning of roots or does the degree of fluorescence depend morhological or anatomical traits of the roots.

Suggested action
Develop flexible type of minirhizotron

Develop protocol for interpretation of images/recording of images

Calibration methods toward volumetric root length density

4.2. Image Analysis

Rapporteur: W. Richner
The participants in this group felt it was necessary to:

- Review the literature, in order to obtain a list of relevant publications dealing with image analysis
- Make an inventory on commercial and PD-software/algorithms and also on hardware
- To contact experts in this field (e.g. Morris Huck) who had plans to establish a database
- Maintain contact with in situ image analysis procedures

For more information and discussion points see Chapter 3.4

4.2.1. Sampling methodology

Rapporteur M. Schenk
Protocol of the working group "Sampling methodology and processing of samples"

The working group divided the problems in four parts
parameters
sampling
storage and processing
statistical analysis

It was decided to list the items to be discussed in detail and to mention related problems. The group did not aim of supplying already "solutions'" or "answers". This has to be done in the next step. The list given in the following might need complementation. The group emphasized that for all decisions on methodological aspects the target of the research has to be specified clearly.

1. Parameters

rooting depth (definition by critical root length density?)

root length density per volume of soil

heterogenity of root distribution (which method?)

soil-root contact

root architecture (root radius distribution, branching pattern, root tips)

root mass (dry matter) (how to clean the sample, loss of plant material, losses of inorganic and organic contents of roots)

growth factors (which data have to be collected for describing the conditions, temperature in the soil, water content, soil structure?)

carbon flow into the root

The available methods to determine the above listed parameters are:

trench-method
core break method
auger method
pin board method
profile wall method
minirhizotron.

It is suggested to discuss the auger method as reference method for evaluation of methods.

2. Sampling

spatial variability (which sampling strategy is supplying valid information?)

positioning of sample (improvement by modelling of root architecture?)

replicates

processing of data (how to aggregate the data over different positions of soil samples and soil depths?)

time schedule for sampling during crop growth (which data are the most valid?)

method of sampling (which auger diameter or other technical details for the mentioned methods)

complementation of methods

3. Storage and processing

soil samples (influence of time temperature and storage conditions in general)

extraction of roots (evaluation of available techniques and their accuracy; from soil samples how to distinguish between dead organic matter and living roots?)

procedure for measuring roots (subsampling, visual scoring method, image analysis)

4. Statistical analysis and models

conventional statistics

Geostatistics (how does this method contribute to increase the information flow from the data?)

4.3. Root activity

Rapporteur: E. George
Summary of work group discussion on root activity

There is an urgent need for practical methods to determine "root activity" under field conditions or for soil-grown plants. While several methods to determine some aspects of root activity are available, they appear to give unsatisfactory results, or are not applicable to the specific research interests of the participants.

The objective most often mentioned was to determine the localisation of uptake along the root or root system. This will be important for water, but also for nutrients (nitrogen, phosphorus, potentially toxic elements such as Cd). A number of questions is linked to this objective: how much can mycorrhizal fungi replace the function of the root in these uptake processes (and how can this be determined), how can the role of root hairs be determined under field conditions, and should priority be given to select methods to determine a general root activty or specific functions of the root? Depending on the research interest, it will also be important to determine transport properties of roots as well as uptake properties.

Soils are very heterogeneous substrates. This spatial heterogenity is not taken into account by most conventional techniques used in experiments for example on effects of soil constraints on root activity. Examples for soil heterogenity were given as mechanical impedance to root growth, soil water distribution or nutrient availabilty. In order to determine the potential activity of a root part, it will also be necessary to know more about the age but also about the "history" of a root. For example, is the root adapted to high or low nutrient supply? Were the rhizosphere conditions actively modified by the root? How does a root system react to changes of supply in time, for example of water? Thus, measurements of the effects of temporal heterogenity on the root system are necessary and will also result in more information on effect of soil environmental conditions on root longevity.

Before root activity can be determined, there must be information on the distribution of the root system in the soil profile (this is a link to groups I and II). Laboratory studies on root activity are necessary to determine the potential uptake rate or longevity of different root zones, but should use conditions (nutrient concentration in the solution, root zone temperature, soil density) similar to those in the field as much as possible. For practical purposes, it needs to be kept in mind that there is a need to consider how many active roots a plant requires (are there maybe too many roots anyhow and we do not need to consider active root length as a limiting factor), and how much of the "active" root length is in actual contact to the soil solution.

The methodology to determine root activity in soil-grown plants is not well established, so that no recommendations for joint experiments could be given at present. Rather, new research should focus on:

- techniques to determine the life history of a root
- methods to describe the reaction of a root to adverse soil conditions (adaptation *versus* critical damage)
- experimental techniques to model heterogenity of soil conditions (for example, split-root system)

In this respect, a coordinated research approach of different laboratories could be to use one plant species and common experimental systems while investigating different aspects of root activity.

5. Conclusions

5.1. Summary of decisions/further activities

A summary of the major points which were brought up during the workshop (sessions & working groups) as relevant for the Concerted Action:

- With the now available tools, including root growth models and statistical procedures like geostatistics, it is possible to investigate in more detail sampling scheme's in relation to expected heterogenity of roots etc.
- Information on the effects of washing and storing root samples on quantity and chemical composition will be collected among the participants and included in the Handbook
- A good description of the technique to install minirhizotrons in the field is needed as well as a protocol for the interpretation of minirhizotron observations
- An international comparison between minirhizotron data and a calibration methods towards volumetric root length density (by core sampling/ trench or pinboard techniques) will be made
- The development of a flexible type of minirhizotron will be in study and if possible initiated.
- A review of the literature will be made in order to obtain a list of relevant publications dealing with image analysis
- An inventory on commercial and PD-software/algorithms and also on hardware will be made
- In the Handbook a description of methods to determine root activity of soil-grown plant will be included, e.g. modern staining technique etc.

It is proposed to work the details for some of the points mentioned above further out on the second workshop

Other activities foreseen

- A newsgroup/discussion list on root research methods will be started on the Internet
- Selection of the Coregroup- Drafting of the titles of the chapters of the Handbook
- Preparing of the second Workshop in September 1995

5.2. Adjusted Workplan (January 1995)

Following steps can be distinguished:	period (y/qu)	participant
Preparation of an overview of present research		
questions involving root studies:		
* inventory of research issues with participants	94/2	1
* inventory of current techniques/facilities	94/2	1
* draft overview of research	94/4	1
* draft overview of relevant root/soil properties	95/1	1
Establishment of Core Group of experts		
* Workshop 1. Start-up and definition activity exchange of data and present position	94/2	User group
* Workshop report	94/4	1
* selection of core group	95/1	1
Implementation of outline of joint activities		
* try-out of draft experimental protocols in (field-) experiments	(94/3)-96/3	User-group
* elaboration of novel methods (i.e.image analysis)	95/1-96/4	wgr RIA
* titles and draft key-words of chapters for Handbook	95/2-95/3	Core-group
* support and editing assistance	95/3-96/2	1
Evaluation of progress and adjustments to protocols		
* Workshop 2: evaluation of activities	95/3	Core-group
* adjustments of protocols, and methods	96/1-96/2	User-group
* definition of key root characteristics	95/2-95/4	1
* workshop report	95/4	1
* try-out in (field-) experiments	95/2-96/3	User-group
Preparation of Handbook on root research methodology		
* drafting and revision of chapters for Handbook	95/3-96/2	Core-group
* meeting of lead-authors	96/3	Core-group
* finalize chapters and editing of Handbook	96/3-96/4	1, Core-Gr.
* printing of Handbook	97/1-97/2	1
* Workshop 3	97/2	User-group Sc.Commun.
* Workshop report	97/3	1

Coordination

Overall coordination, including initiation of inquiries, organisation of workshops, editing of the Handbook and Workshop reports will be done and organised by participant 1 (Van de Geijn and Smit)

6. Root research and literature references from the participating research groups

6.1. Belgium

Laboratory of Soil Fertility and Soil Biology,
Katholieke Universiteit Leuven.
Jan Buysse, Erik Smolders, Roel Merckx.

General

All research activities within the group are linked with the development of a conceptual model describing the bioavailability of nutrients and xenobiotics in the soil. Purpose is to predict levels of soil-born constituents in plants by modelling all plant-physiological and soil chemical processes involved. Up to now, efforts have been concentrating on the description of the processes involved for nitrogen and 137Cs. In the short run, research on Cadmium will be started as well.

Nitrogen

As an important macronutrient, nitrogen nutrition is known to have a strong influence on both total plant growth and on biomass partitioning over roots and shoots. For spinach plants, relations were derived describing the interdepence of growth, biomass partitioning and the level of nitrogen nutrition. The uptake capacity of the root for nitrogen was described in function of the plant's N nutrition state and the external nitrate concentration.

Special emphasis is given to the processes of biomass partitioning. Theoretical growth models (e.g. Thornley, 1972) have hypothesized a determining role for C and N substrates in determining both growth and biomass partitioning. In a series of experiments with different genotypes under different N nutrition levels and different light regimes, it is investigated whether growth and biomass partitioning can indeed be controlled and predicted by the levels of C and N substrates.

Finally, the soil factor is included in the model. At this moment, a model simulating most soil and plant processes at the individual plant scale, is being developed. The simulation model will be used to determine the importance of the different plant and soil parameters on the uptake of nitrate and on the growth of the different plant parts.

Radiocaesium

Agricultural land in Europe has been contaminated with radioactive caesium and strontium after the Chernobyl accident in 1986. Nowadays, the problem is still pertinent in some acid and peaty soils having a small capacity to adsorb Cs irreversibly.

In nutrient solution experiments, the relation between Cs and K uptake was investigated. It was concluded that at K levels higher than 1 mM, Cs uptake was not so much related with K uptake but rather with the external Ca concentration. This was interpreted by considering the Cs uptake dependent on the partial loading of Cs on the root ion exchange complex. However, at external K concentrations lower than 1 mM, the uptake of Cs is seen to increase considerably independently of the external Ca concentration. Influx and efflux experiments for K and Cs were done subsequently to enable interpretation of these results. The internal distribution of Cs in the plant was investigated by measuring the upward and downward flows of Cs and K as influenced by the K nutrition state, the external K concentration and the genotype.

Finally, the importance of the different processes in the soil and the plant will again be weighed by bringing them together in one simulation model.

Cadmium

The bioavailability of Cd is strongly influenced by complexing agents in the soil solution. Experiments relating uptake by the plant and the speciation of Cd in the soil solution are now carried out in cooperation with the CSIRO, Division of Soils, Adelaide, Australia.

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6.2. Danmark

Royal Veterinary and Agricultural University, Copenhagen
N.E. Nielsen

Research

Some Danish studies on root and root function
Development of Methodologies

Studied on plant parameters controlling the efficiency of nutrient uptake from soil which included studies of the kinetics of nutrient uptake by plants from soil, root length and root density in soils were initiated more than 20 years ago (1, 2).

Now we are studying processes in the rhizosphere using a newly developed technique (3, 4 og 5).The technique allow us to study rhizosphere processes of plants at various states of nutrition and growth. According to this method plants are pre-grown and experimental grown under regulated climatic and nutritional conditions. In the experimental set up a root mat develops on a nylon screen (53 µm mesh) in a column of test soil. At termination of experiments the test soil columns are separated from the root mat, quickly frozen in liquid and nitrogen and 'sliced' into thin layers (0.2 mm) using refrigerated microtome.

The results show that the developed plant growing technique allows us to study for instance phosphorus depletion in the rhizosphere of plants grown at various states of nutrition and growth with i) a high degree of rhizophere resolution, ii) control of water and nutrient supply to the plants, iii) control of soil pH in the soil root interface, and iiii) good repeatability.

Using $^{14}CO_2$-pulse-labelling technique rhizodeposition of carbon by field grown barley was studied successfully(7, 8).

Modelling which also includes modelling of the root dynamics is a valuable technique in our simulation of important processes in the soil plant atmosphere system (9, 10 11).

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6.3. France

INRA (Montpellier & Colmar)
F. Tardieu & S. Pellerin

Root elongation as a response to intercepted light, soil temperature and soil water potential.
The objective of the work is to model, with a daily timestep, root elongation, ramification and trajectories as a function of three key environmental variables, namely soil temperature, cumulative light intercepted by leaves and soil water status. A first series of experiments has provided elements for modelling root elongation as a result of intercepted light and soil temperature. On going experiments concern (i) the effects of soil water status on elongation, and (ii) modeling the trajectories of secondary roots

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6.4. Germany

University of Hannover
Institute of Plant Nutrition, (Fachbereich Gartenbau)
M. K. Schenk,

Genotypic differences of N efficiency in cauliflower

Cauliflower takes up large amounts of nitrogen but less than half of it is exported from the field with the curd. It is speculated that genotypes have different N efficiencies.

Aim of the research is to identify genotypes having different N efficiencies and to study causal relationships. Efficiency is differentiated in "N uptake efficiency" and "N use efficiency".

The research programme is in cooperation with the Research Station for Arable Farming and Field Production of Vegetables at Lelystad and a breeding company. The three genotypes used in this programme were preselected by the breeding company and were supposed to have different N efficiencies.

The genotypes were planted in the field in 1993 on two sites, one in Holland the second in Germany on the research station of the Department of Horticulture. The trials had two nitrogen levels: N supply of the soil and 250 kg (N_{min} + $N_{fertilizer}$). To characterize uptake efficiency spatial distribution of roots and nitrate content in the soil were measured.

The results of the first year showed that on both sites one variety was less N-efficient in terms of curd quality. This observation was in accordance with the experience of the breeding company. Other results were:

- The nitrogen level did not affect root length density in the soil in both vertical and horizontal direction.
- Root length density was up to 50 higher next to the plant compared to further distances. The decrease from 12,5 cm to 45 cm distance from the plant was small. Differentiation of vertical distribution of roots in the deeper layers 15-30 cm, 30-45 cm and 45-60 cm was minor.
- Root length densities decreased with depth of the soil. The upper layer contained twice or 4 times more roots than the layers 15-30 or 30-45 cm, respectively. The differentiation between 30-45 cm and 45-60 cm was minor.
- Root length densities on the site in Germany were about 1/3 lower than in Holland. This was partly due to a smaller shoot mass in Germany, because the crop was grown 4 weeks more towards the end of the vegetation period.
- Comparison of the genotypes based on the root:shoot-ratio shows that the more N-efficient genotype had more roots per unit of shoot. This was consistent on both sites and more pronounced than in terms of root length density. However, there was also a tendency towards higher values for the same variety.
- The Nmin residue remaining in the soil at harvest at the N deficiency level was similar for alle genotypes suggesting that variation of root length densities did not affect the NO_3-exhaustion from the soil.

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Christof Engels, Eckhard George, Horst Marschner

Summary of activities
We investigate the effect of soil environmental conditions on root growth, root activity, and root-shoot-relations in several plant species. In specific projects, the influence of different root zone temperatures on root growth, shoot demand for nutrients, and nutrient uptake of maize and wheat is studied. Furthermore, root growth and nutrient uptake of maize is followed during and after a period of drought in the topsoil. This is carried out with a view to determine whether drought in a fertile topsoil also results in plant nutrient deficiency, in addition to direct drought effects on plant water potential. It is also studied how much water roots in deeper soil layers can absorb and transport to the shoot in relation to plant demand. In another experiment, the reaction of Norway spruce, pine, and Douglas fir to local nutrient supply in soil is compared. One of the aims of this project is to study whether increased root growth in a fertilized soil zone is followed by a decrease in root growth in non-fertilized, low-nutrient soil zones. Additionally, at different forest sites, the seasonal time-course of root growth is monitored using "root windows" in Norway spruce and beech stands with different atmospheric input of nitrogen. In most other projects, rhizoboxes with different dimensions are used to determine root growth non-destructively along a transparent front cover. The soil in the boxes can be watered at different soil depths, and soil water potential and nutrient concentrations in the soil solution are monitored at regular intervals. In some experiments, the root zone temperature is controlled by placing the rhizoboxes in large cooling containers. We plan to use image analysis to quantify the root morphology and root growth along the transparent cover. By supply of ^{14}C in some of the experiments, we plan to follow below-ground carbon distribution.

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Humboldt University
Berlin
Senthold Asseng (now CSRIO Australia)

An overview of root projects at Humboldt University Berlin, Germany

A four years root growth modelling project was carried out by Senthold Asseng, Christel Richter (both Humboldt University Berlin, Germany) and Gerd Wessolek (Technical University Berlin, Germany) from 1990 to 1993. To simulate vertical dynamics of biomass, water and nitrogen in a winter wheat crop, an existing wheat crop model and an existing soil model (water, nitrogen, temperature) were linked by a root routine. The root extended model considers the dynamics and feedback's of the major crop characteristics and soil properties. The root routine simulates the following:

- dynamic shoot root ratio controlled by ontogenesis, water and nitrogen stress;
- root growth in horizontal soil layers which considers such soil properties as water, nitrogen, air, density, temperature and the recent root distribution;
- ontogenetically and soil specifically controlled exudation and mortality rate;
- root age and soil water driven root activity;
- water and nitrogen uptake.

The main process of the modelled root growth is a root distribution algorithm according to an "economic principal of the plant". Daily assimilates available for root growth are distributed beginning from the top layer to deeper layers according to soil layer hospitality. A downwards limitation for the assimilate distribution is modelled by calculating daily maximum rooting depth. This principal is confirmed in several comparisons of simulation and field measurements.

The new system model is calibrated with data sets of Müncenberg, Germany in 1989/90. A validation of the model was carried out with further data sets of the same site in the following year of 1990/91. Frequent root measurement data were obtained by using monolith method and hand washing the root samples in both years. Further measurements using minirhizotrons in a water stress experiment with wheat were carried out for model-experiment comparisons at Michigan State University in 1992.

The model is used to simulate water and nitrogen use efficiency of the crop. The complex system behaviour is studied by simulation experiments with different environmental impacts like soil restrictions and climate change assumptions.

The new model is a tested tool for a wheat crop on sandy soil and different tasks concerning the behaviour of the atmosphere-crop-(including root)-soil system. It can be used for further modelling and special analysis in the examination of that complex system.

A further crop modelling project including root growth is supervised by Christel Richter (Humboldt University) with barley. This project started in 1993 and goes through 1996. Root dynamic is followed by frequent auger sampling and hand washing of samples in a field experiment at the experimental station Blumberg of Humboldt University.

6.5. Great Brittain

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RESEARCH ON ROOTS AND RELATED SUBJECTS
The work of the Soil Science Group at the Silsoe Research Institute includes work on root growth, soil structure, soil mechanics, and soil water physics. Other work within the Institute is on aspects of image analysis which are relevant to root research including both hardware and software (e.g. image segmentation algorithm) approaches.

The Soil Science Group has projects and expertise in se6eral aspects of root research. These are described briefly below.

a) Ability of the roots of different varieties of rice to elongate through strong soil.
In this project we are varying soil strength in a controlled way and are measuring the effects of soil strength on the elongation rate of rice roots. The effects of water stress and temperature are also being considered.

   We are attempting to determine the shape of the "response surface" (elongation rate as a function of soil strength, water stress and temperature), and to develop a model for this.

   As part of this work, we have collaborated with Dr G Bengough at the Scottish Crop Research Institute to make direct comparisons between our methods for measuring the maximum growth pressures of roots.

(b) Effects of seedbed conditions on the establishment of seedlings of carrot and onion.
The work includes the determination of the "response surfaces" of the roots of carrot and onion as described for rice in (a), above.

   The physical and mechanical properties of field soils are being determined, and will be combined into an integrated model for plant response to soil conditions.

   The mechanical properties of soil crusts and shoot emergence forces are also being determined.

(c) The effects of soil physical conditions and soil structure on root pathogenic fungi.
This is mainly a "fungus" project, and is examining the effects of soil conditions on the propagation of pathogenic fungi. However, cotton, radish and wheat are being used as host plants.

(d) Effects of soil structural parameters on root behaviour.

   We have no research in progress in this area at present. However, we have many years of research experience and many publications on aspects of this problem.

   Earlier work investigated the effects of seedbed structure and sub-soil structural parameters (such as biopore density and crack patterns) on root environment and dynamlcs.

All the above four areas are in need of further research, both separately and in terms of interactions between them and other factors in the soil-root-crop continuum. We are keen to develop new research programmes on the effects of soil structure, soil strength, and of other physical factors on plant roots.

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Research

Root research at SCRI is integrated within a broader framework of the crop and soil sciences, and in particular with soil microbiology, soil physics, crop physiology, and more fundamental aspects of the physiology of root function. Research is performed at a range of scales, from the physiology of individual cells, through lab and controlled environment experiments, to full scale field experiments.

Some topics which are receiving particular attention at present include

- interactions of plant roots with the soil biology of the rhizosphere.
- the use of stable isotopes to study nutrient and water uptake by old and young roots, carbon flow in the rhizosphere, and the effects of elevated CO_2 on nutrient uptake and carbon cycling.
- effects of soil physical conditions on root growth (including effects of mechanical impedance, aeration and water stresses, and root-shoot communication).
- use of computer simulation of root architecture to study field sampling techniques and strategies (collaborative project with Dr L Pages, INRA, Avignon).

In addition to the above research areas, there are established research groups looking at the physiology of transport within roots, the infection of roots by pathogens, and the applications of non-linear mathematics in biology.

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Research projects

Biogeochemical cycling in agriforestry systems

In this project the effect of climatic factors (UK, Italy, Greece) on root production with time by tree, grass and clover is investigate

- variation in root longevity
- influence of AM infection on root longevity
- influence of root dynamics on N cycling

Root development in a N. Michigan mixed woodland

To asses changes in the hollow ground species development of a recolonising woodland community and especially

- periodicity of production
- root diameter
- root longevity
- AM infection
- development of woody roots

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6.6. Italy

Instituto Sperimentale Agronomico
Bari
D. De Giorgio, D. Ferri, A. Castrignano

Research
Information's on research activity undertaken in our Institute for a Concerted Action in the EC-AIR Programme.

OBJECTIVES

The main objective of our working group is studying root growth and development of herbaceous plants in order to understand and quantify processes influencing root dynamics, such as soil tillage, irrigation, fertilisation and cropping system.

All gathered information, together with the principal meteorological parameters, will be used for validating a simulation model of root growth.

Our activity is included in a more comprehensive research project granted by the Italian Ministry of Agricultural and Forestry and aimed to optimise agricultural management in order to meet opposite requirements, both yield and environment protection ones.

METHODOLOGY

The trial have been carrying out in field on experimental plots, managed with different soil tillage and N fertilisation treatments and cropped with durum wheat, sunflower and grain sorghum.

Root dynamics is studied by using minirhizotron method and monitored with a REES 92 monochromatic video camera.

Some days before sowing, the minirhizotrons are inserted at 45 degree angles to the soil surface and in order to insure an excellent minirhizotron coil contact a 50-51 mm diam core is removed by a tractor mounted hydraulic soil sampler. A 10-cm tube section is left above-ground and wrapped with black plastic tape to exclude light.

The video camera is inserted in each tube at particular times during the crop cycle and the tube number, date and depth are video recorded. The root number is counted on a monitor in the laboratory and recorded using a computer program. Treatment codes are assigned to each tube number and the coded files are then transferred to the statistical and graphics package SAS/SATS and GRAPH and elaborated.

For the whole crop season at quite regular time intervals the following physical and chemical measurements are made: soil water content, bulk density (using a neutron and gamma probe), soil temperature (using PT 100 sensor) and the main soil nutrient contents.

Moreover, in our group is going to study the effects produced by root activity on the fate of soil nutrients. Soil will be then collected form the minirhizotrons at such a distance not affecting root recordings and will be separated form the "bulk soil" by shaking.

Organic C, pH and macro and micro nutrients will be determined on each soil.

The measured parameters will be used in input to a mathematical model that is a new advanced version of CERES-Sorghum model. The program will simulate daily root length density and carbo-hydrate partitioning along the whole rooting depth during the growing season and will also calculate some stress factors, expressing the effects of both dynamic and static constraints on root growth (coarse fragment content; soil strength, temperature, aeration, nitrogen availability).

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Research

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<u>Graduate thesis on root research</u>

Flavio Pinamonti. Effects of the reduction of the soil tillage depth on the root growth in winter wheat (Triticum aestivum L.) (in 1984 and 1985).
Ezio Callegari. Effects of the reduction of the soil tillage depth on root growth in maize (Zea mais L.) in different crop rotations (in 1985 and 1986).
Piecristiano Brazzale. Root architecture in maize and soybean in relation to soil tillage methods and crop rotation (in 1989 and 1990).
Antonio Bonsembiante. Effects of soil type on root architecture in soybean (wall glass method) (in 1991).
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Teofilo Vamerali. Root length density, yield and nitrogen allocation in maize selected for low input (in 1993) (PhD Thesis).
Antonio Rigo. Root distribution in two rapeseed cv in relation to plant densities, nitrogen fertilization and its time of distribution (in 1993-94).

6.7. The Netherlands

AB-DLO Haren
P. de Willigen, G. Brouwer, F. Meijboom, M. van Noordwijk (now Indonesia)

The research is concentrated on quantifying and modelling nitrogen flows under field vegetables. In the project a quantification and mathematical description of nitrogen dynamics is done as a function of different fertilisation levels and supply of crop residues. A special point of interest is the interaction between root distribution and uptake.

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AB-DLO Wageningen
S. v.d. Geijn, J. Groenwold, A. Haverkort, J. Schröder, A.L. Smit

Research

- Utilisation of nitrogen in relation to rooting characteristics (maize and field grown vegetables)
- Effects of nematodes (potato cyst nematode, root knot nematodes) on root growth and root functioning (peas, potatoes)
- CO_2-effects on rooting dynamics

The experiments are carried out in the field and in a modern rhizotron facility (The Wageningen Rhizolab)

Methods to assess the root distribution and intensity:

- minirhizotrons
- Auger sampling
- trenchwall method

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6.8. Portugal

UNIVERSITY OF EVORA -
Oliveira, M.R.G

Research

In the last years we have been carrying out field experiments in association with mainly two programs on irrigation.

A - Experiments made in association with the research program on " The technological quality of processing tomatoes in the Mediterranean countries".

Under drip irrigation, a small soil-root volume per plant causes problems if application of water or nutrients is delayed for even a short period of time. In the context of this program we have developed two trials:

TRIAL 1 - a 2-year trial to evaluate the effects of four different water regimes on root growth and distribution of tomato, under drip irrigation.

Treatments: irrigation at -10; -20; -40; and -60 kPa corresponding to 85; 65; 55; and 50 % of field capacity, respectively.

Methodological approach used in this trial was the trench profile method (Bohm) and a 5 x 5 cm grid.

The paper about this experiment was submitted for publication in the Journal of the American Society for Horticultural Science.

TRIAL 2 - a 2-years trial to study water regimes and nitrogen levels on tomato root growth at four dates along the growing season.

Treatments - besides water regime, nitrogen levels (50, 150, and 250 kg ha), were applied with drip irrigation.

Methodological approach- used in this trial was the collection of soil-root samples, with an auger, at different depths. Samples were taken in three different places, in relation with plants near the emitter and replicated for plants between emitters, until maximum rooting depth..

B - Experiments made in association with the research program on "The technology of surface irrigation, with long furrows, in Mediterranean Brown soils".

Surface irrigation systems are adequate methods for Mediterranean regions, but Mediterranean brown soils have an heterogeneous infiltration process and the control of water along the furrow is important to prevent water waste at low end and non uniform soil water content.In the context of this program we have studied maize root growth under two field experiments:

TRIAL 1- to study maize root growth in relation with water distribution along the furrow and between furrows.

Treatments - Distances from the irrigation pipe (70, 170 and 270 m) Furrows were located between each two rows (1.5 m apart). Methodological approach - in this trial soil-root cores were taken on five dates along the growing season. Samples were located in four different places.

TRIAL 2 - with the objective of studying the effects of loosening the B horizon by subsoiling on maize root growth.

Treatments - A - control; B - subsoiled; C - subsoiled with mole draining.

Methodological approach - the minirhizotron technique , endoscope and camera.

Papers, from these two trials were accepted for oral presentation at the conference on

Agricultural Engineering (Italy - 29th Aug. to the 1st of Sep., 1994).

A full report will be sent for publication in the "Journal of the Agric. Eng. Res.".

THIS YEAR we are trying to improve our experience with the minirhizotron technique. A new field experiment with the objective of studying sunflower root growth in relation with water distribution in the soil profile, resulting from furrow irrigation, is being carried out.

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6.9. Spain

Instituto de Recursos Naturales y Agrobiologia, CSIC
Dr Felix MORENO Investigador Cientifico, Head of the Department of Sustainability of the Soil-Plant-Atmosphere System
Dr Enrique FERNANDEZ, Colaborador Cientifico, memher of the mentioned Department

Research activity concerning the root system:
General aim: influence of different management systems concerning irrigation and tillage on the root system.

Species: species of agricultural interest in the area (olive tree, maize, cotton, wheat).

Experimental conditions: normally field conditions, with some lab experiments.

Studied aspects: Root distribution, root activity, root dynamics and root histology. Most of the experiments has been integrated studies concerning several of those aspects.

Main actual interest: Sap flow through roots of different kind and under different soil conditions.

Root distribution:
Influence of the soil water regime on the root distribution of olive trees. Field experiments with trees under dry-farming, drip-irrigation and pond irrigation.
Root distribution on cotton cropped on saline soils. Field experiments.
Root distribution on wheat under different tillage systems. Field experiments.

Root depth of maize cropped at the traditional way of the Guadalquivir Valley (Sevilla). Field experiments.

Methods: The trench method and the auger-sampling method. Determinations of weight, diameter, length (intersection line method)

Root activity:
Uptake of water and nutrients from different areas of the soil explored by the root system of olive trees under drip-irrigation and dry-farming conditions. Field experiments.

Method: Labelling with 32p; liquid scintillation counter.

Root dynamics:
Influence of the soil water regime on the dynamics of the root system of olive trees. Field experiments with trees under dry farming, drip-irrigation and pond irrigation.

Root dynamics on cotton cropped on saline soils. Field experiments.

Methods: Minirhizotron observation tubes. Endoscope for direct observation .

Root Histoloqy:
Influence of the soil water regime on the root histology of olive trees grown in containers.

Method: Optical microscope observations.

Sap flow measurements (experiments to start on Sep. 94):
Measurement of the root activity and root sap flow on olive trees under different water conditions. Field experiments.

Method: The heat-pulse technique; combination with soil water depletion measurements.

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6.10. Sweden

Research

References

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6.11. Switzerland

Institute of Plant Sciences
Swiss Federal Insitute of Technology (ETH, Zürich
Hubert Buergi, Dr. Boy Feil, Markus Liedgens, Dr. Walter Richner, Dr. Alberto Soldati, Prof. Peter Stamp.

Research

Stress physiology in maize

a) Chilling-stress
These investigations are mainly focussing on root morphology, root internal anatomy, and water uptake of maize seedlings as influenced by low temperatures. A better knowledge

of seedling root growth at low temperatures is beneficial for maize breeding for cool climates and for the design of sustainable cropping systems (e.g., mulch systems with a lowered topsoil temperature due to plant residues). Previous field and growth chamber studies were mainly dealing with the investigation of morphological and anatomical root traits and their suitability for yield prediction.

Applied methods: Monolith methods, growth-chamber based system that allows for vertical temperature gradients in the root zone, image analysis of washed roots, manual measurement of anatomical traits.

b) Drought stress
Cooperative Projects are undertaken with Kasetsart University (Bangkok, Thailand) to get a better understanding of mechanisms limiting maize growth at low water supply.

Previous root studies focussed on the effects of low water supply on seedling root growth.

Applied methods: Manual measurement of root morphological and anatomical traits, time domain reflectometry (TDR).

Root growth of maize seedlings as influenced by localized supply of ammonium and nitrate

Main objective of these investigations is to study the response of root morphology and physiology to an enhanced ammonium supply. For ecological reasons, NH4 should be the preferred source of nitrogen in plant nutrition.

Applied methods: Split-root system, image analysis of hydroponically grown roots, analysis of nitrogen contents in shoots and roots.

Investigation of sustainable cropping systems

The main objective of these project is to investigate the response of plant roots to adverse conditions often encountered in sustainable systems. Such knowledge will be useful in the development of ameliorated and new systems. Research focuses primarily on the investigation of root growth and interspecific competition in intercropping systems.

a) Maize mulch systems
Previous and ongoing projects are investigating spatial and temporal patterns of maize root growth and root competition for water and nitrogen in living-ryegrass mulch systems. Experiments are conducted in the field and in a non-weighing lysimeter system.

b) Other systems
Planned projects will investigate root growth and competition in cassava-bean intercropping systems in Columbia and in perennial ryegrass-white clover stands in a free-air carbon dioxide enriched experiment (FACE).

Applied methods: Auger sampling, image analysis of washed roots, minirhizotrons in the field and in a now-weighing lysimeter system, suction lysimetry, TDR.

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Appendix I:

List of participants and cooperating research groups

Group	Name	Participant 1st workshop	Adres
B_1	dr. J. Buysse	yes	Cath. University Leuven Lab. voor Bodemvruchtbh. en Bodembiol. Kardinaal Mercierlaan 92 B-3001 Leuven Belgium tel +32 16 321609 jan.buysse@agr.kuleuven.ac.be fax +32 16 321997
B_1	dr. R. Merckx	no	Cath. University Leuven Lab. voor Bodemvruchtbh. en Bodembiol. Kardinaal Mercierlaan 92 B-3001 Leuven Belgium tel +32 16 321605 roel.merckx@agr.kuleuven.ac.be fax +32 16 321997
CH_1	dr. W. Richner	yes	ETH Institüt für Pflanzenwissenschaften Universitätsstrasse 2 CH-8092 Zürich Switserland tel +41 1 26323878 richner@ipw.agrl.ethz.ch fax +41 1 2620496
CH_1	dr. P. Stamp	no	ETH Institüt für Pflanzenwissenschaften Universitätsstrasse 2 CH-8092 Zürich Switserland
D_1	dr. E George	yes	Hohenheim University Inst. of Plant Nutrition (330) Postfach 7005 62 70593 Stuttgart 70 Germany tel +49 711 4593664 george@rs1.rz.uni-hohenheim.de fax +49 711 4593295

Group	Name	Participant 1st workshop	Adres
D_1	dr. Barbara Dinkelaker	no	Universiteit Hohenheim Inst. für Pflanzenernährung Postfach 700562 70593 Stuttgart tel. - fax +49 62303 68336
D_1	dr. H. Marschner	no	Universität Hohenheim Inst. of Plant Nutrition (330) Postfach 7005 62 70593 Stuttgart 70 Germany tel +49 711 4593664 fax +49 711 4593295
D_2	dr M. Schenk	yess	Universität Hannover Inst. für Pflanzenernährung Herrenhäuserstrasse 2 D-30419 Hannover Germany tel +49 511 7622626 fax +49 511 7623611
D_3	dr. S. Asseng	yes	CSIRO Division of Plant Industry Private Bag PO Wembley WA 6014 Australia tel + 61 99 387 0615 s.asseng@ccmar.csiro.au fax + 61 9 387 8991
DK_1	dr N.E. Nielsen	yes	Royal Veterinary and Agricultural University Soil, Water and Plant Nutrition 40, Thorvaldsensvej DK-1871 Frederiksberg C Copenhagen Denmark tel +45 35 283480 Niels Erik Nielsen Pagsci. kvl.dk Tara.S.Gahoonia@agsci.kvl.dk fax +45 35 283460

Group	Name	Participant 1st workshop	Adres
E_1	dr. E. Fernandez	yes	Inst.de Recur. Natur. Y Agrobiol. de Sevilla Campus de Reina Mercedes S/N Aptdo 1052 41080-Sevilla Spain tel. +34 5 4624711 jefer@cica.es fax +00 34 5 4624002
E_1	dr. F. Moreno	no	Inst.de Recur. Natur. Y Agrobiol. de Sevilla Campus de Reina Mercedes S/N Aptdo 1052 41080-Sevilla Spain tel. +34 5 4624711 jefer@cica.es fax +00 34 5 4624002
F_1	dr. S. Pellerin	no	INRA Laboratoire D'Agronomie 28 rue de Herrlisheim B.P. 507 68021 Colmar Cedex France tel +33897 24923 pellerin@colmar.inra.fr fax +33897 24933
F_2	dr. F. Tardieu	no	INRA Lab. d'Ecofysiologie 34060 Montpellier France +33 67 61 26 17 tardieu@msdos. montpellier.inra.fr fax + 33 67 52 21 16
F_3	dr. P. Denoroy	no	INRA Station de Bioclimatologie 78850 Thivernal-Grignon France + 33 130 81 55 60 denoroy@bcgn.grignon.inra.fr fax + 33 130 81 55 63

Group	Name	Participant 1st workshop	Adres
F_4	dr. L. Pages	no	INRA, Centre d'Avignon Ecophysiologie et Horticulture Site Agroparc, Domaine St-Paul 34914 AVIGNON CEDEX. 9 Loic @ AGRO1.AVIGNON.INRA.FR Fax: (33) 90 31 60 28
GB_1	dr. D. Atkinson	no	SAC West Mains Road Edinburgh EH9 3JG UK + 44 31 667 1041 fax + 44 31 667 2601
GB_1	dr. J. Hooker	yes	SAC Land resources Crabstone estate Bucksburn Aberdeen ab91tq UK + 44 224 715455 fax + 44 224 713423
GB_2	dr G. Bengough	yes	Scottish Crop Research Institute Invergrowie Dundee DD2 5DA Scotland UK tel +44 382 562 731 cepgb@scri.sari.ac.uk fax +44 382 562 426
GB_3	dr. A. R. Dexter	yes	Silsoe Research Institute Wrest Park, Silsoe Bedford MK45 4HS UK tel + 44 525 860 000 dexter@bbsrc.ac.uk fax +44 525 860 156
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Group	Name	Participant 1st workshop	Adres
GB_4	dr. L. Simmonds	yes	Univ. of Reading Dept. Soil Science London Road Reading UK + 44 734 318911 asssimmd@reading.ac.uk fax + 44 734 869858
GB_4	dr. M. Rounsevell	no	Cranfield Univ. Soil Survey and Land Research Centre Silsoe, Bedfordshire MK45 4DT UK + 44 525 860 428 ssm@silsoe.cranfield.ac.uk fax +44 525 86 1147
GR_1	dr. N. Papamichos	no	University of Thessolonika 54006 Thessolonika Greece tel + 30 31 998924 fax +00 30 31 472633
I_1	dr. A.M. Castrignano	yes	Instituto Sperimentale Agronomico via C. Ulsiani 5 70 125 Bari Italy tel +39 80 5366922 romito@.evm.uniba.it??? fax +39 80 5563020
I_1	dr .D. De Giorgio	no	Instituto Sperimentale Agronomico via C. Ulsiani 5 70 125 Bari Italy tel +39 80 5366922 fax +39 80 5563020
I_2	dr. S. Bona	yes	Univ. di Padova Dipartimento di Agronomia Ambientale e Produzionii Vegetali Via Gradenigo 6 35131 PADOVA Italy tel +39 49 8071945 colterb@ipdunix.unipd.it fax +39 49 8070850

Group	Name	Participant 1st workshop	Adres
I_2	dr G. Mosca	no	Univ. di Padova Dipartimento di Agronomia Ambientale e Produzionii Vegetali Via Gradenigo 6 35131 PADOVA Italy tel +39 49 8071945 agra09@ipdunivx.it fax +39 49 8070850
NL_1	G. Brouwer	yes	AB-DLO vestiging Haren P.O. Box 129 9750 AC Haren (Gr) Holland + 31 50 337361 brouwer@ab.agro.nl fax + 31 50 337291
NL_1	J. Groenwold	yes	AB-DLO vestiging Wageningen P.O. Box 14 6700 AA Wageningen Holland + 31 8370 75873 groenwold@ab.agro.nl fax +31 8370 23110
NL_1	dr. M van Noordwijk	No	ICRAF/SARRP Forest Research Centre Jalang Gunung Batu 5 p.o.box 161 Bogor 16001 Indonesia + 62 251 315 567 ICRAF-Indonesia@cgnet.com fax + 62 251 315 234
NL_1	ir. J. Schröder	yes	AB-DLO vestiging Wageningen P.O. Box 14 6700 AA Wageningen Holland + 31 8370 75965 schroder@ab.agro.nl fax +31 8370 23110

Group	Name	Participant 1st workshop	Adres
NL_1	dr. A.L. Smit	yes	AB-DLO vestiging Wageningen P.O. Box 14 6700 AA Wageningen Holland + 31 8370 75977 a.l.smit@ab.agro.nl fax +31 8370 23110
NL_1	dr. S.C. van de Geijn	yes	AB-DLO vestiging Wageningen P.O. Box 14 6700 AA Wageningen Holland + 31 8370 75850 geijn@ab.agro.nl fax +31 8370 23110
NL_1	dr. P. de Willigen	yes	AB-DLO vestiging Haren Oosterweg 92 P.O. Box 129 9750 AC Haren (Gr) Holland + 31 50 337304 willigen@ab.agro.nl fax +31 50 337291
P_1	dr M. R. Oliveira	yes	Universidade de Evora Dep. de Fitotechnia Apartàdo 94 7001 Evora Codex Portugal tel +351 66 711188 mrol@evunix.uevora.pt fax +351 66 711163
S_1	dr. T. Lundborg	yes	Univ. Agric. Sciences Plant Breeding S-268 31 Svalov Sweden + 46 418 67077 tomas.lundborg@vff.slu.se fax + 46 418 67081

Appendix II:
Program First Workshop

First Workshop EEC-Concerted Action AIR3-CT93-0994
The dynamics of rooting patterns in relation to nutrients and water in soils
Development, standardisation and documentation of methodologies
14-16 June 1994
Kasteel Hoekelum, Edeseweg 124, 6721KE Bennekom
tel. + 31 8380 32124
fax + 31 8380 32208

PROGRAM

Tuesday 14 June

- 12.30h	**Registration**
12.30 - 13.30h	**Lunch**
13.30 - 14.50h	**Contributions of Participants I** Everybody is invited to inform the other participants about his/her research project(s) with the emphasis on the methodological aspects or methodological difficulties. 13.30-13.50 v.d. Geijn 13.50-14.00 Dexter/Whalley 14.00-14.10 George 14.10-14.20 Richner 14.20-14.30 Lundborg 14.30-14.40 Hooker 14.40-14.50 Asseng
14.40 -15.20h	**Tea/Coffee break**
15.20 -17.30h	**Contributions of Participants II** 15.20-15.30 Bengough 15.30-15.40 Simmonds 15.40-15.50 de Willigen/Brouwer 15.50-16.00 Nielsen 16.00-16.10 Buysse **16.10-16.30 Break** 16.30-16.40 Bona 16.40-16.50 Schenk 16.50-17.00 Castrignano 17.00-17.10 Oliveira 17.10-17.20 Fernandez 17.20-17.30 Smit/Groenwold/Schroder
19.00h-	**Dinner**

Wednesday 15 June

Based on the inventory (see annex Table 2) we have chosen 6 subjects which shall be treated in *more detail. In each session we would like to have i) a broad outline of the techniques now* available and the degree of standardization (or lack of standardization) ii) identification of existing problems which impair further development iii) identification and initiation of relevant coöperative activities in the next years

8.30	- 10.00h	Session 1 **Sampling methodology (dr. G. Bengough)** Statistics of sampling in the field Errors in washing and storage procedures Scaling problems
10.00	- 10.30h	Break
10.30	- 12.30h	Session 2 **Minirhizotron technique (dr. A.L. Smit)** Installation technique Conversion of minirhizotron data to volumetric root length density More efficient recording storage and retrieval of images Distinction between species
12.30	- 13.30h	**Lunch**
13.30	- 15.00h	Session 3 **Assessment of physiological functioning of roots (dr. E. George)** Distinction between dead and alive roots in samples Effect of soil structure and physical conditions on root functioning
15.00	- 15.30h	**Break**
15.30	- 17.30h	Session 4 **Image analysis in root research (dr. W. Richner)** New possibilities with image analysis for quantitative measurements of root length, diameter distribution, root morphology/architecture
17.30	- 19.00h	**Excursion to the Wageningen Rhizolab (J. Groenwold)**
19.00	-	**Dinner**

Thursday 16 June

8.30	- 10.00h	Session 5 **Model development (dr. P. de Willigen)** Modelling of root architecture/morphology Modelling of uptake Interactions betwen root distributions and root activity Standardization of root parameters
10.00	- 10.15h	**Break**
10.15	- 11.00h	Session 6 **New methods in root research (dr. S.C. v.d. Geijn)**
11.00	- 13.00h	**Working groups**
13.00	- 14.00h	**Lunch**
14.00	- 15.00h	**Operational plan**
15.00		**Closure of the workshop**

Appendix III:

Inventory Form

EEC-Concerted Action AIR3-CT93-0994

The dynamics of rooting patterns in relation to nutrients and water in soils

Development, standardization and documentation of methodologies

1. Name: ....
2. Institute/University: ....
3. Adress: ....
4. City: ....
5. Country: ....
6. Telephone: ....
7. Fax: ....
8. E-mail: ....

- In this inventory you are asked to give details on those projects in your research group in which the physiological role of roots is one of the key factors. In the case of more than one project please give the details **for each project separately** by filling in Appendix A.

9. Could you mention (in order of priority, number 1(high) to 5 (low)) some of the techniques in root research (or the main technical drawbacks) which in your opinion should receive attention in the concerted action, e.g.
   - installation of minirhizotrons in the field
   - conversion from minirhizotrondata to volumetric root length density
   - development of new methods
   - use of image analysis (purpose?)
   - assessment of physiological functioning of roots, also separation between dead and live roots
   - washing procedure of soil auger samples
   - model development of root growth/distribution/morphoplogy
   - other
     1. ....
        ....
     2. ....
        ....

10. **Could you mention other research groups in your country which deal with root research as described in the concerted action?**
    ....
    ....
    ....

Appendix A

Inventory

First Inventory EEC-Concerted Action AIR3-CT93-0994
The dynamics of rooting patterns in relation to nutrients and water in soils
Development, standardization and documentation of methodologies

1. Title of project:
....
....
....
....
....

2. Projectidentification (e.g. number):
3. Project leader: ....
4. Institute/University: ....
5. Labour in scientists_months/year/project: ....
6. Start of the project (year): ....
7. End of the project (year): ....
8. Indicate the main research question(s) for this project:
....
....
....
....
....
....
....
....
....
....

10. Plant species involved: ....

11. Indicate in which environment(s) the experiments are done (more than one tick is possible):
Field.. ❑; soil type(s): ....
Rhizotron-like facility........................... ❑; with undisturbed soil ❑ with disturbed soil ❑
Greenhouse.. ❑; potsize < 2l ❑ <10l ❑ >10 l ❑
Growth chambers................................ ❑
Pot experiments.................................. ❑
Other, viz. .... ❑
.... ❑
.... ❑
.... ❑

12. Indicate the level of integration at which the research is done:
field/crop-level .... ❑
plant level .... ❑
organ (root) level .... ❑
cellular level .... ❑

13. Which technique(s) are used in this project to quantify root dynamics or root morphology:

Minirhizotrons .... ❑
Profile-wall .... ❑
Auger sampling .... ❑
Core-break method .... ❑
Rhizotron-facility .... ❑
Split-root. .... ❑
Other, viz. ....
....
....
....
....
....

14. Which root parameters are quantified?

Root dynamics

❑ Length of roots per plant/volume soil /area soil/
Description of method: Line Intersect Method/ ....
....
....
....
....

❑ Mass of roots
❑ dry weigh per plant/volume soil/area soil/ ....
❑ fresh weight per plant/volume soil/area soil/ ....

❑ Diameter of roots Method ....
....
....
....
....

❑ Area of roots Method ....
....
....
....
....

❑ Turnover of roots Method ....
....
....
....
....
....

❑ Morphology of roots Method ....
....
....
....
....
....

Functioning of roots

Uptake of water .... ❑
N .... ❑
P .... ❑
K .... ❑
other, viz. .... ❑

Influence of external factors on root growth:
Pathogens .... ☐ viz. ....
Soil physical factors .... ☐ viz. ....
Soil chemical factors .... ☐ viz. ....
Other, ... ☐ viz. ....
....
....
....

15. Is image analysis used in the project? .. ☐yes ☐ no
If yes, please indicate briefly hard- and software, and how it is used
....
....
....
....
....
....
....
....

16. Please describe briefly any on-going field experiments in 1994 which could possibly be used for methodological comparisons in this concerted action.
....
....
....
....
....

Appendix IV:

Inventory results (Research Questions)

Question number 8: Main research questions per project

Number	Name	Titel of project	Research question	Plant species
1	Atkinson/Hooker	Biogeochemical cycling in agriforestry systems	The effect of climatic factors (UK, IT, Gr) on root production with time by tree, grass and clover	*Acer, Prunus avium, Holium perenne, Trifolium repens*
2	Atkinson/Fogel	Root development in a N- Michigan mixed woodland	Assess changes in the hollow ground species development of a recolonising woodland community and especially; periodicity of production, root diameter, root longevity, AM infection, development of woody roots	*Prunus penns., Pteridium aq, Rubus sp. Hieraccum sp., Acer saccharium*
3	Atkinson/ Millard	Effect of elevated CO_2 on carbon flux into roots and associated microorganisms	Effect of N and CO_2 levels on the growth of roots, the flow of C to the roots, flow of C to AM fungi, root exudates	*Lolium perenne, Plantago lanceolata*
4	Bengough/Pages	Studying methods for measuring root length (minirhizotrons, root mapping, core sampling) using simulation models of root architecture in 3 dimensions	What are effects of root system architecture and sampling position on the root lengths calculated using minirhizotrons, root mapping, auger and core-break techniques	several
5	Robinson/Fitter/ Raven	Integrating the effects of elevated CO_2 on linked processes in the plant-soil-microbe system using multiple stable isotopes	Does elevated CO_2 influence roots system architecture (length, branching, turnover)	wheat

Number	Name	Titel of project	Research question	Plant species
6	de Giorgio	Root growth dynamics of durum wheat and nutrient evolutionin the soil	Relationship between nutrient availability and uptake and root distribution (minirhizotrons) Model development to simulate root length density and carbohydrate partitioning along the whole rooting depth	durum wheat
7	Dexter	Factors influencing seedling establishment	mechanical impedance and water stress on root elongation rates and shoot emergence forces	carrot, onion
8	Dexter	The physiological and cellular basis for the growth of rice roots through strong soil	genetic variability in the ability of different varieties of rice to penetrate strong soil	rice
9	Dexter/Gilligan	Spatial dynamics of soil borne plant pathogens	Effects of soil physical conditions and structure (esp. in the rhizosphere) on propagation of plant pathogenic fungi	cotton, wheat, radish
10	Moreno/ Fernandez	Study of the system Soil-Plant-Atmosphere on Olive and Almond Crops under different water regimes and drip irrigation	Response of root system to water regime (root distribution, root activity, root dynamics and root histology). Assessment of water balance, response of the aboveground part of the plant	olive, *almond*
11	Lundborg	Genetic variation in uptake and transport of cadmium in wheat and oats	Importance of root growth/morphology/activity for uptake of cadmium in the soil profile Genetic variations in root growth/morphology/activity	wheat, oats
12	Marschner	Effects of nutrient supply on root growth, root turnover and carbohydrate metabolism of trees	Effect of local supply of nutrients (N, Mg) on root growth and longevity *of spruce and pine*	spruce, pine
13	Marschner	Effects of soil drying and rewetting on root growth and nutrient uptake of maize	Plasticity of root growth under conditions on non-homogenous water and nutrient supply Factors involved in the regulation of root growth in different soil zones Limiting factors for nutrient uptake in drying soil Recovery of root growth and nutrient uptake after rewetting the soil	maize

Number	Name	Titel of project	Research question	Plant species
14	Marschner	Root growth dynamics and nitrogen uptake of roots and mycorrhizal hyphae at forest sites with different atmospheric nitrogen input	Time pattern of root growth at forest sites with different climatic conditions Nitrogen uptake by different root forms and at different times of the year	spruce, beech
15	Merckx	Soil-to-plant transfer of nutrients:development of an availability concept	Influence of nutrient conditions on root growth and root distribution; influence of plant growth on the "nutrient supply capacity" of the soil	spinach, wheat, bean
16	Mosca	Molecular basis of the interaction between rhizobia and leguminous plants, optimization of biological nitrogen in cropping systems	Exploitation of N_2-fixation in soybean in a crop rotation with cerals (maize and bread-wheat). Study of root exploration in different crops, soils and with different	
17	Nielsen	Rhizosphere processes of various plant species and genotypes controlling the efficiency of P and Trace elements uptake from soil	why do palnt species and cereal genotypes differ?	lupin, pea, sugar beet, linum, quinoa, rape, rye, maize, barley, wheat
18	Nielsen/Jensen	Use of root contact concept in calculating root water uptake under field conditions	Simulation of water utptake in relation to contact roots and soil	rape
19	Oliveira	The dynamics of rooting patterns of crops under surface irrigation technology in Mediterranean soils	Study of rooting patterns in relation to: soil water distribution and the effect of loosening the B-horizon	maize, sunflower
20	Papamichos/ Alifragis	Biogeochemical cycling in agroforestry systems	quantification of partitioning and cycling of NPC within trees at different locations and climates Interaction between mycorrhiza and roots in relation to transfer of nutrients and C and the influence of temperature on these processes	*acer, pinus, trifolium repens, lolium perenne*

Number	Name	Titel of project	Research question	Plant species
21	Soldati/ Richner	Root growth and competiion in maize mulch systems	- investigate root growth of maize in a living mulch rye-grass crop - investigate root competition for N, water and space - investigate root turnover and longevity	maize and It. *ryegrass mulch*
22	Schenk	Genotypic differences of N-efficiency in cauliflower	Genotypic differences in N-uptake efficiency and N-use efficiency	cauliflower
23	Schröder	Growth and functioning of maize roots as related to the utilisation and losses of nitrogen	Improvement of nutrient utilisation of maize. Identification of factors that may improve synchronisation and synlocalisation of roots and nutrients such as nutrient placement and increase soil temperature (i.e. postponement of planting dates)	maize
24	Smit/Groenwold	Effects of heterogenity (roots, water, nutrients) on uptake and utilisation by vegetable crops	Improvement of nitrogen utilisation of field-grown vegetables by improvement of synchronisation and synlocation (with special emphasis on succession of crops within a year)	spinach, beet root
25	de Willigen	Quantifying and modelling nitrogen flows under field vegetables	Quantification and mathematical description of nitrogen dynamics as a function of different fertilisation levels and supply of crop residues. Special point of interest : interaction between root distribution and uptake	Brussels sprouts, leek spinach broccoli
26	Tardieu	Root elongation as a response to intercepted light, soil temperature and soil water potential	Model root elongation, ramification and trajectories as a function of temperature, light and soil water status	sunflower, maize
27	de Willigen/ Dijksterhuis	Production Sudano Sahilienne (PSS)	Development of sustainable agricultural systems in the Sahel Region, improvement of pastures with leguminoses. Special interest in the role of woody species in natural savannes (hydraulic lift, pumping up of nutrients	cowpea, stylosanthes, andropogeen, trees

Appendix V:

Inventory results (Techniques to be treated in the workshop)

Question nr 9 Techniques of root research which should be treated in workshop

Name	Papa-michos	Atkin-son	Merckx	Dex-ter	Fernan-dez	Marsch-ner	de Giorgio	Lund-borg	Mosca	Oli-veira	Rich-ner	Schenk	Tar-dieu	Niel-sen	Ben-gough	Smit	de Willigen	Schrö-der	#	aver. priority
Assessment of physiol. functioning of roots (dead and alive)	3		2	1		1	2	2	4		4	2	2[1]	5	1	1	3	2	15	2.3
Model development	5	5[2]	1	2		5		5		3	5	3	1	3	3	4	2	1[3]	15	3.2
Conversion minirhizotron data to Lrv and mass	2	2			1	3	4		2	2			3[4]	1	4	2[5]		3	12	2.4
Use of image analysis		3	5			2	3		1		1			4		3	4	4	10	3.0
Development of new methods	1		4		2	4	5		5					2	2		1[6]		9	2.9
Installation of minirhizotrons	4						1	4	3	1						5			6	3.0
Washing/storage procedures of soil/root samples			3							4		1					2[7]		4	2.5

1 The use of ^{14}C and ^{13}C tracers to assess root growth, C-partitioning, dead/alive roots
2 Development allometric relation ships for tree root systems
3 including modeling the effects of root distribution on nutrient and water availability and the consequences for production
4 The use of rhizotrons and mapping in the field
5 Including assessment of turnover
6 Updating old methods (e.g. pinboard) to obtain data for models
7 Loss of dry matter and nutrients during washing and storage

Name	Papa-michos	Atkin-son	Merckx	Dex-ter	Fernan-dez	Marsch-ner	de Giorgio	Lund-borg	Mosca	Oli-veira	Rich-ner	Schenk	Tar-dieu	Niel-sen	Ben-gough	Smit	de Willigen	Schrö-der	#	aver. priority
Sampling methodology (Scaling problems + Statistical problems)		1									3[8]								2	2.0
Distribution of root activities in the root system according to root development								1											1	1.0
Modification of minirhizotron technique (more efficient recording, storage and retrieval of images											2								1	2.0
Effect of soil structure and physical conditions				3															1	3.0
Root architecture/Root morphology								3											1	3.0
Interactions root distribution/-root activity					3														1	3.0
Distinction between different root species		4																	1	4.0
Standardization of root parameters										5									1	5.0

8 Statistical issues in root research (e.g. analysis of problematic data; use of non-traditional techniques like geostatistics

Appendix VI:

Inventory results (Techniques currently used)

Question nr 13: Techniques used to quantify root dynamics and morphology

		Mini-rhizotrons	Profile wall	Auger Sampling	Core-break	Rhizotron	Splitroot	Other
1	Atkinson/Hooker	x		x				
2	Atkinson/Fogel					x		
3	Atkinson/Millard	x						direct extraction from pots
4	Bengough/Pages	x	x	x	x			
5	Robinson/Fitter/Raven							Roots grown in long thin tubes (1m x 0.02 x 0.04 m) with one removable side. Roots visible beneath removable side. Destructive harvests taken
6	de Giorgio	x						
7	Dexter							Special apparatus in the laboratory under different combinations of mechanical and water stress
8	Dexter							Special apparatus in the laboratory under different combinations of mechanical and water stress
9	Dexter/Gilligan							Visilog, x-ray, ct-scan
10	Moreno/Fernandez	x	x	x				
11	Lundborg						x	Root boxes
12	Marschner							Root boxes
13	Marschner					x	x	
14	Marschner							Ingrowth cores, root windows

		Mini-rhizotrons	Profile wall	Auger Sampling	Core-break	Rhizotron	Splitroot	Other
15	Merckx							Washing of whole plant system
16	Mosca	x	x	x		x		
17	Nielsen							Studying of rhizosphere processes in thin soil layers of different proximity to roots
18	Nielsen/Jensen			x				
19	Oliveira	x		x				
20	Papamichos/Alifragis	x		x	x			
21	Soldati/Richner	x		x				
22	Schenk			x				
23	Schröder		x	x		x		
24	Smit/Groenwold	x		x		x		
25	de Willigen		x				x	Pinboard
26	de Willigen/ Dijksterhuis							Pinboard
27	Tardieu					x		Following root elongation in hydroponics and windows in the field Incorporation of ^{14}C

Appendix VII:

Inventory results (Root parameters)

Question nr 14: Root parameters

nr.	Project leader	Length	Mass of roots	Diameter	Area	Turnover	Morphology	**Other**
1	Atkinson /Hooker	IA[9] and C.Map root program	dw and fw	from IA and microscope	IA	Cohort analysis (seq. images of video frame)		
2	Atkinson /Fogel	LI[10] and IA		microscope		Acetate tracings/videoimages		
3	Atkinson/ Millard	IA	dw			video cohort analysis		
4	Bengough/ Pages	length and number intersecting with planes					branching intervals, lag times before branching, root growth rate	Generation of root system model
5	Robinson/ Fitter/Raven	IA	dw	IA		video cohort analysis	IA, length internodes, # root tips	
6	de Giorgio	conversion from # roots with minirhizotrons						
7	Dexter	direct measurement with callipers		microscope & callipers				
8	Dexter	direct measurement with callipers		microscope & callipers				
9	Dexter/ Gilligan							
10	Moreno/ Fernandez	LI	dw	manual classification			minirhizotrons	Histology of roots using the paraffin inclusion method

[9] *IA= Image analysis*
[10] *LI= Line Intersect Method*

nr.	Project leader	Length	Mass of roots	Diameter	Area	Turnover	Morphology	**Other**
11	Lundborg	computer calculation					root boxes	
12	Marschner	IA	dw fw			staining		branching (IA)
13	Marschner	IA	dw fw					branching (IA) along the wall of the rhizotron
14	Marschner	IA						IA
15	Merckx	LI	fw	microscope	calcula ted			
16	Mosca	Comair	dw	microscope	calcula ted			
17	Nielsen				special metho d			
18	Nielsen/ Jensen	LI	dw		calcula ted			
19	Oliveira	LI	dw					
20	Papamichos /Alifragis	minirhizotrons & core break	dw	core-break	core-break	core-break		
21	Soldati/ Richner	conversion from minirhizotron data	dw	IA	calcula ted	minirhizotron images and ROOTS software		
22	Schenk	LI?						
23	Schröder	LI & conversion from minirhizotron data						
24	Smit/ Groenwold	LI & conversion from minirhizotron data & IA	dw	IA		cohort analysis		UV-fluorescence
25	de Willigen	LI	dw	microscope	calcula ted		classification in thin (< 1mm) and thick roots	
26	de Willigen/ Dijksterhuis	LI	dw					
27	Tardieu	IA						

Appendix VIII:

Inventory results (The use of Image Analysis)

Question 15: Use of image analysis in the project

	Name	Software/Hardware
1	Atkinson /Hooker	C.Map Root Magiscan
2	Atkinson /Fogel	C.Map Root Magiscan, also used on dynamics of fungi associated with roots
3	Atkinson/ Millard	C.Map Root Magiscan
5	Robinson/ Fitter/ Raven	Magiscan hardware, software written at York Univ. For length of internodes, # of root tips, mean diameter
9	Dexter/ Gilligan	VISILOG, X-ray and CT-scanning. CT-scans are downloaded in Visilog
12	Marschner	Image-C by Imtronic (Berlin) with CCD Vision Camera Model XC-711P and PACOMP 486 computer
13	Marschner	Image-C by Imtronic (Berlin) with CCD Vision Camera Model XC-711P and PACOMP 486 computer
14	Marschner	Image-C by Imtronic (Berlin) with CCD Vision Camera Model XC-711P and PACOMP 486 computer
16	Mosca	not yet
20	Papamichos/ Alifragis	80286 microprocessorwith TARGA 16 image board (Truevision Inc) and C-Map Roots software. To assess changes in individual roots in time
21	Soldati/ Richner	Host Computer (Sun SparcServer 470), Image processing hardware and IMCO/S software (KONTRON) München), Prog Res camera with resolution 3000 by 2300 pixels (Kontron) Robotic Camera system. Used for morhoplogical traits and automated analysis of digitised Minirhizotron images (in development)
24	Smit/Groenwold	Hardware: Macintosch fx (20/160 Mb). High resolution 3D-scanner (3600 x 4000 pixels); Optical rewritable disk. Software: NIH-Image, Scil-Image, TCL-Image. Used for length of root samples, turnover

Appendix IX:

Inventory Results (Research on functioning of roots)

Question nr 14: Functioning of roots

nr.	Project leader	H_2O-uptake	N-uptake	P-uptake	K-uptake	Other	Influence of external factors on root growth
1	Atkinson /Hooker						
2	Atkinson /Fogel	x	x				
3	Atkinson/ Millard	x					CO_2
4	Bengough/ Pages						
5	Robinson/ Fitter/ Raven	x	x				water, N, CO_2
6	de Giorgio		x				physical and chemical factors
7	Dexter						mechanical and water stress (temperature)
8	Dexter						Mechanical resistance, temperature
9	Dexter/ Gilligan						Gaummannomyces gram., Rhizoctonia, structure and water stress
10	Moreno/ Fernandez	x		x			water content, impedance, irrigation system (pond, drip)
11	Lundborg					Cd	Cd
12	Marschner		x			Mg	nutrient supply
13	Marschner	x	x	x	x		soil water content, nutrient availability
14	Marschner		x				temperature
15	Merckx						nutrient availability

nr.	Project leader	H_2O-uptake	N-uptake	P-uptake	K-uptake	Other	Influence of external factors on root growth
16	Mosca		x				
17	Nielsen	x	x	x	x		
18	Nielsen/ Jensen						
19	Oliveira	x					soil water distribution and strength
20	Papamichos/ Alifragis	x	x	x			pathogens, physical and chemical factors, mycorrhizal infections, species interations
21	Soldati/ Richner	x	x				different water regimes, different nitrogen levels, competition with living mulch crop
22	Schenk						
23	Schröder	x	x	x			N-placement, slurry placement, temperature
24	Smit/Groenwold	x	x				water, temperature, nitrogen, nematodes
25	de Willigen		x				
26	de Willigen/ Dijksterhuis	x	x	x			depth of water front, soil pan
27	Tardieu	x					soil water potential, temperature, carbon flux tot the root