

KOMMISSIONEN FOR DE EUROPÆISKE FÆLLESSKABER  
KOMMISSION DER EUROPÄISCHEN GEMEINSCHAFTEN  
COMMISSION OF THE EUROPEAN COMMUNITIES  
COMMISSION DES COMMUNAUTÉS EUROPÉENNES  
COMMISSIONE DELLE COMUNITÀ EUROPEE  
COMMISSIE VAN DE EUROPESE GEMEENSCHAPPEN

**EURATOM**

Årsberetning 1975

**PROGRAM BIOLOGI - SUNDHEDSBESKYTTELSE**

Jahresbericht 1975

**PROGRAMM BIOLOGIE - GESUNDHEITSSCHUTZ**

Annual Report 1975

**PROGRAMME BIOLOGY - HEALTH PROTECTION**

Rapport Annuel 1975

**PROGRAMME BIOLOGIE - PROTECTION SANITAIRE**

Relazione Annuale 1975

**PROGRAMMA BIOLOGIA - PROTEZIONE SANITARIA**

Jaarverslag 1975

**PROGRAMMA BIOLOGIE - GEZONDHEIDSBESCHERMING**

**1975**

**II**



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The annual reports in this volume were prepared under the responsibility of the heads of the research teams, set up under the various contracts, and were submitted in this form to the Commission and its contractual partners.

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Membres du Comité consultatif en matière de gestion  
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soil-plant relations, radiation analysis, food  
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\* Bericht 1975 verspätet eingetroffen, siehe Ende Band II.  
Report 1975 arrived late, see end of volume II.  
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FORSCHUNGSTÄTIGKEIT ANWENDUNGEN LANDWIRTSCHAFT

RESEARCH ON APPLICATIONS IN AGRICULTURE

RECHERCHES RELATIVES AUX APPLICATIONS AGRONOMIQUES



MUTAGENESE, BODEN-PFLANZEN BEZIEHUNGEN,  
STRAHLENANALYSE, LEBENSMITTELKONSERVIERUNG

MUTAGENESIS, SOIL-PLANT RELATIONS,  
RADIATION ANALYSIS, FOOD CONSERVATION

MUTAGENESE, RELATIONS SOLS-PLANTES,  
ANALYSE PAR RAYONNEMENT, CONSERVATION ALIMENTS



Contractant van de Commissie: Institute of the Association EURATOM-ITAL, Wageningen, the Netherlands.

Nummer van het contract: 094-72-1 BIAN

Hoofd van de groepen voor onderzoek: Dr. Ir. D. de Zeeuw.

Algemeen onderwerp van het contract:

APPLICATIONS OF NUCLEAR METHODS IN AGRICULTURE AND BIOLOGY

- behaviour of natural chemical elements and pollutants in soils.
  - uptake, transport, accumulation, redistribution of mineral elements and pollutants in plants.
  - mutation breeding, incompatibility, mutagenesis.
  - food irradiation.
  - genetic control of insect pests.
  - development of nuclear methods for agriculture, environmental and plant-biological research.
- 

Algemene omschrijving van de uitgevoerde werkzaamheden:

In the soils and plant parts of the 1975 programme, the following topics were dealt with:

- the working out of a preliminary simulation model of the behaviour of the fumigant methylbromide in soils and first experiments on the uptake of  $\text{Br}^-$  by lettuce.
- a mathematical model describes the influence of the nutrient concentration of the solution in the surroundings of a root, of root growth and plant growth on uptake rates. Appropriate mixtures of synthetic resins, simulating a soil; became available. Maize plants were grown under controlled conditions for the purpose of collecting basic data (1) in relation to the working out of a further improved model and (2) about rates of growth, transpiration and ion absorption. A two compartment growth chamber has been built.
- further study of various aspects of the kinetics of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake and of the redistribution of N in plants for protein filling of seeds. Uptake of major nutrients is now better understood on the basis of an improved theoretical model.
- preliminary experiments comparing the uptake by spinach of free Cd and of Cd complexed by a ligand-clay.

Topics of interest for mutation breeding, incompatibility and mutagenesis research were:

- interesting new mutants i.e. in ornamentals by the combination of the adventitious bud technique and mutation induction by irradiation.
- the effect of DTT (dithiothreitol) upon radiosensitivity, mutation frequency and mutation spectrum, and its interference with the dose-fractionation effect on radiation resistance in *Saintpaulia ionantha* H. Wendl cv. Utrecht.
- In *Achimenes*, autotetraploids produce a much higher mutation frequency and probably a (much) wider mutation spectrum as compared to the parental diploid.
- further control and improvement of the adventitious bud technique in *Saintpaulia* and particularly in *Chrysanthemum*, using *in vivo* detached leaves and *in vitro* mainly pedicel explants.

- continuation of the study on the generation of new self-incompatibility alleles, which in *Lycopersicon peruvianum* Mill. and *Nicotiana glauca* Link et Otto apparently originate in the style or pistil resp. of inbred populations. In *Nicotiana glauca* Link et Otto the largest nonsatellited acrocentric chromosome is the S-bearing one.
- further studies concerning the involvement of peroxidase isoenzyme 10 in the rejection of incompatible pollentubes in styles of *Nicotiana glauca* Link et Otto.

In relation to food-irradiation, the following topics are of interest:

- increase of the radiation resistance of bacterial spores by loading them with specific metals might be associated with the oxygen effect. Disulphide bonds in the outer coat of spores play a role in determining their resistance to combined radiation and heat treatments.
- direct and indirect effects of wateractivity characteristics of different media on radiation resistance of a *Citrobacter* species.
- increase of phenolic compounds in fruits and vegetables, as the most typical response to radiation stress.
- continuation of the study of the technological aspects of the preservation of e.g. cut endive, cabbage, mangoes, onions, steampeeled potatoes, by means of ionizing radiation.
- further extension of the practical application of the food irradiation procedure, paying also attention to legislation, marketability and international trading.
- study of the reproductive function of Groot Yorkshire sows in relation to the wholesomeness of irradiated total diets.

Topics in the field of radiation genetics of insect pests were:

- structural chromosome mutations in *Hylemya antiqua*, Meigen induced either by X-rays or fast neutrons concerned: X-linked translocations, pericentric inversions. Besides cytological observations and the crosses for the production of homozygous lines, experiments were done on inbreeding, in fieldcages and concerning competition and density effect among larvae.
- with respect to *Tetranychus urticae* C.L. Koch research concentrated on viability of structural chromosome mutations in homozygous conditions, on re-irradiation of such lines for inducing new complex rearrangements and release techniques for sterile and substerile insects.

In the methodology part of the programme topics were:

- improvement of the embedding technique of plant material in relation to microlocalization of soluble substances.
- irradiation treatment of sludge reduces its specific resistance to filtration, increases the compressibility of the sludge solids. Laboratory experiments contributed to explaining these effects.
- developments aiming at automation of operation, data collection, recording and analysis in several experimental set-ups.
- measurements of density in granular material in an elevator system, of moisture transport in cattle-food pellets, on liquid/liquid displacement from packed beds, of residence time of food in the stomach of a sheep.
- improvement of Cherenkov-counting of  $\beta$ -emitters.
- promotion of nuclear methods, advice, courses, edition of newsletters.



The programme for 1975 has once more been carried out in close cooperation with other scientific institutes and organizations.

Examples of this collaboration are:

- on different aspects of the application programme within working-groups of the European Society of Nuclear methods in Agriculture (ESNA);
- on pollution, radioactive and other, with the Biology group at Ispra and institutes in the Netherlands, Belgium and Germany;
- on mutation breeding (vegetatively propagated crops, protein improvement, disease resistance) and incompatibility in higher plants in the Mutation Breeding Contact Group;
- cooperation to projects concerning the testing of irradiated food, wholesomeness testing set up by the Organization for Economic Cooperation and Development (OECD) and the International Atomic Energy Agency (IAEA). In this respect also collaboration exists with institutes in the Netherlands, Germany, Denmark, Belgium.
- research on genetic control of insect pests, coordinated in Section VII of the TNO working group "integrated control of Insect Pests" and in the joint European Working Group of the "Organisation Internationale de la Lutte Biologique" (OILB). Cooperation with projects of the IAEA and of the entomology programme of the Biology Division.

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P.B. 48, Wageningen, The Netherlands.

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Changes in the Scientific Staff

Ir. P.W.F. de Vrijer left the Institute to accept research duties elsewhere. Temporary members (post-graduate fellows) responsible for particular aspects of the programme: ir. M.A. Daelemans, dr. A.W. Spanjers, G. Zurlini, M.Sc., dr. E.M. Perea Dallos, dr. T. Luczkiewicz.  
Several guest-workers have spent 6 to 12 months at the Institute.

Resultaten van het project No.

Hoofd van het team en wetenschappelijke medewerkers:

F. van Dorp, M.J. Frissel, P. Poelstra and J. Sinnaeve.

Titel van het project:

The ionic-composition of the soil solution as a function of time and place, with respect to the ion uptake by plant roots.

---

Beschrijving van de resultaten:

Data from maize (*Zea mays* L., cv. Ona) grown on nutrient solution in gravel (see project No. 6) are used for the simulation of the nutrient concentration of the solution around the root.

The following terms are used: 1. experimental uptake rates which are for water calculated from the water consumption and for nutrients from measured nutrient contents of harvested plants. 2. massflow rates which are the uptake rates of water multiplied by the concentrations of nutrients in the solution around the roots, and 3. transfer coefficients which are the experimental uptake rates of nutrients divided by the computed massflow rates.

A transfer coefficient larger than 1 indicates that massflow is not sufficient to match the measured uptake of the plant, so depletion of the nutrient around the root and diffusion towards the rootsurface will occur; if massflow is larger than the measured uptake, the transfer coefficient will be less than 1 and accumulation around and diffusion away from the root will occur.

The simulation model is divided into three submodels for easier handling and for a more economic use of computer time.

The first submodel (model A) calculates, from observed data, water and nutrient uptake rates as a function of time. For a first approximation ion concentrations in the nutrient solution are taken to be constant. The root environment is not divided into compartments. Fig. 1 shows typical transfercoefficients for K, Ca, Mg, N and P. The first days the transfercoefficients are all larger than unity, because transpiration and thus massflow are small. After day 25 they become less than unity; when growth reaches the exponential stage the transfer coefficients of N, P and K are again larger than unity, whereas those of Ca and Mg remain smaller.

In the second submodel (model B) the rooting medium is divided into 10 equal layers of 7.5 cm thickness. The experimentally determined root distribution is introduced and the possibility to use root activities as a function of the age of the roots is built in. With the uptake rates of the total plant, taken from submodel A, uptake rates of water and nutrients as function of time and depth are calculated. Uptake of water causes a decrease of the watercontent in the layers above the watertable, until a water content is reached below which plants cannot absorb water and nutrients; watertransport in these layers is supposed to be impossible. In the layers below the watertable, wateruptake causes a watertransport from the bottom of the growth cylinder up to the layer where the water is absorbed. Nutrients move either by convection with the water or by diffusion following a concentration gradient between the layers. The latter is here unimportant because of the dimensions of the layers. Four times a day the growth medium is aerated and the solution in all layers is homogenized; every second day the nutrient solution is removed. After homogenization or renewal, all layers are brought to their original water content. The changes in concentrations caused by uptake of water and nutrients are calculated for each layer. Massflow rates and transfer coefficients are calculated. Fig. 2 shows typical data for K. Massflow rates, transfercoefficients and concentrations in the layers 1, 3 and 5 (numbered from the top) are given from day 20 till 22.5. After homogenization (four times a

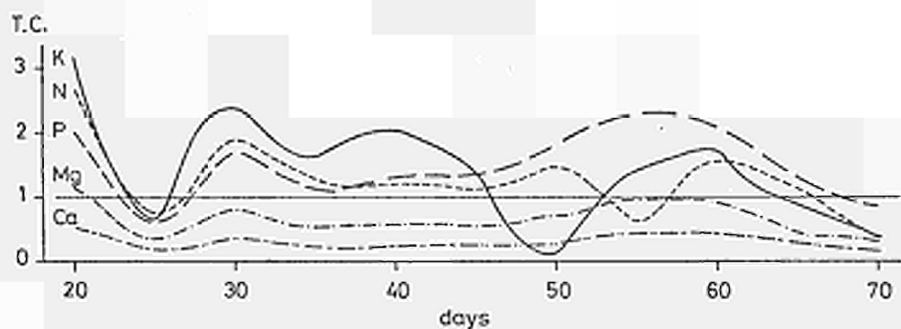


Fig. 1 - Transfer coefficients (T.C.) for Ca, Mg, P, N and K.

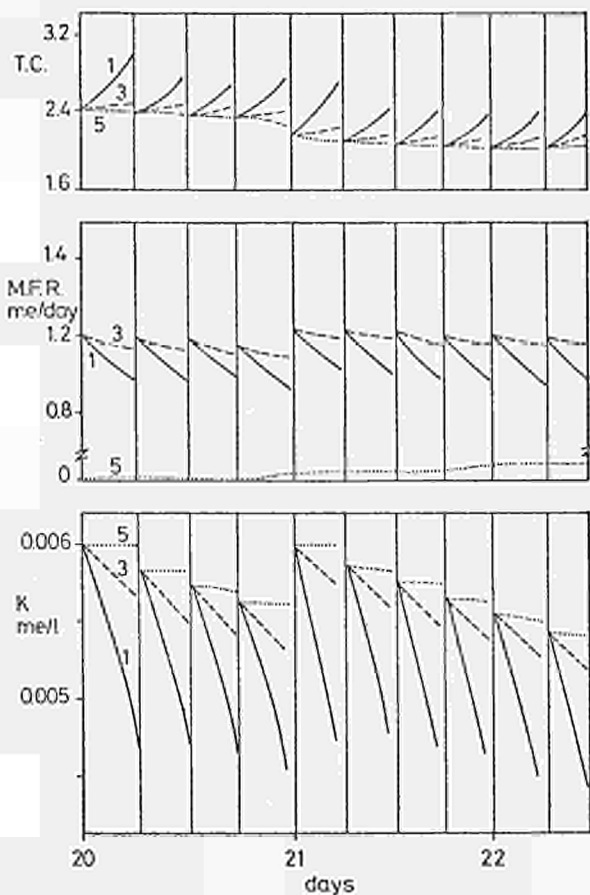


Fig. 2 - Transfer coefficients (T.C.), massflow rate (M.F.R.) and potassium concentration in layer 1, 3 and 5.

day) the transfer coefficients and concentrations become equal for all layers. After renewal (at day 21) the original concentration level is restored. The amounts of roots in the layers 1 and 3 are similar but in layer 1 the volumetric ratio air/solution is higher, which causes a faster depletion of potassium. Equal amounts of roots in the layers 1 and 3 result in the same massflow rates at the start, but a faster decrease of concentration in layer 1 during absorption reduces it. In layer 5 almost no roots were present from day 20 to 22.5, so nutrient depletion and massflow rate are negligible.

The third submodel (model M) describes the process in the surroundings of one root for short periods during the growth of the maize plant. The amount of medium in the chosen layer available for 1 cm active root is calculated taking into account a homogeneous distribution of roots in this layer. This amount is assumed to surround the root and may be divided into different peels of which the first has a thickness equal to the measured length of the root hairs. The water uptake rate calculated in model B is now partitioned over a day and night period; the transpiration rate during the day being ten times higher than during the night (experimental observation). Fig. 3 shows uptake data when it is assumed that the nutrient uptake rate is similar during day and night. Concentration gradients caused by water and nutrient uptake in function of the distance to the root and of time are calculated. The concentration changes at greater distance from the root are of course smaller. The nitrogen concentration rises somewhat during daytime while, during the night, it decreases, the overall tendency being a decrease caused by a continued absorption.

The water uptake rate, massflow rate and transfer coefficient of nitrogen in layer 4 between day 35 and 37.5 are given in fig. 3. The ion uptake rate of nitrogen rises gradually from  $6.3$  to  $6.9 \times 10^{-5}$  me/day for the considered period.

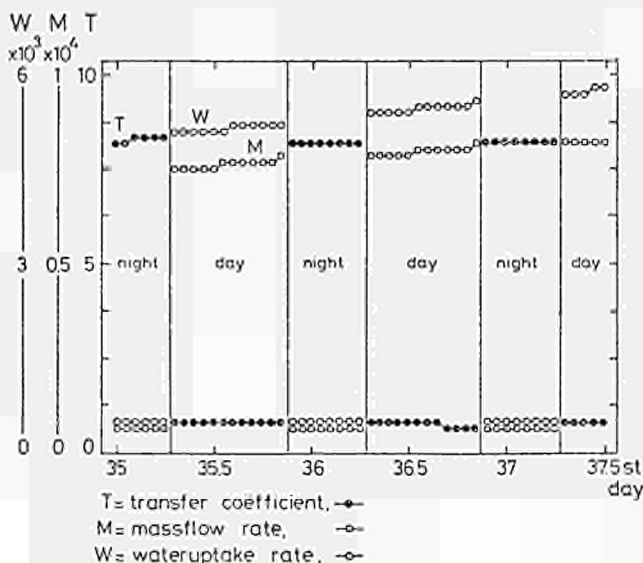


Fig. 3 - Wateruptake (W), massflow rate (M) and transfer coefficient (T) of nitrogen between day 35 and 37.5 for one cm root in layer 4.

Publications - 1975.

- SINNAEVE, J., F. VAN DORP, M.J. FRISSEL. Description of a recently developed phytotron for the study of plant-soil interactions aiming at an optimal combination of experimental work and simulation studies. Presented at the Annual ESNA Meeting, Cadarache, France, September 1975.
- SINNAEVE, J., F. VAN DORP. Description of two mixtures of synthetic resins as a growth substrate medium for the study of plant-soil interactions. Evaluation of the experimental advantages. Presented at the Annual ESNA Meeting, Cadarache, France, September 1975.
- SINNAEVE, J., F. VAN DORP. Physicochemical aspects of the plant-soil relation and of the supply of nutrients to the root. Presented at the Annual Scientific Meeting of the Nederlandse Bodemkundige Vereniging on the Plant Root and its Environment. Wageningen, The Netherlands, November 1975. To be published as an External Report.

Resultaten van het project No.

Hoofd van het team en wetenschappelijke medewerkers:

H. Siebering, A. Daelemans, M.J. Frissel.

Titel van het project:

Transport of pesticides and herbicides in soils.

---

Beschrijving van de resultaten:

The subject of investigation is the behaviour of the fumigant methyl bromide (MB) in soils and its possible influence on plants. This extremely poisonous compound is usually applied in greenhouse horticulture as a gas under a soilcover in dosages varying from 50 to 100 g.m<sup>-2</sup>. After removal of the soilcover the compound may volatilize into the atmosphere. The most important decomposition product is Br<sup>-</sup>, which is easily taken up by many horticultural crops. Possible pollution of soil and atmosphere by MB and Br<sup>-</sup>, are of great concern and the present study is meant to obtain a better insight in the probability of such pollution as well as to study Br<sup>-</sup> uptake by vegetables (e.g. lettuce).

Behaviour of methyl bromide in soils.

This part of the study aims at the mathematical description of the interactions of the fumigant with the soilsystem and the working out of a simulation model for the transport processes. The following interactions between fumigant and soilsystem are of interest: adsorption on the solid phase, dissolution in the aqueous phase, decomposition and chemisorption.

- adsorption.

Adsorption measurements carried out at 20°C on an airdried humic sandy soil, obtained from the Glasshouse Crops Research and Experiment Station at Naaldwijk resulted in a linear adsorption isotherm over the range of investigation (up to 35 μmol cm<sup>-3</sup> in the gasphase). The following relation between the amount of MB adsorbed per gram dry soil (S) and the concentration in the gasphase per cm<sup>3</sup> (C<sub>g</sub>) was obtained:

$$S = 1.2 C_g$$

The coefficient 1.2 measures the "distribution ratio" K s/g.

The adsorption isotherm is given in fig. 1. Preliminary adsorption measurements on a peat soil indicated that the distribution ratio may increase to K s/g = 3.8.

- dissolution in the aqueous phase.

From the vapourpressure of MB at 20°C (1816 mbar) and its watersolubility at 20°C (168 μmol.cm<sup>-3</sup>), the distribution ratio between the gasphase and the waterphase may be calculated: K w/g = 4.1.

Experiments carried out in demineralized water confirmed this value over the concentration range of investigation (up to 35 μmol.cm<sup>-3</sup> in the gasphase).

The relation between the concentration in the waterphase (C<sub>w</sub>) and the concentration in the gasphase (C<sub>g</sub>) therefore is: C<sub>w</sub> = 4.1 C<sub>g</sub>

However, there are indications that the distribution ratio may significantly increase when considering soilmoisture instead of demineralized water.

- decomposition.

At this stage a first order equation, relating the decomposition (P<sub>d</sub>) to the amount of fumigant per cm<sup>3</sup> bulksoil (Q), is used:

$$P_d = K_d \cdot Q$$

where K<sub>d</sub> is a constant, expressed in day<sup>-1</sup>.

From data, reported in literature, K<sub>d</sub> = 0.05 day<sup>-1</sup> seemed to be a reasonable value.

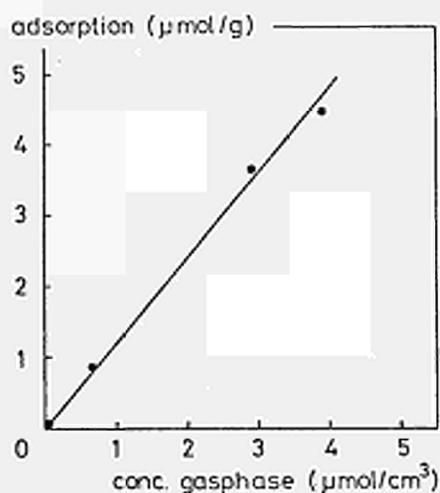


Fig. 1 - Adsorption isotherm at 20°C on a humic sandy soil (airdried).

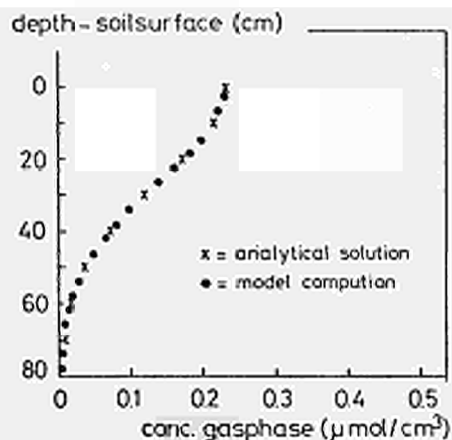


Fig. 2 - Concentration of methylbromide in the soilatmosphere at different depths, 2 days after fumigation started.  
 x x analytical solution  
 • • model computation.

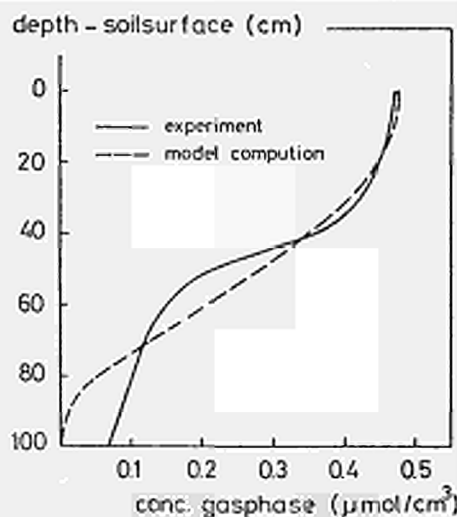


Fig. 3 - Concentration of methylbromide in the soilatmosphere at different depths, 2 days after fumigation started.  
 — field experiment  
 - - - - model computation.



- chemisorption.

In a similar way, the following relation between the chemisorption ( $P_C$ ) and  $Q$  may be used:

$$P_C = K_C \cdot Q.$$

No data are available to verify this relation; the rate constant  $K_C$  has been given the arbitrarily value of  $0.005 \text{ day}^{-1}$ .

On this basis the transportrate of MB in soil may be described by the following equation:

$$\delta Q / \delta t = \delta (D_p \cdot \delta C_g / \delta x) / \delta x + P,$$

where  $D_p$  is the diffusion coefficient in the porous medium, which value may be derived from the diffusion coefficient in air ( $D_0 = 8500 \text{ cm}^2 \text{ day}^{-1}$ ) and where  $P$  is a production term.

Applying the distribution ratios  $K_w/g$ ,  $K_s/g$ , the bulkdensity  $\rho$ , the volume fraction gasfilled pores  $\epsilon_g$  and the volume fraction waterfilled pores  $\epsilon_w$ , the following relation between the amount MB per  $\text{cm}^3$  soil ( $Q$ ) and the concentration in the gasphase ( $C_g$ ) may be written:

$$Q = \epsilon_g \cdot C_g + \epsilon_w \cdot K_w/g \cdot C_g + \rho \cdot K_s/g \cdot C_g.$$

The production term  $P$  contains a decomposition part  $P_d$  and a chemisorption part  $P_C$ :  $P = -(P_d + P_C) = -(K_d + K_C) \cdot Q$ .

A simulation model may now be developed, based on the models used in the former studies on bromacil and propyzamide. In this model, the soilprofile is divided into compartments of 4 cm thickness; between soilcover and soil-surface an aircompartment of 4 cm thickness is assumed to exist.

At  $t = 0$  the dosage is supposed to be present in this compartment.

The soilcover is considered to be impervious to MB, although some permeability with different covers is reported in the literature. After removal of the soilcover the concentration in the aircompartment is kept at zero in the model.

Verification of the model, with respect to the applied mathematical relations, may be done by comparing its results with an analytical solution. This requires, of course, a simplification of the system. Assuming all parameters to be independent of time and position and assuming the dosage at  $t = 0$  to be present in the first soilcompartment, the transport equation may be solved. The transportsystem is defined in detail in table 1.

Table 1 - Characterization of the transportsystem of MB in soils, in the case of mathematical verification. (Dosage =  $50 \text{ g m}^{-2}$ ; covertime = 2 days)

	$D_p$ ( $\text{cm}^2 \text{ day}^{-1}$ )	$K_w/g$	$K_s/g$	$K_d$	$K_C$	$\epsilon_g$	$\epsilon_w$	$\rho$
depth (0-100 cm)	1000	4.1	4.1	0.05	0.005	0.2	0.2	1.2

Figure 2 shows the concentration of MB in the gasphase at different depths in the soilprofile, two days after the start of the fumigation, both calculated by the analytical approach and modelcomputation.

The results fit quite well, showing that programme errors were absent, rather than correctness of the results.

A physical verification of the model may be done by comparing its results with field experiments. A field experiment reported by Drosihn (Diss. 1967, Technische Hochschule, Hanover), was chosen for this purpose.

A characterization of the system is given in table 2.

Table 2 - Characterization of the transportsystem of MB in soils; in the case of physical verification. (Dosage = 50 g m<sup>-2</sup>; covertime = 2 days)

Depth(cm)	D <sub>p</sub> cm <sup>2</sup> .day <sup>-1</sup>	K <sub>w</sub> /g	K <sub>s</sub> /g	K <sub>d</sub>	K <sub>c</sub>	ε <sub>g</sub>	ε <sub>w</sub>	ρ g cm <sup>-3</sup>
0	1935	4.1	1.0	0.05	0.005	0.45	0.05	1.15
10	1550					0.38*	0.06*	1.29
20	840					0.252*	0.128*	1.45
30	665		1.0			0.22*	0.125*	1.5
45	545		0.2			0.215*	0.155*	1.51
60	335					0.16*	0.192*	1.56
80	85					0.10*	0.235*	1.59
100	35	4.1	0.2	0.05	0.005	0.08*	0.245*	1.62

\* reported by Drosihn.

Missing data were estimated.

By tuning adsorption coefficients within a reasonable range, it was tried to fit the model results and field experiments. Concentration-depth profiles, two days after the start of the fumigation are given in fig. 3. In the upper 50 cm of the soil profile there is good agreement. In the lower zone the results deviate significantly. There are several possible causes for this deviation such as incorrect presuppositions with the computation or inaccurate measurement of MB concentrations at the low levels concerned. However, considering the number of estimates that had to be made concerning the interaction of MB with the soil, the result of this first model computation seems reasonable.

#### Br<sup>-</sup> uptake by vegetables.

This part aims at the study of Br<sup>-</sup> uptake from a disinfected soil. In the literature uptake of bromide from soil after addition of a bromide salt was described as a passive process, a linear relationship being found between bromide concentrations in the plant and in the soil-solution. (Kempton R.J. and G.A. Maw: Ann. Appl. Biol., 72, 71-79 (1972); Chao T.T.: Agron.J., 52, 595-596 (1966)). Therefore the relation between Br<sup>-</sup> uptake from the soil solution in a disinfected soil and transpiration has to be known under normal growth-conditions.

#### - bromide concentration in soil solution.

Some adsorption measurements and leaching experiments indicated that, in a humic sandy soil obtained from Naaldwijk, after addition of different amounts of potassiumbromide (30, 60 µg Br<sup>-</sup>g<sup>-1</sup>) nearly all bromide ions are in the soil solution. Column leaching experiments and adsorption-desorption isotherm have to be done.

#### - Total bromide uptake compared to transpiration.

Lettuce (*Lactuca sativa* L. cv. Amanda Plus) was grown in disinfected soil under controlled climatic conditions (day (14 h) temperature 22°C, night 16°C, relative humidity 65%). As a result of disinfection and KBr application the concentration of Br<sup>-</sup> in the soil solution was calculated at 40 µg Br<sup>-</sup>g<sup>-1</sup>. Comparing dry weight production, transpiration and Br<sup>-</sup> uptake at

different stages during a total growth period of 38 days (total dry weight 4 g) it was shown that:

1. at the various stages, the amount transpired per g dry matter produced remains the same: approx. 200 ml per g.d.w.
2. approx. 0,001 mg  $\text{Br}^-$  is taken up over ml transpired. This result compares quite well with literature data.

This preliminary result is in agreement with the existence of a direct relationship  $\text{Br}^-$  uptake - transpiration, but has to be confirmed at various concentration levels of the soil solution.

Publications - 1975.

- LEISTRA, M. and M.J. FRISSEL. Computations on the material balance of pesticides in soil. In "Environmental Quality and Safety", George Thieme Verlag, Stuttgart, April 1975.
- ROLLAND, J.-P. and M.J. FRISSEL. Leaching of Bam in soil columns and evaluation of the results by simulation. In "Environmental Quality and Safety", George Thieme Verlag, Stuttgart, April 1975.

Resultaten van het project No. 6

Hoofd van het team en wetenschappelijke medewerkers:

J. Sinnaeve, F. van Dorp, M.J. Frissel.

Titel van het project:

Ion uptake by intact plants from the soil or from the equilibrium solution of a synthetic resin. Experimental and simulation approach.

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Beschrijving van de resultaten:

a. Study of mixtures of synthetic resins as growth substrates.

The plants (*Zea mays* L. cv. Ona; *Hordeum vulgare* L. cv. Aramir; *Pisum sativum* L. cv. Allround and *Spinacea oleracea* L. cv. Winterreus) grown on resin mixtures X till XVII (all mediums with and without micronutrients) have been analysed.

All plants grown on mediums without micronutrients showed deficiency symptoms. A mixture of a strong acid and a strong base exchanger (mixture XV) gave very poor results due to a shortage of divalent cations, what could have been caused by the limited volume of substrate (250 ml for three plants). The mineral composition of the plants grown on mixture XVII was near to the one observed for the reference plants grown on a potting soil. However, direct comparison of the results is deceiving as, due to slower growth rates on the resin mixtures, plants harvested at the same age are not in the same stage of physiological development. Based upon these results, a final experiment permitting statistical analysis of the results, was set up, using one litre of substrate per plant. The composition of the mixtures used is given in table 1. This experiment has led to several definitive conclusions:

- it was definitively proven that a combination of a weak acid and a strong base exchanger is not suited for long term growth experiments.
- mineral composition and data characterizing the physiological development of the plants grown on the other mixtures have proven their suitability as a growth substrate.
- the cation/anion ratio of the plant material was not changed by replacing part of the weak base exchanger by a strong one. The modification, however, retarded the growth in a severe way.
- agraperlite as well as silversand can be used as a dilutant. Silversand stimulates root development when a weak base exchanger is used whilst only minor differences are observed when the mixture contains a weak as well as a strong base exchanger.
- analysis of the equilibrium solution and percolation experiments have shown that fairly low ionic concentrations in the equilibrium solution can be maintained for several months.
- the sensitivity for iron deficiency can be decreased by using a weak acid exchanger for the iron.

During the past months, these synthetic substrates have been used to grow two varieties of *Vicia faba* L. with very good results. They will be used now for studies regarding the mineral nutrition of crops.

b. Gravel - nutrient solution experiments with maize.

The long term growth experiment with maize plants (*Zea mays* L. cv. Ona) reported last year has been completed with a new experiment over three months carried out under apparently identical experimental conditions in a climate controlled growth chamber. Both series of data have been used for the development of simulation models describing the mineral nutrition

Table 1 - Composition of the resin mixtures used for the final experiment.

Mixture	Resin types	Inert material	me per litre mixture						pH
			Ca	Mg	K	NO <sub>3</sub>	SO <sub>4</sub>	H <sub>2</sub> PO <sub>4</sub>	
IXX	Aw1+Bs	Agraperlite	260	30	40	120	30	20	6.9
XX	Aw1+Bw	Agraperlite	260	30	40	120	30	20	6.4
XXI**	Aw1+Bw+Bs	Agraperlite	260	30	40	120	30	20	6.3
XXII	Aw1+Bs	Silversand	260	30	40	120	30	20	7.3
XXIII	Aw1+Bw	Silversand	260	30	40	120	30	20	6.8
XXIV**	Aw1+Bw+Bs	Silversand	260	30	40	120	30	20	6.7

\*\* 70% of the anions is adsorbed on the weak base exchanger.

30% of the anions is adsorbed on the strong base exchanger.

P.S. Micro-nutrients added to all mixtures (me per litre mixture)

3.6 me Fe, 1.8 me Mn, 1.8 me Zn, 1.8 me Cu and 1.8 me Mo as Dowex 50 W (As type)

2.6 me B as Dowex 1 (Bs type).

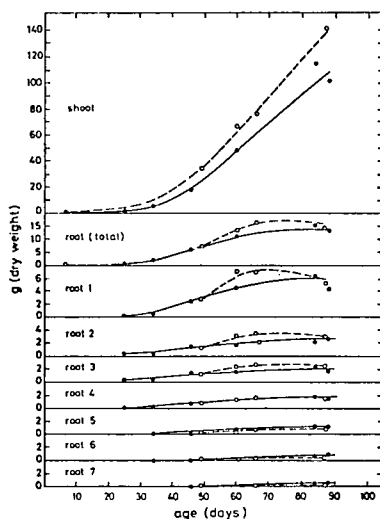


Fig. 1 - Growth experiment with maize plants (Gravel culture).  
Roots 1 till 7 are in different layers (thickness 7.5 cm).

— series A

o----o series B

of the crop (see project 3). Some of the experimental data are given in figures 1, 2 and 3. Figure 1 gives the increase of the dry weight of root and shoot as well as the depth penetration of the roots in successive layers of 7.5 cm thickness. A different rate of development of the shoot is observed (for each series average values of two or four observations). The same statement can be made for the root development and for the rooting intensity in the three upper layers of the column. Figure 2 indicates, at 80 days, a leaf area for series 2 being 35% higher than the one observed for series 1. The most striking difference, however, concerns the transpiration rate: it exceeds for series 2 by 25% the transpiration rate observed for series 1 (in all cases it was computed for several plants (at least 4 observations) as a mean value a period of 24 hours). This may indicate an unnoticed change of the microclimate around the leaves. It is obvious that a higher transpiration rate combined with a higher leaf area must result in a higher mineral content of the plants of series 2 what is confirmed by the data given in figure 3.

Such observations make it very difficult and also very dangerous to compare two experimental runs. The very few results presented here emphasize the necessity to control more adequately the experimental conditions and indicate that a measurement of the metabolic activity of the plant is a real necessity. This has led us to the development of a specially designed growth chamber.

c. Development of a two compartment growth chamber.

The quantification of plant responses, i.e. the reaction of a plant towards a combination of external parameters, requires adequate control and exact measurement of the climates of roots and shoots. The realization of a growth chamber fulfilling the imposed criteria was technically difficult and very time consuming as commercially available equipment answering the purpose did not exist! The most important characteristics of the developed growth chambers are:

- two separated compartments for respectively roots and shoots.
- independent temperature control of both compartments.
- soil columns (depth 75 cm, total volume 4.8 litres) are used.
- double control of the water management over the profile. By one system, drained soil columns can be used; by the other one a constant water level between -15 and -70 cm can be maintained.
- the wind speed in the shoot compartment is very homogeneous and can be varied between 0.15 and 0.51 m.sec<sup>-1</sup>.
- the light intensity is homogeneous all over the shoot compartment and reaches a maximum level of 61 klux.
- the consumption or production of carbondioxide can be measured and concentrations between 200 and 2000 ppm can be maintained.
- all climatic parameters are semi-continuously recorded by digital multiplexing with a selected time step.

With exception of the carbondioxide measuring device, the equipment is finished and since the end of November, a first experiment with maize (*Zea mays* L. cv. Ona) is running.

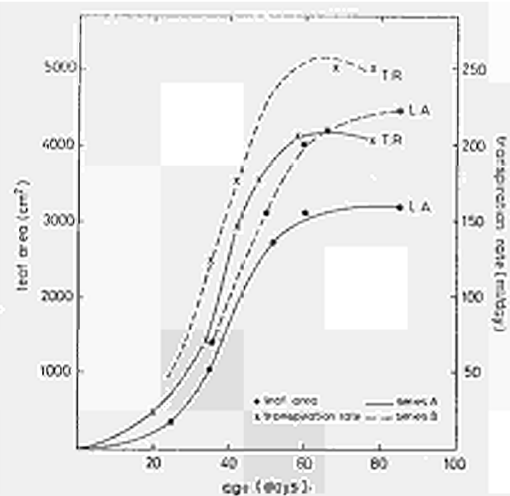


Fig. 2 - Growth experiment with maize plants (Gravel culture).

— series A      ○ Leaf area  
 - - - series B      x Transpiration rate

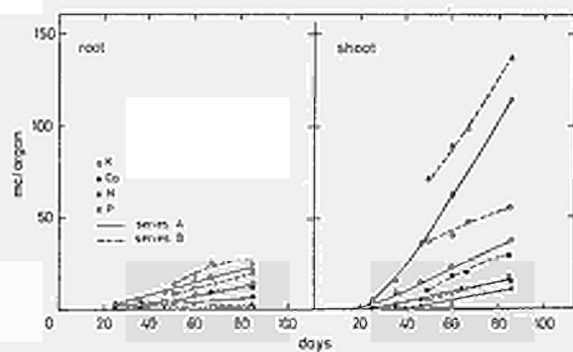


Fig. 3 - Growth experiment with maize plants (Gravel culture)

— series A      ○ K  
 - - - series B      ● Ca  
                     △ N  
                     x P

Publications - 1975.

- SINNAEVE, J., F. VAN DORP, M.J. FRISSEL. Description of a recently developed phytotron for the study of plant-soil interactions aiming at an optimal combination of experimental work and simulation studies. Presented at the Annual ESNA Meeting, Cadarache, France, September 1975.
- SINNAEVE, J., F. VAN DORP. Description of two mixtures of synthetic resins as a growth substrate medium for the study of plant-soil interactions. Evaluation of the experimental advantages. Presented at the Annual ESNA Meeting, Cadarache, France, September 1975.
- SINNAEVE, J., F. VAN DORP. Physicochemical aspects of the plant-soil relation and of the supply of nutrients to the root. Presented at the Annual Scientific Meeting of the Nederlandse Bodemkundige Vereniging on the Plant Root and its Environment, Wageningen, The Netherlands, November 1975. To be published as an External Report.



Resultaten van het project No. 7

loofd van het team en wetenschappelijke medewerkers:

i. Verfaillie, Ch. Hänisch ten Cate, H. Breteler.

Titel van het project:

Study of the kinetics of ion-uptake by intact plants.

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Beschrijving van de resultaten:

i. Theoretical interpretation.

In relation to the previous work on the kinetic analysis of gas and ion exchange phenomena in rice plants (*Oryza sativa* L.) an analytical expression of the rate of apparent photosynthesis (net CO<sub>2</sub> consumption) as a function of the CO<sub>2</sub> concentration and of the light intensity has been introduced.

The intrinsic effects of the uptake of H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> ions on the acidity of the nutrient solution has been calculated in function of the total concentration of both ions and of the pH. Theoretical results found and plotted in figure 1 confirm the experimental observations (1972).

The interferences of the matrix ions of a Hoagland-Arnon I nutrient solution with the proton uptake from a ternary HPK system (1973) have been better described by computing the evolution of molar uptake ratio  $\Delta H/\Delta P$  after injection of various chemical compounds into the HPK solution (see figure 2).

. Nitrogen uptake.

. Usefulness of a gaseous ammonia specific electrode (Orion) for the determination of NH<sub>4</sub><sup>+</sup> concentration.

Straight standard lines were obtained when the logarithm of the NH<sub>4</sub><sup>+</sup> concentration ( $5 \times 10^{-6}$  to  $10^{-1}$  M) was plotted against the electrode potential. The response of the electrode was stable within 15 to 30 seconds, depending on the NH<sub>4</sub><sup>+</sup> concentration level in the test solution. Among the interfering substances were glutamine and volatile amines. The electrode is suitable for the assay of NH<sub>4</sub><sup>+</sup> in nutrient solutions and, with certain restrictions, also for the analysis of free ammonium in plant tissue extracts.

. Kinetic survey of NH<sub>4</sub><sup>+</sup> uptake by detached roots of maize (*Zea mays* L. cv. CIV-2 Prior).

Calculation of the intake of NH<sub>4</sub><sup>+</sup> at steady state from NH<sub>4</sub>Cl solutions containing 0.05 mMol CaCl<sub>2</sub> per liter, measured by medium depletion with an NH<sub>3</sub> electrode, seemed to reveal the existence of a single isotherm between  $5 \times 10^{-6}$  and  $5 \times 10^{-2}$  M NH<sub>4</sub><sup>+</sup> with a K<sub>m</sub> of approx.  $10^{-5}$  M. At extremely high and low concentrations the uptake data were very inaccurate. Experiments will be repeated using <sup>15</sup>N tracing in the plant. Satisfactory results with emission spectrographic assay for <sup>15</sup>N have been obtained with <sup>15</sup>NH<sub>4</sub>Cl standards. To obtain a faster and more reliable preparation of the samples, a new vacuum line is under construction.

. Assay of adenosine phosphates in plants, using a liquid scintillation counter.

For the determination of ATP, ADP and AMP in plant extracts, see report of project No. 9 of the Radiation Protection programme.

. Ammonium-rubidium uptake interaction in detached roots of maize (*Zea mays* L., cv. CIV-2 Prior).

As compared with nitrate nutrition, ammonium nutrition is known to result in lower K<sup>+</sup> contents in plants. Short term experiments were conducted to assess whether NH<sub>4</sub><sup>+</sup> affects the uptake of K<sup>+</sup> in a different

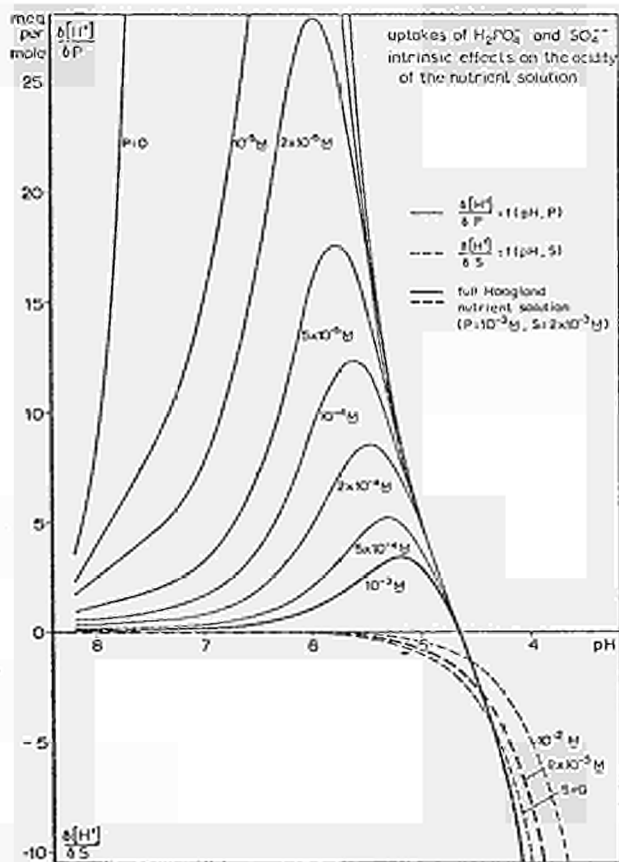


Fig. 1 - Variations of the acidity of a nutrient solution due to selective uptake of  $H_2PO_4^-$  and  $SO_4^{2-}$  ions as a function of the pH and of the respective ion concentrations.

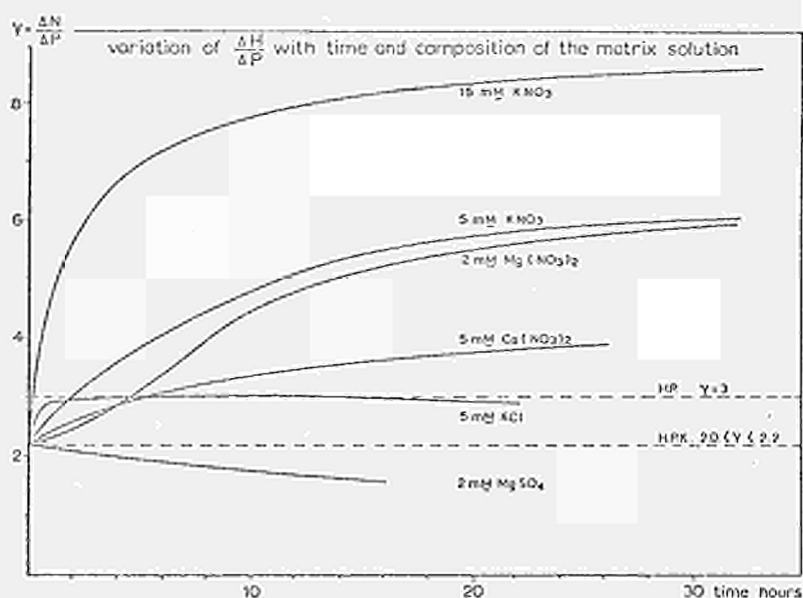


Fig. 2 - Evolution of the ratio of molar uptake of proton and phosphate after injection of various compounds in the nutrient solution.  $\Delta N$  and  $\Delta P$  represent the cumulative proton and phosphate uptakes.

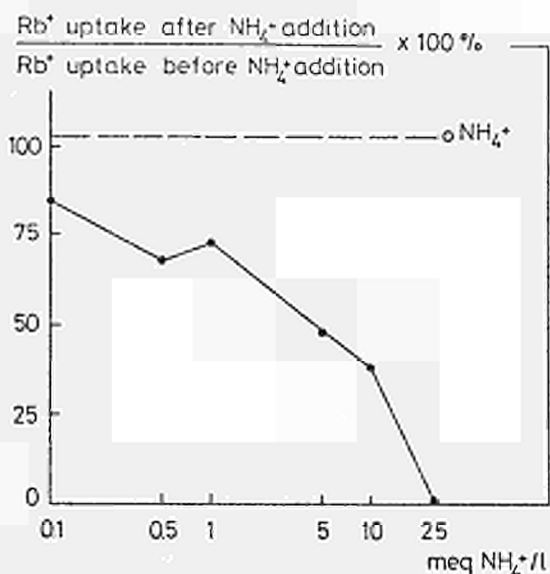


Fig. 3 - Effect of  $\text{NH}_4^+$  concentration on active  $\text{Rb}^+$  uptake by excised maize roots.  $\text{NH}_4^+$  added as  $\text{NH}_4\text{Cl}$  to 1 mM  $\text{RbCl}$ , 0.05 mM  $\text{CaCl}_2$  solutions.

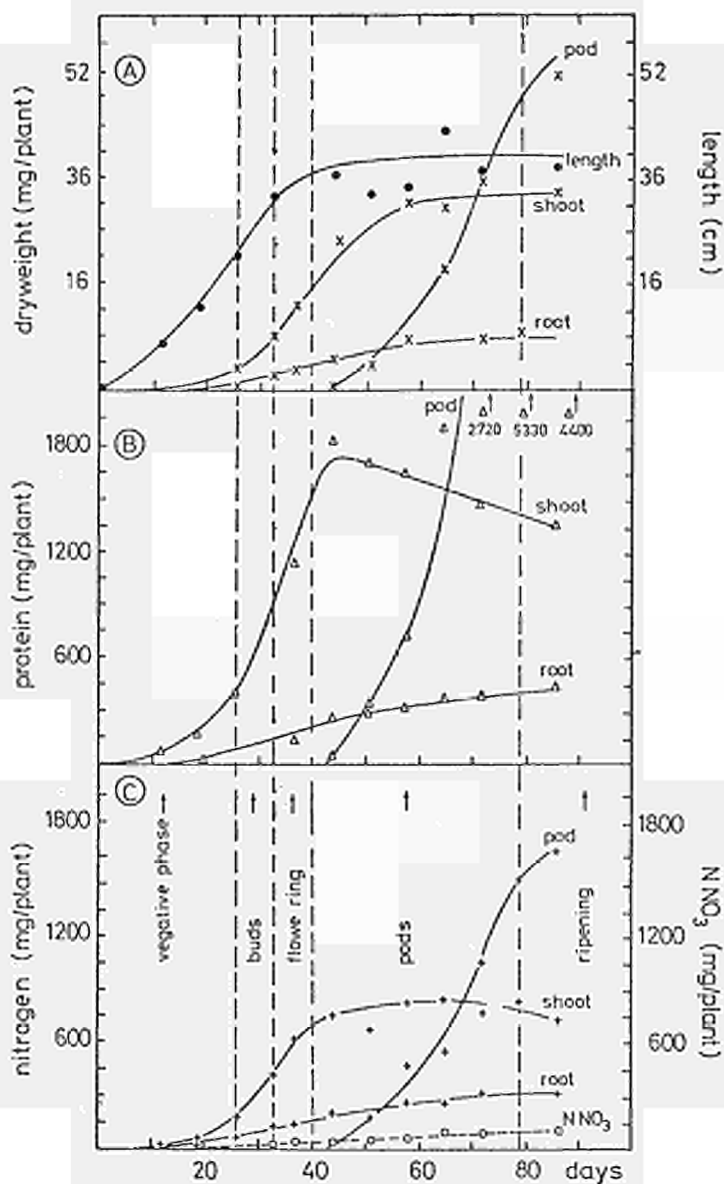


Fig. 4 - Length (cm. ●), dry weight (g, x), total nitrogen content (mg, +), nitrate nitrogen (mg, o) and protein (mg, Δ) expressed per plant in the course of time of *Phaseolus vulgaris* L. cv. Witte Krombek.

way than other alkaline cations, and whether the effects of  $\text{NH}_4^+$  on  $\text{K}^+$  uptake and of  $\text{K}^+$  on  $\text{NH}_4^+$  uptake are symmetric.

$^{86}\text{Rb}$  was used as tracer for  $\text{Rb}^+$  which is a suitable substitute for  $\text{K}^+$  in short term experiments with maize. Working with submerged detached root systems was allowed, because the contribution of  $\text{Rb}^+$  recovered in the collected vascular exudate to total  $\text{Rb}^+$  uptake was less than 1% over a period of 5 hours. Preliminary results show that the uptake of  $\text{NH}_4^+$  from 1 mM  $\text{NH}_4\text{Cl}$ , 0.05 mM  $\text{CaCl}_2$  solution cannot be reduced by more than 20 to 30% by  $\text{Rb}^+$  concentrations ranging from 1 to 100 mM. None of the other alkaline cations acted in a more repressive way at 25 mM, the effect of  $\text{Li}^+$  even begin negligible. Roots from  $\text{NH}_4^+$  - precultured plants absorbed  $\text{Rb}^+$  only by pure physical-chemical processes. Cultivation of plants on an  $\text{NO}_3^-$  containing solution stimulated the  $\text{Rb}^+$  absorption. Addition of  $\text{NH}_4^+$  to  $\text{Rb}^+$  absorbing roots reduced the  $\text{Rb}^+$  and therefore the  $\text{K}^+$  uptake to zero at 25 mM  $\text{NH}_4^+$  (figure 3).

## 2. Redistribution of nitrogen.

### 1. Redistribution of nitrogen for protein filling of seeds.

A suitable plant species, having seeds with a high protein content, a short life cycle, and easily growing in water culture in a climate-controlled room, has been looked for. The french dwarfbean (*Phaseolus vulgaris* L., cv. Witte Krombek) met most of the requirements. Its culture on nutrient solutions has been improved.

The length, dry weight, nitrogen content (Kjeldahl), nitrate content ( $\text{NO}_3^-$  electrode) and protein content (Lowry) of roots, shoots and pods during the vegetative and generative development of the plant was measured and the results are given in figure 4. The length of the shoots increased up to day 35 (flowering). Dry weight, nitrogen and protein contents in roots and shoots increased up to day 45 (start of seed filling), while the weight of the pods increased very much after 45 days. This increase did not take place at the expense of the vegetative parts of the plant.

### 2. Methodological work on protein degrading enzymes.

Optimum conditions for the extraction and the assay of peptidase and protease activities was 7.5 and 7.0 respectively. KCN (0.2 mM) intensified the ninhydrin colour reaction for the protease activity assay.

## Publications - 1975.

BRETELIER, H. Carboxylates and the uptake of ammonium by excised maize roots. Agric.Res.Rep. 837, 1-99 (1975).

VERFAILLIE, G. Nouvelle approche cinétique adaptée à l'étude des échanges gazeux et ioniques chez *Oryza sativa* L. en aquiculture. Ph.D. thesis, Université Catholique de Louvain, (June 1975).

Resultaten van het project No. 8

Hoofd van het team en wetenschappelijke medewerkers:

J. Sinnaeve, G. Desmet, M.J. Frissel, G.R.M. Verfaillie.

Titel van het project:

Ion uptake by roots from dilute solutions.

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Beschrijving van de resultaten:

a. Double labelling ion-uptake experiments.

The controversial results obtained in double labelling experiments using cesium ( $^{134}\text{Cs}$  and  $^{137}\text{Cs}$ ), an ion not yet present in the plant (see Annual report 1974), have been further analysed and discussed at various meetings (e.g. ESNA, Cadarache, September 1975) but the obvious discrepancy observed could not be explained.

Physical tests (e.g. adsorption experiments on glass, resins or clays; evaporation with paper strips), tests on the reliability of counting procedure and the computation routine, and new absorption experiments with and without blocking of the metabolically linked absorption step are in the planning stage.

b. Absorption of cadmium by spinach plants.

In collaboration with Prof. A. Cremers from the Catholic University of Louvain (Belgium) some preliminary experiments regarding the absorption of free and complexed cadmium by spinach (*Spinacea oleracea* L. cv. Verbeterd Breedblad) have been carried out. The stability of some metal complexes with aluminosilicates and for instance polyamines is raised by some three to four orders of magnitude. Three plant nutrition treatments were compared. The first one was an absorption at a free cadmium concentration of  $10^{-5}$  M; the second one with the same amount of cadmium but complexed by the ligand-clay; the third one with a free cadmium concentration of  $1.1 \times 10^{-5}$  M during a first period (4 days) followed by a second period during which the plants were transferred to a cadmium free nutrient solution containing non-saturated ligand-clay. In the first treatment, the cadmium concentration in the nutrient solution decreased from  $1.0 \times 10^{-5}$  M at the start of the experiment to  $6.9 \times 10^{-6}$  M at the end. In the second experiment the concentration of free cadmium in the solution was  $4.6 \times 10^{-8}$  M at the start and  $7.6 \times 10^{-6}$  M at the end, indicating a slow release of cadmium. This explains how the plants of treatment 2 absorbed as much cadmium as those of treatment one. (See table 1). The most interesting results, however, are those of treatment 3. After 4 days the absorbed amounts of cadmium are comparable to the quantities observed for the first treatment. At the end of the second period, the concentrations in root and shoot were reduced by 40 % and 60 %, respectively, indicating important decontamination of the roots and subsequent decontamination of the shoot by internal redistribution and continued release.

Table 1 - Absorption experiments with free and complexed cadmium.

Absorption period	Cadmium in the plant	Nutrient solution data with regard to cadmium			
		free Cd 10 <sup>-5</sup> M	bound Cd 10 <sup>-5</sup> M	free Cd 1.1x10 <sup>-5</sup> M	without Cd with ligand-clay
4 days	ppm Cd in the root	474	424	461	
	ppm Cd in the shoot	139	115	115	
7 days	ppm Cd in the root	458	437		168
	ppm Cd in the shoot	148	143		'66

Publications - 1975.

- FRISSEL, M.J. Report from collaborators in a joint project of ESNA. Some absorption and translocation experiments in tomato plants *Lycopersicon esculentum* Mill. cv. Marettte VF to check the reliability of radioactive tracing methods as an international joint project of ESNA. Proc. XIXth Int. Hort. Congress, Warszawa, 1975.
- FRISSEL, M.J. The above mentioned ESNA report is also published in Newsletter of the Indian Soc. for Nuclear Techniques, 1974, 3, 41 (published March 1975).
- MINNAEVE, J. Present state and future perspectives of the ESNA joint project on double labelling. Proposal of further experiments. Presented at the Annual ESNA Meeting, Cadarache, September 1975.
- MEEN, H., and M.J. FRISSEL. Simulation of hormone transport in petiole segments of COLEUS. *Physiol. plant.* 1975, 34, 208-215.

Resultaten van het project No. 10

Hoofd van het team en wetenschappelijke medewerkers:

C. Broertjes, S. Roest and G.S. Bokelmann.

Titel van het project:

The development of adventitious bud techniques *in vivo* or *in vitro* for mutation breeding.

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Beschrijving van de resultaten:

The use of the adventitious bud technique, namely the development of adventitious plantlets on detached leaves, has proven to be a powerful tool in mutation breeding because usually a high percentage of solid mutants is obtained after irradiation of leaves. This has been demonstrated in a number of crops such as *Achimenes*, *Begonia*, *Streptocarpus* and others, which aspect of the project is discontinued and replaced by the development of *in vivo* and *in vitro* propagation techniques for mutation breeding (c).

Interesting results were obtained in a few new experiments in which the effect of DTT (dithiothreitol) upon radiosensitivity, mutation frequency and mutation spectrum is being studied, using *Achimenes* as a test plant (a) (Broertjes). In another experiment with *Achimenes* it was demonstrated that autotetraploids indeed produce a much higher mutation frequency and probably a (much) wider mutation spectrum as compared to the parental diploid (b) (Broertjes).

The second and increasingly important part of the project (c) (Roest and Bokelmann) is the development of the method in crops which do not spontaneously produce adventitious buds. Besides *in vivo* techniques also *in vitro* methods are studied since some of the latter look more and more promising, either as a tool in plant breeding or as a (fast) propagation method.

In *Chrysanthemum* adventitious bud techniques were developed *in vivo* with detached leaves and *in vitro* using explants of flower heads, pedicels and leaves. In addition, the significance of these methods was evaluated for mutation breeding. Experiments have just been started to develop adventitious bud techniques *in vitro* for *Begonia* and the important food crop the potato.

- a. DTT treatment of seeds, prior to irradiation with X-rays or fast neutrons, has proven to have a protective effect in *Arabidopsis*. This is explained on the basis of the prevention and/or the efficient repair of chromosome breaks and the resulting (gross) chromosome aberrations. This has been tested in the asexual propagated species *Achimenes* cv. Cupido. The maximum permissible DTT concentration and application time was 1% during 2 hours without irradiation. With subsequent irradiation 0.5% during 2 hours was preferred, giving the same or even a better protection as compared to 1%. A considerable protective effect was observed: 4 krad X-rays, being almost lethal without pretreatment, is comparable with 8 krad X-rays with pretreatment, concerning survival. (With fast neutrons this is 1½ and 2 krad, respectively). The mutation frequency was (much) lower with pretreatment. The final effect was that a pretreatment with DTT decreases the mutation frequency compared on the basis of production of large rhizomes, which is correlated with survival of the leaves. The mutation spectrum did not differ with that of non-pretreated leaves. The results suggest that a) most mutations, drastic or non-dramatic ones, are in fact chromosome aberrations, b) DTT only reduces the number of aberrations and c) no conclusions can be drawn whether or not DTT is a general radical scavenger or preferentially repairs or prevents chromosomal damage.



- b. The diploid cv. Tarantella and its autotetraploid cv. Tango were used to test the phenomenon (observed earlier in *Saintpaulia* and *Streptocarpus* on a non-significant small scale) that autotetraploids can produce extremely high mutation frequencies. Leaves of the diploid "Tarantella" were irradiated with a few X-ray and fast neutron doses. Among 570 adventitious plantlets, produced by these leaves, only 6 mutants were found (1.1%) with slight variations in flower colour and flower size. One mutant was more distinct, having a compact growth habit. The low mutation frequency could be explained on the basis of a relatively low heterozygosity (which will be investigated). Similar treatments were given to leaves of the tetraploid cv. Tango. Among 970 adventitious plantlets, so far approx. 300 mutants were found. A surprisingly high mutation frequency ( $\pm 30\%$ ), as compared to the diploid "Tarantella", for which no explanation is at hand at present. Moreover, a (very) wide mutation spectrum has been observed (dwarfing plants, probably based upon drastic chromosomal aberrations, but also fairly normal plants with a relatively large variety of different pink and red-(dish) flower colours).
- c. Using pedicel explants of the cultivars Super Yellow and Bravo of *Chrysanthemum morifolium* Ram. *in vitro* a very fast propagation could be achieved. In practice this method attracted attention and two chrysanthemum nurseries have just started to use this *in vitro* procedure for vegetative propagation. This method further appeared to be attractive for storage; pedicels of the cultivars Super Yellow and Bravo could be stored for at least 15 and 12 months, respectively, at 2°C, after which periods isolated shoots were subcultured and still easily initiated adventitious roots and consequently complete plantlets at 20°C. In view of mutation breeding, in a final experiment pedicels of the cv. Bravo were irradiated with 800 rad X-rays, being approximately the optimum dose. It was again demonstrated that via an *in vitro* culture almost exclusively solid (non-chimeral) mutants were produced, with a flower colour and/or size and form of the flowers differing from the original "Bravo" type. In addition, rather often more than one phenotypically identical solid mutant was found, which always was derived from the same explant. Consequently, they ultimately originate from a single mutated cell (Broertjes, Roest and Bokelmann, 1976). The solid nature of these mutants was confirmed in an experiment in which 5 striking mutants were propagated asexually in two ways, namely by rooting shoot cuttings and by propagation *in vitro*, using pedicel explants. Plants of both groups were subsequently irradiated with a high dose of X-rays (2000 rad) and exposed to short day conditions and induced to flowering. If the mutated plants would be of a periclinal chimeric nature, after irradiation uncovering of the apex had to be expected and flower heads of the "Bravo" genotype would become visible. In view of the fact that this phenomenon has never been observed it was proven that via this *in vitro* method solid mutants were produced.

The *in vivo* formation of adventitious shoots on detached leaves of cv. Bravo could be further stimulated by a mineral nutrition with  $\text{KNO}_3$  or  $\text{NH}_4\text{NO}_3$ ; 25 cc per week per leaf of a solution containing 2 gr/l being approximately optimal. In a first experiment with irradiated leaves of cv. Bravo it was determined that the *in vivo* production of adventitious shoots on detached leaves is not the method of propagation which we are looking for to be used for mutation breeding, amongst others because a substantial percentage of chimeral plants was observed.

A final experiment has been started, under more optimum conditions, in which the significance of this *in vivo* method will again be evaluated using approximately 3500 leaves irradiated with 0, 750, 1000 and 1250 rad X-rays. From these leaves about 1000 adventitious shoots were derived, which were grown up to plants and subsequently induced to flowering under short day conditions.

In view of the fact that space is limited in the "short day" growth chamber, so far not all 1000 plants did flower yet.

However, it can already be concluded that, in contrast with the first experiment, almost all mutants, characterized by a different colour, form or size of the flower heads, were of a solid nature, which phenomenon was also observed after irradiation of pedicel and leaf explants and the subsequent *in vitro* culture.

Furthermore research has been started with the monectochimeric potato clone EM 52 of the cultivar Désirée. Preliminary experiments proved to be rather successful and via adventitious bud and root formation *in vitro* the first plantlets were produced, which could be transplanted into soil and subsequently grown up to plants.

Our experiments with the potato (*Solanum tuberosum* L.) will be continued using different explants of several cultivars.

In addition experiments have been started to develop adventitious bud techniques for the triploid *Begonia* clones "Schwabenland" and "SO<sub>1</sub>". So far the first adventitious shoots were produced using different explants cultivated *in vitro*.

#### Publications - 1975

- BROERTJES, C. The development of (new) *in vivo* and *in vitro* techniques of significance for mutation breeding of vegetatively propagated crops. In: Improvement of Vegetatively Propagated Plants through Induced Mutations, IAEA (No. 173), Vienna: 23-31 (1975) (Meeting at Tokai, Japan, 1974).
- BROERTJES, C. Improvement of vegetatively propagated plants by mutation breeding. In: XIXth Int. Hort. Congress (Warszawa, 1974), II: 201-205 (1975).
- BROERTJES, C. Mutation breeding of autotetraploid *Achimenes* cultivars. *Euphytica* 25(2) (1976) (in press).
- BROERTJES, C. Is DTT a means to improve the mutation spectrum of vegetatively propagated plants? *Euphytica* 25(2) (1976) (in press)
- BROERTJES, C., S. ROEST and G.S. BOKELMANN. Mutation breeding of *Chrysanthemum morifolium* Ram. using *in vivo* and *in vitro* adventitious bud techniques. *Euphytica* 25(1) (1976) (in press).
- ROEST, S. and G.S. BOKELMANN. Vegetatieve vermeerdering van *Chrysanthemum morifolium*. *Vakblad Bloemisterij* 30(32); 29 (1975).
- ROEST, S. and G.S. BOKELMANN. Vegetative propagation of *Chrysanthemum morifolium* Ram. *in vitro*. *Scientia Hort.* 3; 317-330 (1975).
- ROEST, S. and G.S. BOKELMANN. Mutatieveredling van *Chrysanthemum morifolium*. *Vakblad Bloemisterij* (1976).
- ROEST, S. and G.S. BOKELMANN. Vegetative propagation of *Solanum tuberosum* L. *in vitro*. *Potato Research* (1976).

Resultaten van het project No. 12

Hoofd van het team en wetenschappelijke medewerkers:

A.J.G. van Gestel.

Titel van het project:

Induction of self-compatibility in dihaploid *Solanum tuberosum* L.

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Beschrijving van de resultaten:

In 1975 no experimental work has been carried out.

The final report concerning the induction of self-compatibility in dihaploid *Solanum tuberosum* L. is in preparation and will become available in 1976.

Resultaten van het project No. 13

Hoofd van het team en wetenschappelijke medewerkers:

A.J.G. van Gastel, D. de Nettancourt.

Titel van het project:

Spontaneous and induced mutations at the S-locus: A comparative analysis on the origin and nature of constructive and negative mutations.

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Beschrijving van de resultaten:

The generation of new self-incompatibility alleles.

In order to increase our knowledge on the generation of new S-alleles experiments were conducted with the aim to distinguish between the recombination theory and the inactivation-reactivation hypothesis, both possible explanations for the occurrence of new alleles in *Lycopersicon peruvianum* Mill.

The results, which should be considered as very preliminary, because the testcrosses were not always conclusive, indicate (table 1) that different S-specificities are present in the inbred progenies. Among these, two are the parental alleles, S<sub>1</sub> and S<sub>2</sub>, which are of course expected to be present in the populations, and the others are S<sub>3</sub>, S<sub>4</sub>, S<sub>5</sub>, S<sub>6</sub> and S<sub>7</sub>. Hence, it seems that a plant can give rise to an inbred progeny which is segregating for several S-alleles. The new specificities were only detected in the pistil which, in some cases, could be shown to contain simultaneously 5 different specificities. The majority of the plants with the new S-alleles were analysed to see if these incompatibility relationships did not result from a single state of sterility. This is not the case because each plant was normally cross-compatible with tester stocks not carrying the S-alleles which had been detected in their styles.

Not a single case of a new specificity was found in the pollen and therefore it seems that new specificities are first generated in the pistil.

The crosses, performed on each inbred plant, aimed at testing the hypothesis on intracistronic crossing-over and the reactivation-inactivation theory derived from the concept of EDSTRÖM concerning the storage of genetical variability. If S<sub>3</sub> (the new specificity originated after inbreeding S<sub>1</sub>S<sub>2</sub> genotypes) appears from inbred S<sub>1</sub>S<sub>2</sub> plants it would have been demonstrated that the specificity of a new S-allele depends upon the identity of the parental alleles and that, crossing-over may be involved in the generation of a new S-specificity. The appearance of S<sub>6</sub> or S<sub>7</sub> (this means the specificity of the *L. peruvianum* plant used as grandmother) in the inbred progeny would substantiate the hypothesis on the resurgence of new specificities which were present in the ancestral clones.

The results obtained showed that S<sub>3</sub>, S<sub>6</sub> and S<sub>7</sub> appeared as new specificities and one might conclude that apparently the two hypotheses were correct. One must, however, consider the fact that the inbred population, which normally should have segregated for only S<sub>1</sub> and S<sub>2</sub> alleles, was found to contain, in at least some plants, all the S-alleles which could be tested by the tester stocks.

Hence, one wonders if other new alleles, completely unrelated to the family history of the inbred population, would not have been found if appropriate tester stocks had been available for detecting such alleles. The possibility needs to be verified, but it is tempting to consider the self-incompatible plant as the carrier of the complete collection of S-alleles segregating in the species and to consider inbreeding as the agent for switching on and off some of these alleles in a particular genetic background.

Similar results have been obtained with inbred plants of *Nicotiana glauca* Link *et* Otto at our laboratory. On the basis of these studies it appears, although on a preliminary basis, that:

- new specificities are generated in inbred populations.
- the appearance of these new specificities is at first restricted to the style and occurs in such a manner that a diploid style can express more than two alleles of the S-locus.
- this manifestation of polyallelism in diploid styles excludes the possibility that the results obtained in the present experiment simply derived from contamination by stray pollen. Such contamination could possibly explain the occasional appearance of one new specificity but it can not result into the presence of several new S-alleles in an inbred population, nor lead to the accumulation of several different S-alleles, within a single pistil.
- the occurrence of new specificities will pass unnoticed by the experimenter, unless specific attempts with appropriate tester stocks are made to detect such new alleles.

Table 1 - S-genotype in the pollen and the style of inbred plants of *Lycopersicon peruvianum* Mill. (genotype  $S_1S_2$ ).

Genotype in		no.
style	pollen	
$S_2S_2$	$S_2S_2$	2
$S_1S_2$	$S_1S_2$	4
$S_2S_7$	$S_1S_2$	1
$S_1S_2S_3$		1
$S_1S_2S_3S_7$	$S_1S_2$	1
$S_1S_2S_3S_6$	$S_1S_2$	1
$S_1S_2S_3S_5$	$S_1S_2$	2
$S_1S_2S_3S_4$	$S_1S_1$	1
$S_1S_2S_3S_5S_7$	$S_1S_1$	1

Publications - 1975

- GASTEL, A.J.G. VAN and D. DE NETTANCOURT. The effects of different mutagens on self-incompatibility in *Nicotiana alata* Link and Otto. II. Acute irradiations with X-rays and fast neutrons. *Heredity* 34, 381-392 (1975).
- GASTEL, A.J.G. VAN and D. DE NETTANCOURT. The sensitivity of the pollen and stilar component of the self-incompatibility reaction to chronic gamma irradiation. *Radiat.Bot.* 15, (1975).
- GASTEL, A.J.G. VAN and D. DE NETTANCOURT. The generation of new incompatibility alleles. *Incompatibility Newsletter* 6, 66-69 (1975).
- NETTANCOURT, D. DE, M. DEVREUX, F. CARLUCCIO, M. CRESTI, E. PACINI, G. SARFATTI and A.J.G. VAN GASTEL. Facts and hypotheses on the origin of S mutations and on the function of the S gene in *Nicotiana alata* and *Lycopersicon peruvianum*. *Proc.R.Soc. Lond.B.*, 188, 345-360 (1975).
- GASTEL, A.J.G. VAN. Mutability at the self-incompatibility locus and identification of the S bearing chromosome in *Nicotiana alata*. Ph.D. thesis, Agric. University, Wageningen (in press).

Resultaten van het project No. 14

hoofd van het team en wetenschappelijke medewerkers:

A.J.G. van Gastel, F. Engels, F. Carluccio and D. de Nettancourt.

Titel van het project:

Establishment of linkage relationships with the S-locus of self-incompatible plants and identification of the S-bearing chromosome.

Beschrijving van de resultaten:

Identification of the S-bearing chromosome in *Nicotiana alata* Link et Otto.

The chromosome bearing a given gene can be determined with the help of trisomic series and by the analysis of segregation ratios in their progenies.

When all the chromosomes can be distinguished morphologically, karyotype analysis allows, in combination with the genetic data, identification of the chromosomes bearing the various genes characterized by their trisomic inheritance.

With regard to the identification of the S-bearing chromosome the situation is, however, more simple. Due to the fact that a style does not accept pollen grains with a matched S-allele, aneuploid individuals with three different S-alleles will reject three different classes of pollen grains and can be selected by means of simple testcrosses with appropriate homozygous tester clones.

Such aneuploid plants with 3 different S-alleles were produced by crosses between a triploid (SaSaSb) and a diploid (ScSd) *N. alata* plant.

All individuals which displayed in their styles three different S-specificities were cytologically examined in order to identify the chromosome(s) which are present three times.

The idiogram (see Fig. 1) and the armlength ratios for the haploid set of *N. alata* chromosomes have been established by Carluccio *et al.* (1974).

In Table 1 the results of the karyotype analysis are summarized, taking into account the fact that the chromosomes within a group (St<sub>1</sub> and St<sub>2</sub>; Sat<sub>1</sub> and Sat<sub>2</sub>; M<sub>1</sub> and M<sub>2</sub>; M<sub>3</sub> and M<sub>4</sub>) can hardly be distinguished.

Table 1. Karyotype analysis of aneuploid plants which display three different S-specificities.

aneuploid with three S-specificities	number of chromosomes	chromosomes which are present 3 times				
		Telo	St <sub>1</sub> and/or St <sub>2</sub>	Sat <sub>1</sub> and/or Sat <sub>2</sub>	M <sub>1</sub> and/or M <sub>2</sub>	M <sub>3</sub> and/or M <sub>4</sub>
C191 A-22	24	x	x		x	x
C191 A-26	23		x		x	x
C191 A-3	25		x	x	x	x
C191 A-18	22		x		x	x
C191 A-19	19+1			x		
C191 A-30	23		x		x	x
C191 A-35	20		x			
C191 A-42	24		x	x	x	x

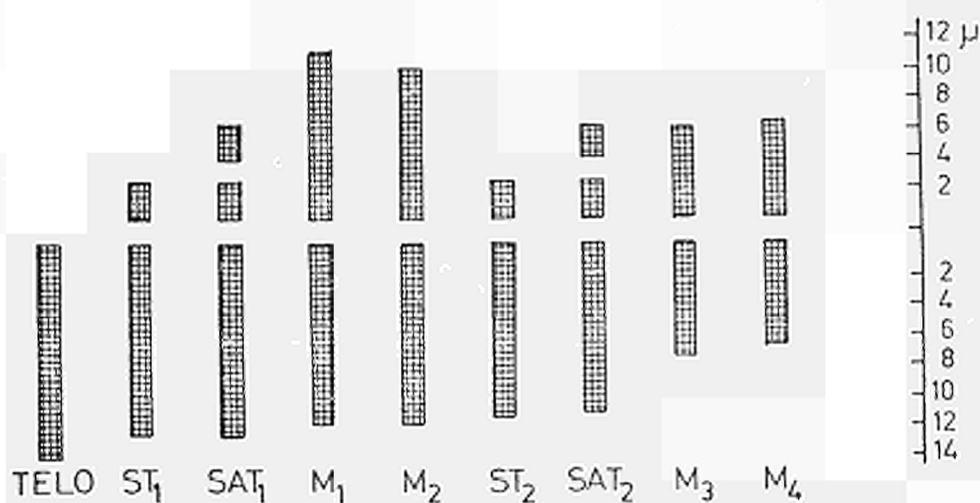


Fig. 1 - Idiogram of *Nicotiana glauca* Link et Otto (Carluccio, De Nettancourt and Van Gastel, 1974).

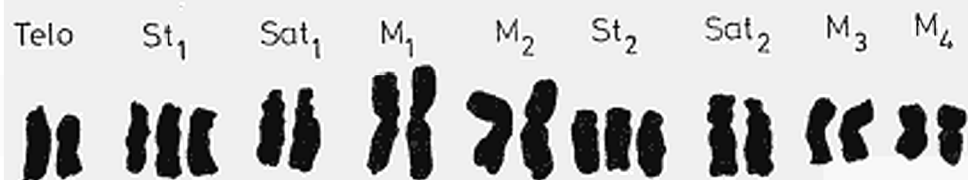


Fig. 2 - Idiogram of plant C191 A-35.



Plant C191 A-19 could have been the most important one because it only displayed 19 chromosomes. However, besides three Sat<sub>1</sub> chromosomes, a centric fragment was present in addition to the 19 chromosomes. Since an S-allele may be situated on such a fragment the results of the karyotype analysis can not be used for the identification of the S-bearing chromosome. Since in the remaining aneuploids (see Table 1) the nonsatellited acrocentric chromosomes (St<sub>1</sub>, St<sub>2</sub>) are the only ones which are always present three times it can be concluded that one of these chromosomes is the S-bearing chromosome.

Analysis of the nonsatellited acrocentric chromosomes of C191 A-22 indicated that in this plant probably only St<sub>1</sub> is present three times. Moreover it appeared that in C191 A-26, C191 A-3, C191 A-18, C191 A-30, C191 A-35 (see Fig. 2) and C191 A-42 both nonsatellited acrocentric chromosomes were present three times. Therefore it can be concluded that the largest nonsatellited acrocentric chromosome is the S-bearing one in *Nicotiana alata* Link et Otto.

To confirm this conclusion and because the two nonsatellited acrocentric chromosomes differ only slightly in their morphology, staining of the chromosomes of *N. alata* by means of banding techniques has been initiated. The results showed that the Giemsa C-banding technique is very promising and may allow a clear distinction between morphologically identical chromosomes in *N. alata*.

#### Publications - 1975

GASTEL, A.J.G. VAN and F. CARLUCCIO. Identification of the S-bearing chromosome in *Nicotiana alata*. Incompatibility Newsletter 6, 87-90 (1975).

GASTEL, A.J.G. VAN. Mutability at the self-incompatibility locus and identification of the S-bearing chromosome in *Nicotiana alata*. Ph.D. thesis, Agric. University, Wageningen (in press).

Resultaten van het project No. 15

Hoofd van het team en wetenschappelijke medewerkers:

G.M.M. Bredemeijer.

Titel van het project:

Biochemical aspects of self-incompatibility in *Lycopersicon peruvianum* Mill and *Nicotiana alata* Link and Otto.

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Beschrijving van de resultaten:

In a previous study it has been suggested that peroxidase isoenzyme 10 in styles of *Nicotiana alata* might be involved in the rejection of incompatible pollen tubes because the tips of these tubes grow in a stylar part with a high peroxidase 10 activity, whereas compatible tube tips grow in a part with a very low activity of this isoenzyme. In order to confirm or reject this hypothesis, the influence of various techniques available for obtaining a temporary breakdown of the self-incompatibility reaction on the activity of peroxidase 10 should be studied. During the past year the effect of bud self-pollination and delayed self-pollination was studied.

The pollination-induced increase in peroxidase 10 activity, observed in mature styles, does not take place in immature styles unless the pollinations are carried out within one day before anthesis. A study of pollen tube growth and seedset revealed that the incompatibility character increases clearly during the last day before anthesis. The fact that the shift from compatibility to incompatibility corresponds to the change in capacity to induce peroxidase 10 activity after pollination supports the hypothesis of an involvement of this isoenzyme in the rejection reaction.

Further evidence that peroxidase 10 plays a role in the inhibition of incompatible pollen tubes was obtained by studying the influence of the age of the mature flower on pollen tube growth and pollination-induced increase in peroxidase 10 activity. The rate of pollen tube growth of incompatible pollen in aged flowers (fig. 1; A4) is, at a first stage of about one and a half day, + 40% of the growth rate of compatible pollen tubes. Subsequently, the rate of incompatible pollen tube growth is much more reduced suddenly. In flowers selfed at anthesis (fig. 1; A0) the inhibition during the first 2 days after pollination is as strong as that observed during the first inhibition phase (+ 1½ day) in flowers selfed at 4 days after anthesis; afterwards the inhibition of pollen tube growth increases gradually instead of abruptly as observed in the aged flowers.

Comparison of peroxidase 10 activity in the various segments of styles selfed at anthesis and 4 days following anthesis (fig. 2) revealed a clear difference at 31 h after pollination when the two growth curves of incompatible pollen tubes (fig. 1) begin to diverge. In segment 2 peroxidase 10 activity is much higher when selfing was carried out 4 days after anthesis than when selfing was performed on the day of flower opening. This segment corresponds exactly to the stylar part in which differences in pollen tube growth are initiated (fig. 1). In the segments 1 peroxidase 10 activity is low in both cases and the rates of pollen tube growth remain identical. The fact that the two incompatible pollen tube growth curves in fig. 1 diverge at a moment that the pollen tubes have just entered stylar parts which differ in peroxidase 10 activity supports the hypothesis that this isoenzyme is involved in the rejection of incompatible pollen tubes. This result also demonstrates that the action of peroxidase 10 is not the only inhibiting principle because incompatible pollen tubes are already retarded in segment 1 (fig. 1) in which peroxidase 10 activity is very low.

In spite of the fact that both the bud-pollination experiments as well as the delayed self-pollination experiments revealed a positive correlation between peroxidase 10 activity and the strength of the rejection reaction one

does not yet know if peroxidase 10 indeed inhibits incompatible pollen tubes. Therefore, a study of the effect of isolated peroxidase 10 on pollen tube growth *in vitro* and *in vivo* was initiated. The first attempts to isolate and purify peroxidase 10 caused a considerable loss of enzyme activity because a low molecular weight activator or co-factor is lost.

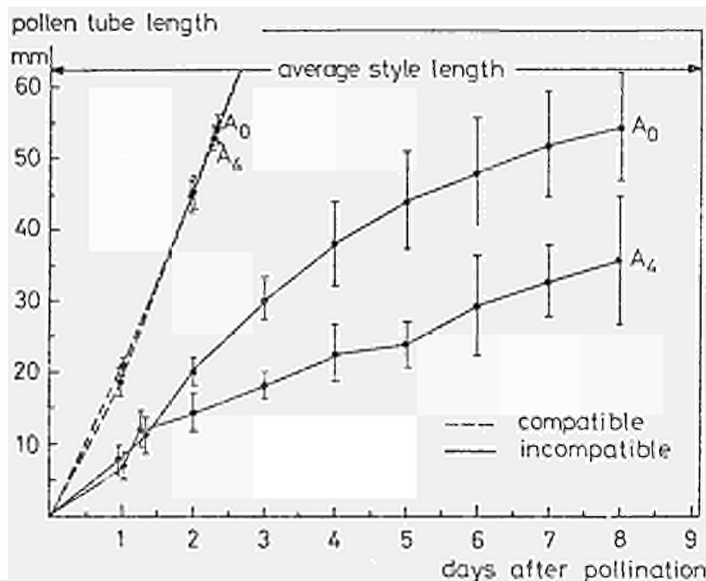


Fig. 1 - Growth curves of compatible and incompatible pollen tubes in attached flowers pollinated at anthesis (A<sub>0</sub>) or 4 days following anthesis (A<sub>4</sub>). Each value is an average of pollen tube lengths observed in at least 20 styles.










seg- ment	anthesis	post anthesis
1		
2		
3		
4		
5		
6		

Fig. 2 - Distribution of peroxidase 10 activity in styles at 31 h after selfing attached flowers at anthesis or 4 days after anthesis. Minimum and maximum pollen tube lengths out of at least 20 styles are given.

#### Publications - 1975

BREDEMEIJER, G.M.M. and J. BLAAS. A possible role of a stylar peroxidase gradient in the rejection of incompatible growing pollen tubes. *Acta Bot. Neerl.* 24: 37-48 (1975).

BREDEMEIJER, G.M.M. The effect of peroxidase on pollen germination and pollen tube growth *in vitro*. *Incompatibility Newsletter* 5: 34-39 (1975).

BREDEMEIJER, G.M.M. Effect of bud-pollination and delayed self-pollination on the induction of a possible rejection peroxidase in styles of *Nicotiana glauca*. *Acta Bot. Neerl.* (in press) (1976).

resultaten van het project No. 16

oofd van het team en wetenschappelijke medewerkers:

M.G. Ebbens-Groot, F.M. Engels, C. Broertjes, K.H. Chadwick, H.P. Leenouts, K.J. Puite.

titel van het project:

he radiation dose-fractionation effect in *Saintpaulia*.

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beschrijving van de resultaten:

. Dose fractionation experiments with detached leaves of *Saintpaulia*.

leaves of *Saintpaulia ionantha* H. Wendl. cv. Utrecht develop a higher radioresistance against a second X-ray dose when exposed to a low initial radiation dose, which effect reaches a maximum 8 to 24 h after the first dose.

Concerning this dose-fractionation effect, the influence of the chemical protector" DTT (dithiothreitol) upon subsequently irradiated leaves, as studied. Modification of the dose-fractionation effect by means of this compound is considered as being helpful for a better understanding of the dose-fractionation effect. It has been demonstrated, that DTT gives approx. the same "protection" against a high X-ray dose as the low initial dose of 500 rad does, whereas the combination of these two factors (DTT given just before the second dose) gives some additional "protections" hereupon. The modification of the effect of the initial dose by means of DTT has also been investigated, applying DTT-treatments either before/after the 500 rad treatment or without 500 rad treatment, followed by a series of (second) doses, 24 h thereafter. Leaves treated with DTT following the 500 rad irradiation showed a higher radiation resistance against a second dose than leaves treated with DTT before this low-dose irradiation. The comparable DTT-treated and acutely irradiated series and the non DTT-treated, fractionated series showed nearly equal radiation sensitivities. These sensitivities were higher than those found for the treatments with DTT before or after the 500 rad dose, followed by a series of second doses.

DTT-treatment, given before the 500 rad irradiation, therefore, seems to interfere with the 500 rad-effect, with the result that the 500 rad irradiation cannot induce the usual amount of "protection" against a second (high) dose given later-on.

Concerning the research on this part of the project, carried out in the period 1972-1975, a thesis of Mrs. H.M.G. Ebbens-Groot is in preparation.

1. Cytological and morphological investigation on the onset of reactivity of epidermal cells in detached leaves of *Saintpaulia*.

The onset of mitotic activity in epidermal cells at the base of the petioles of detached leaves shows up within 4-5 days of cultivation in water. After 7 days of cultivation, meristems have been formed at the base of the etiole. These meristems are exactly the same as those found in the centre of the motherplants producing these leaves. The meristems can be considered as potential plantlets. These adventitious plantlets can be counted with the naked eye after 9 weeks of cultivation. It was observed that:

- . the distribution of meristems near the base of the petiole is not uniform;
- . the number of epidermal cells, participating in the formation of the meristems, varies considerably;
- . the number of meristems, observed after 17 days of cultivation, is twice as large as the number of plantlets found after 9 weeks of cultivation;
- . At increasing age of the leaves a characteristic decrease in the number of cells, which can be reactivated, has been observed;
- . the length of the petiole determines the time needed for reactivation at the base as well as the size of the region where reactivation takes place;
- . within different leaves the region of reactivation may have the same width around the petiole, but the number of cells in the length of the

petiole varies with a factor 2-3. This is related to different stages of cell elongation between the different leaves.

In irradiation experiments (acute or fractionated) we observed, being aware of the above mentioned facts, that:

1. the number of reactivated epidermal cells increases approximately 2x by low dose irradiation;
2. in both types of experiments a dip in the dose-effect curve was found at 4,5 krad, which coincides with a visible change in the morphology of the meristems. In the range 0 - 4,5 krad, very large meristems, and between 4,5 - 9,0 krad very small meristems are formed.

Such studies on the effects of radiation at cellular and meristematic level are considerably affected by all factors influencing the reactivation process in epidermal cells and the growth of meristems. Reproducibility therefore should be improved by minimizing the effects of such factors.

With respect to the preceding findings it seems necessary to standardize an area of cell reactivation and meristem formation.

A method has been worked out of wounding the epidermis of detached leaves. Somewhere between the base of the petiole and the leaveblade, small holes were made by glass needles of varying diameter (touching 1 to 30 epidermal cells). Reactivation, meristem and plantlet formation normally occurred near the hole. The reactivated area is surrounded exclusively by still inactive  $G_0$  cells. It was, however, found in repeated experiments that the number of dead cells was not correlated to the number of destroyed cells caused by the wounding with the glass needle and the reactivated cells were not homogeneously distributed around the hole, when countings were made after 5 days of cultivation. When the number of reactivated cells was counted at 8 days of cultivation, the first reactivated cells were already dividing and their original number could only be estimated. Another difficulty was the injury to the epidermal strip due to the presence of the holes, at the time it was made from the petiole. Part of the reactivated cells, in the surroundings of the hole, remained in fact on the petiole.

Unicellular wounding of the epidermis may be obtained by shaving off the epidermal hairs with an electric razor. The hairbase in the epidermis consists of a number of small cells (in fact originating epidermal cells). When the hair is shaved off only the base cells of the hair are reactivated. The groups of base cells are all surrounded by inactive  $G_0$  cells. The whole area of the epidermis that has been shaved, is covered with small meristems after 3 weeks of cultivation. Although these results were very promising we had to reject this method for the following reasons:

1. Shaving off all epidermal hairs at the same level (very critical) appeared to be unreproducible.
2. By the shaving action several hairs are broken, causing the base cells to die.
3. Where the surface of the petiole is touched by the shaving apparatus, all epidermal cells are reactivated.
4. An important variation in the number of base cells has been found.

At present areas of reactivation on the dorsal face of the petiole, near the base, where the disturbing influence of most of the factors mentioned above may be avoided, are considered for investigation.

esultaten van het project No. 18

oofd van het team en wetenschappelijke medewerkers:

. Broertjes.

itel van het project:

rganization and coordination of applied mutation breeding.

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eschrijving van de resultaten:

uring 1975 many scientists and commercial plant breeders used the  
ervice of the Association, namely:

- . information about the possibilities of mutation breeding in solving  
problems in specific crops.
- . requests for literature.
- . irradiation of plant material.

n cooperative projects between breeders and the Association, 17 new  
rogrammes were started, the majority of which with vegetatively  
ropagated plants (*Anthurium*, *Chrysanthemum*, carnation, *Euphorbia fulgens* (2x),  
*Planchon*, *Malvaviscus*, *Scilla tubergeniana*, *Stephanotus*, *Sinningia*,  
*Treptocarpus*, tulip and potato) and 4 with seed propagated species  
*Cyclamen*, *Pennisetum* (Kenia), wheat and "megurk", a crossbreeding  
roduct between melon and gherkin).

his brings the total number of projects, started since 1959, at 223.  
Several mutants, resulting from some of these projects, were  
ntroduced into commerce, such as: *Alstroemeria* cv. Rosali and cv. Zebra  
lr. Verboom, v.Staaveren, Aalsmeer), *Begonia* cv. Tiara (Doorenbos and  
rper, Dept. of Horticulture, Agric. Univ., Wageningen) and the  
*Irysanthemum* cultivars Blue Winner, Bronze Winner, Coral Winner, White  
inner, Yellow Winner and Yellow Fiducia (lr. van der Knaap, Fides, De Lier),  
he Winner-types apparently being quite successful.

he Mutation Breeding Contact Group had its annual meeting at Cadarache,  
rance, for the first time jointly with the European Society of Nuclear  
ethods in Agriculture (ESNA), in particular with the working group  
Applied Mutagenesis". This will be repeated every two years, when ESNA  
ets in a West-European country.

#### ublications - 1975

ROERTJES, C. Minutes of the meeting of the Mutation Breeding  
Contact Group, held at Wageningen, October 1974.  
(Ext. Report No. 23 of the Association Euratom-ITAL);  
86 pp. (1975).

Resultaten van het project No. 20

Hoofd van het team en wetenschappelijke medewerkers:

H. Stegeman.

Titel van het project:

The radiation and heat resistance of bacterial spores.

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Beschrijving van de resultaten:

Role of metal content in radiation resistance

As mentioned in the annual report 1974 spores of *Bacillus subtilis* ATCC 6633 with a high manganese content, obtained by increasing the manganese content of the sporulation medium or reducing its calcium content, were more resistant to irradiation. This study has been continued with spores of the thermophilic strain *Bacillus stearothermophilus* 1518R. Spores were produced in a chemically defined liquid sporulation medium with 1 ppm  $\text{Ca}^{2+}$  and 10 ppm  $\text{Mn}^{2+}$ , 25 ppm  $\text{Ca}^{2+}$  and 10 ppm  $\text{Mn}^{2+}$ , 25 ppm  $\text{Ca}^{2+}$  and 50 ppm  $\text{Mn}^{2+}$ , respectively. The heat resistance was significantly higher for spores formed in media with 1 ppm  $\text{Ca}^{2+}$  and 10 ppm  $\text{Mn}^{2+}$  or 25 ppm  $\text{Ca}^{2+}$  and 50 ppm  $\text{Mn}^{2+}$  than for spores formed in the other medium. There was no difference in radiation resistance under anoxic conditions. Under oxic conditions the shoulder of the survival curve of spores produced in media with 1 ppm  $\text{Ca}^{2+}$  and 10 ppm  $\text{Mn}^{2+}$  or 25 ppm  $\text{Ca}^{2+}$  and 50 ppm  $\text{Mn}^{2+}$  was larger.

Different kind of radiosensitizers were used to get more information on the mechanism of protection and about the localization within the spores of those metals which were able to increase the resistance to irradiation. Hydrated spores in water get essentially 99% of their damage from indirect action via radiolysis products of water ( $\cdot\text{OH}$ ,  $\cdot\text{H}$  and  $e^-$  aq). Under anaerobic conditions the main damaging radical responsible for indirect action is thought to be the hydroxyl radical. The increase of radiation resistance of sodium loaded spores was only measured under aerobic conditions. An increase in hydroxyl radical concentration resulted in sensitization to the same degree of sodium loaded and untreated spores. These results indicate that the protection by loading the spores with some specific metals might be associated with the oxygen effect. It was remarkable, in this respect, that also the hydrogen radical increased the radiation sensitivity of sodium loaded spores; the hydrated electron seemed to play no significant role. Data about the other kind of sensitizers and about spores with other properties are not yet available.

Synergistic effect of irradiation and heat (in cooperation with F. Engels).

Bacterial spores are more easily destroyed by heat when they are first subjected to a sub-lethal dose of gamma radiation. The correlation between the ionic state of bacterial spores and the synergistic effect, as reported in annual report 1974, suggest that the sensitizing mechanism of pre-irradiation is likely located in the spore cortex and associated with the role of the peptidoglycan polymer in maintaining the heat resistance of the spore. The aim of this study was to get basic information on the role of the spore coat in the synergistic effect of a combined radiation and heat treatment. The disulphide-rich protein, probably located in the outer coat regions of spores, is important in determining the spore resistance to enzymes and to lysis with  $\text{H}_2\text{O}_2$ , because spores treated with reagents which rupture S-S bonds become sensitive to lysozyme and to lysis with  $\text{H}_2\text{O}_2$ . The results of our experiments with mercaptoethanol demonstrate that the disulphide-rich layer is also important in protecting the spores to the sensitizing effect of pre-irradiation to subsequent heat treatments. The mercaptoethanol treatment did not alter the heat resistance of *B. subtilis* ATCC 6633 spores in water or in buffer but the sensitizing effect of pre-irradiation increased markedly (fig. 1).



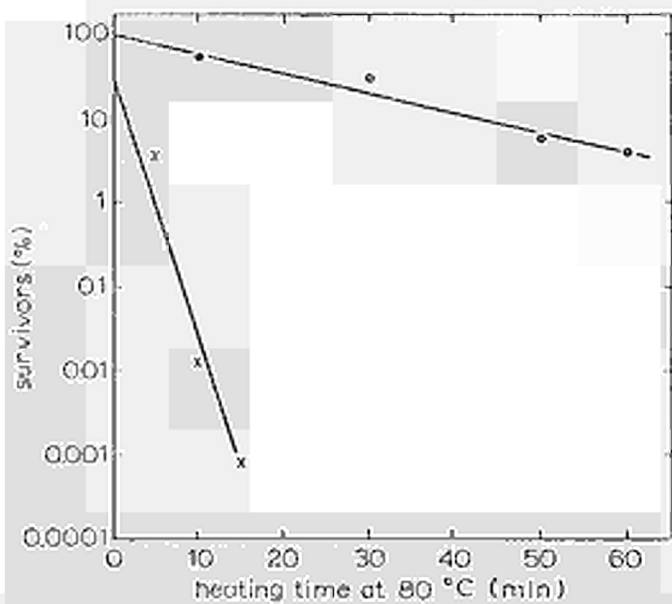


Fig. 1 - Effect of preirradiation with 100 krad on heat resistance of *B. subtilis* ATCC 6633 spores treated with mercaptoethanol.

Qualitatively similar results were obtained with spores of *B. stearothermophilus* NCA 1518R, however, their heat resistance decreased to a certain extent (table 1). Electron microscopical examination of thin sectioned spores showed that mercaptoethanol caused no detectable changes in the coats of *B. subtilis* spores. Examination of *B. stearothermophilus* spores suggested that the mercaptoethanol treatment removed the thin outer coat layer (fig. 2a and b). A possible explanation for the role of disulphide bonds in determining the spore resistance to a combined radiation and heat treatment might be that the disulphide can serve as a donor source of electrons for repairing the peptidoglycan damage caused by ionizing radiation.

In the literature it was noted that extraction of spores with dithiothreitol plus sodium laurylsulfate could remove both the inner and the outer coat without affecting viability or heat resistance. We found with *B. subtilis* ATCC 6633 that a first dithiothreitol treatment resulted in partial removal of the inner coat layer (fig. 3a and b). Further removal of the inner coat could be obtained by repeating the extraction (fig. 3c). Complete removal of the spore coat was not achieved. Similar results were obtained with *B. stearothermophilus* NCA 1518R. One extraction treatment with dithiothreitol resulted in the removal of the outer coat and a reduction of the inner coat (fig. 2c). Further treatments with dithiothreitol affected strongly the viability of *B. stearothermophilus* spores.

The heat and radiation resistance of treated *B. subtilis* spores was unchanged. The heat-sensitization of treated spores by irradiation increased strongly and was exactly identical to the heat-sensitization for *B. subtilis* spores treated with mercaptoethanol. There was no difference in level of sensitizing between once and thrice treated spores.

The radiation resistance of treated spores of *B. stearothermophilus* was also unchanged but the heat resistance in water decreased. The synergistic effect of irradiation and heat increased remarkably (table 1). One might expect that a reduction of the coat is responsible for a stronger attack on the peptidoglycan by the radiolytic products of water. The difference in heat-sensitization by irradiation between *B. subtilis* and *B. stearothermophilus* might be due to the greater protective role of the cortex of *B. stearothermophilus* in maintaining the higher heat resistance of these spores.

Table 1 - Effect of pretreatment on heat sensitization of *B. stearothermophilus* 1518R spores by irradiation.

pretreatment	heat resistance without pre-irradiation	heat resistance with pre-irradiation (100 krad)
untreated control	$D_{115^{\circ}\text{C}} = 7 \text{ min}^*$	$D_{115^{\circ}\text{C}} = 5 \text{ min}^*$
mercaptoethanol-urea	$D_{105^{\circ}\text{C}} = 18 \text{ min}$	$D_{105^{\circ}\text{C}} = 2 \text{ min}$
dithiothreitol-SLS	$D_{105^{\circ}\text{C}} = 18 \text{ min}$	$D_{95^{\circ}\text{C}} = 5 \text{ min}$

\*Value calculated from the linear part of the survival curve.

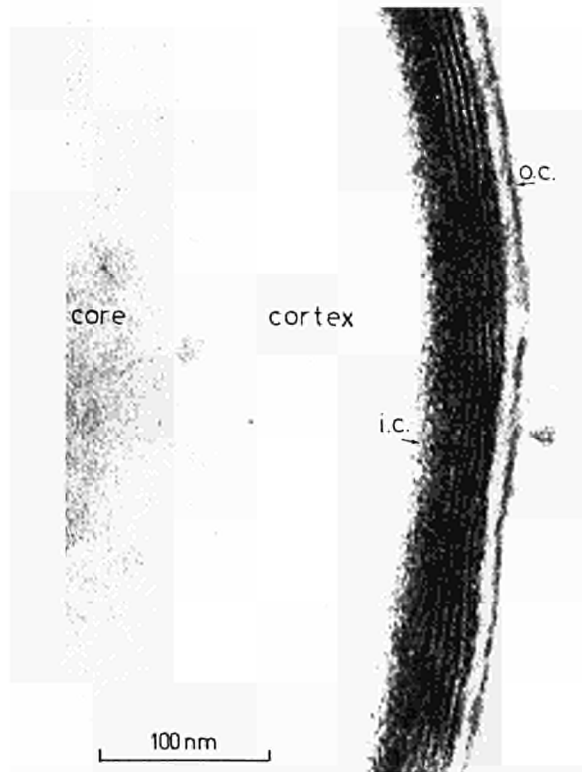


Fig. 2a - Spore coat of *B. stearothermophilus* untreated showing the multilayered inner coat (i.c.) and the thin outer coat layer (o.c.)



Fig. 2b - Spore coat of *B. stearothermophilus* treated with mercaptoethanol showing the removal of the thin outer coat layer.



Fig. 2c - Spore coat of *B. stearothermophilus* treated once with dithiothreitol showing the removal of the thin outer coat layer and the reduced inner coat layer.



Fig. 3a - Spore coat of *B. subtilis* untreated showing the multilayered layers.



Fig. 3b - Spore coat of *B. subtilis* treated once with dithiothreitol showing a partial removal of the inner coat layer.

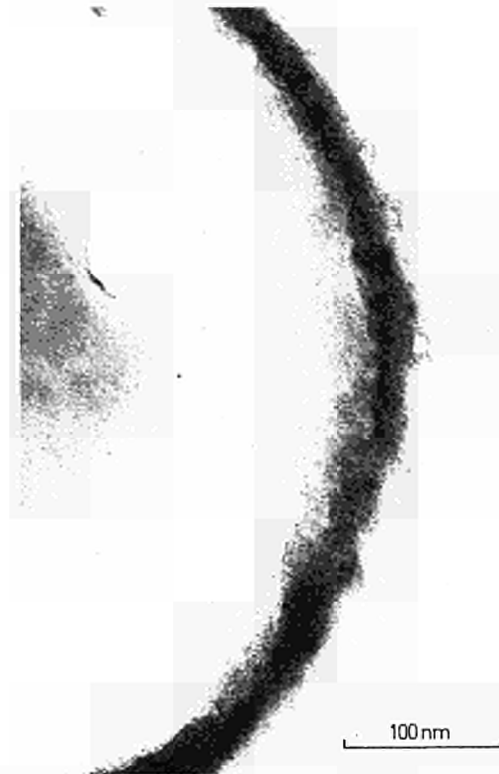


Fig. 3c - Spore coat of *B. subtilis* treated thrice with dithiothreitol showing the persistent outer coat layer.

Resultaten van het project No. 21

Hoofd van het team en wetenschappelijke medewerkers:

J.G. van Kooij, H.B. Leveling.

Titel van het project:

The influence of environmental factors on the radiation resistance of microorganisms. The influence of the wateractivity of the medium.

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Beschrijving van de resultaten:

A number of products have been used for a study of the wateractivity effects on the radiation resistance of a *Citrobacter* species.

The incorporation of the test microorganism into media of different water-activity was done in several ways. *Citrobacter* was allowed to grow in compost of  $A_w = 0.99$ . The *Citrobacter* enriched seed culture was either directly transferred to dry, sterile compost (cf. Figure 1, compost direct), or first lyophilized and then transferred to compost (cf. Figure 1, compost lyophilized). Conditioning of the inoculated samples of compost to different moisture contents (corresponding with different  $A_w$  values) was done by adding and thoroughly mixing of pre-calculated amounts of sterile water. Fishmeal as well as compost were inoculated with a suspension of *Citrobacter*; during mixing a pre-determined amount of sterile water was added, and finally the samples of fishmeal and compost were conditioned to different  $A_w$  values above saturated salt solutions. Samples prepared in this way are indicated in Figure 1 by "Fishmeal" and "Compost". Furthermore, the radio-resistance of *Citrobacter* species was determined in mixtures of glycerol-phosphate-buffer of different  $A_w$ , denominated in Figure 1 "Glycerol".

The  $D_{10}$ -values in krad of *Citrobacter* species, obtained from several samples of irradiated media at various  $A_w$  values are depicted in Figure 1.

Some interesting remarks can be drawn from the graphs. For the solid media the radio-resistance of *Citrobacter* reaches a maximum in the range of  $A_w$  values between 0.4 and 0.7. For "compost lyophilized" the maximum was shifted towards a higher  $A_w$ . Recovery of a smaller number of viable cells at comparable  $A_w$  values under the condition where lyophilized cells are used, does not necessarily reflect a cellular characteristic but rather the influence of a stress effect. For solid media the radioresistance of *Citrobacter* decreases in the lower range of  $A_w$  values. Recovery of viable cells from compost media is always smaller than from fishmeal, which most probably can be ascribed to a much higher protective effect on the bacteria present in a protein rich medium.

The "glycerol" graph is quite different from the solid media graphs. The lowest  $D_{10}$  is found if the glycerol/water mixture does not contain any or only small amounts of glycerol and the highest protective effect is obtained in the absence of water. A second but much lower maximum in the  $D_{10}$  value was found at  $A_w$  value of approx. 0.7. The different graphs reflect the radiochemical effects related to indirect and direct effects of ionizing radiation. In case of higher  $A_w$  values corresponding with larger amounts of available water the indirect effects are superior; in the lower range of  $A_w$  values the indirect effects predominate, but at the same time protection of bacterial cells becomes operative, the magnitude of which depends on the chemical composition of the medium.

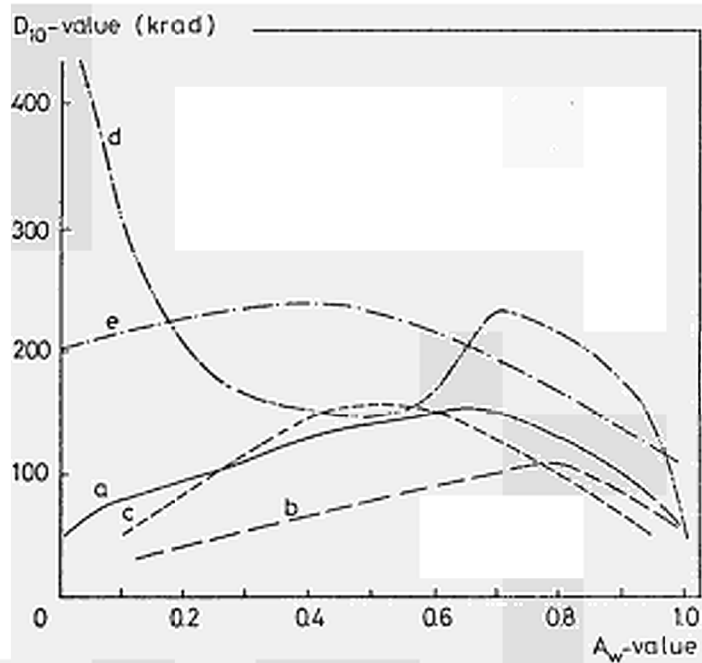


Fig. 1 - Relationship between D<sub>10</sub> value and different A<sub>w</sub>-values for some media of different composition.

- compost
- - - compost (lyophilized)
- - - - - compost above salt mixtures
- · - · - fishmeal above salt mixtures
- · · · · glycerol-phosphatebuffer mixtures

Resultaten van het project No. 22

Hoofd van het team en wetenschappelijke medewerkers:

W.W.A. Bergers.

Titel van het project:

Radiation and heat effects on enzymes.

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Beschrijving van de resultaten:

An extensive literature study was completed on the effect of gamma irradiation on enzymes in living plant tissues with regard to the biochemical changes, that occur in fresh fruit and vegetables during storage after irradiation. In order to evaluate the changes in irradiated vegetable foods a comparison was made with the biochemical changes under general storage and under stress conditions.

The effect of gamma irradiation in biochemical activities induced in fruit and vegetables during subsequent storage, generally declines progressively from metabolically active to senescent plant tissues, (e.g. climacteric fruits near the climacteric maximum, fully ripe fruits and senescent leafy plant organs).

The induced biochemical activities in metabolically more active plant tissues may introduce, after irradiation, either "favourable" effects, such as sprout inhibition and ripening delay, or stress effects proportionally to the doses applied. This induced stress by gamma irradiation disrupts normal metabolism and has many features in common with storage conditions causing stress, e.g. chilling injury and ethylene. They probably have in common that cellular membranes and hormone regulated enzyme systems are damaged. Increase of phenolic compounds is the most typical response to radiation stress in fruit and vegetables and this increase is mediated by the induction of the key enzyme in phenolic biosynthesis: Phenyl Ammonia Lyase (P.A.L.).

The extent of increase in phenolic compounds needs further investigation for two reasons: (1) increase of phenolic compounds affects quality of commodities with regard to discolourations and changes in taste, (2) increase of phenolic compounds raises the problem of naturally occurring toxic compounds in vegetable foodstuffs.

It was decided to study the effect of radiation stress on those vegetable foodsystems, in which the amount of naturally occurring toxic compounds is known to be influenced by environmental stresses and an experimental outline has been prepared for studies on changes in phenolic compounds and solanine in the potato.

Publications - 1975.

BERGERS, W.W.A. Physical and biochemical changes of fresh fruit and vegetables during storage and the effect of gamma irradiation. External Report No. 41, Association Euratom-ITAL, December 1975.



Resultaten van het project No. 24

Hoofd van het team en wetenschappelijke medewerkers:

J.G. van Kooij, H.G. Heins, D. Is. Langerak.

Titel van het project:

Coordination of food irradiation research.

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Beschrijving van de resultaten:

1. Studies to promote practical application of food irradiation.

Legislation of food irradiation.

A category II petition for the irradiation of "peeled potatoes" was requested in order to enable a study of irradiated peeled potatoes on pilot plant scale.

In close cooperation with the Institute for Poultry Research "Het Spelderholt" additional microbiological information on the occurrence of pathogens in poultry became available. These data, in fact, were requested by the Ministry of Health and Environmental Hygiene in order to effectuate clearance in category III of irradiated chicken. A request for unlimited sale has been petitioned, and the possibility for unlimited sale of irradiated chicken is expected early 1976.

Category II clearances for the irradiation of fillets of round and flat fish as well as for the irradiation of cooked, benzoic acid treated shrimps have been requested.

The National Health Council has appointed a Working Party upon request of the Committee of food irradiation and the Committee of toxicology of the Health Council. The terms of reference of the Working Party are:

- to review the acceptability of food irradiation with doses up to 300 krad as a preservation process without demanding complete wholesomeness testing of each individual product;
- to review the acceptability of the general clearance of food, irradiated with doses up to 50 krad without demanding any additional wholesomeness testing.

This concept shifts from wholesomeness testing of individual irradiated foodstuffs towards more broadly based testing procedures, which would allow extrapolation of data to foods having common basic chemical constituents. The ESNA Food Irradiation Working Group has proposed to organize in Spring 1976 in the Netherlands an international meeting on procedures for clearances of a number of irradiated food products of common interest in member countries of ESNA. This meeting will be held under auspices of the Dutch General Inspection for foodproducts of the Ministry of Health and Environmental Hygiene.

2. Development of food irradiation outside the Association's Institute.

Upon advice of the Institute for Fishery Products TNO a working group was formed to elaborate on the possibilities of a practical application of the irradiation of fillets of flat and round fish, and of shrimps.

The Institute for Processing and Storage of Agricultural Products has evaluated the data on low energy electron-beam irradiation of potatoes with the result that electron irradiation does not prevent sprouting satisfactorily, and the problem of after cooking discoloration can not be solved by this technique. It was decided to prepare a proposal for further studies on the mechanism, responsible for the after cooking discoloration in irradiated potatoes.

The strength of the gamma-source of the Pilot Plant for Food Irradiation was increased up to 180.000 Curies  $^{60}\text{Co}$ , and the 6 box-transport system was changed into a 5 box system.

Publications - 1975.

- ANON. Toelatingsaanvraag voor het bestralen en in de handel brengen van beperkte hoeveelheden (categorie II) voorverpakte geschilderde aardappelen. April 1975.
- ANON. Toelatingsaanvraag voor het bestralen en in de handel brengen van onbeperkte hoeveelheden drooggeslachte kuikens (categorie III). November 1975.
- ANON. Toelatingsaanvraag voor het bestralen en in de handel brengen van beperkte hoeveelheden schol en kabeljauwfilets (categorie II). November 1975.
- ANON. Toelatingsaanvraag voor het bestralen en in de handel brengen van beperkte hoeveelheden garnalen (categorie II). November 1975.
- HEINS, H.G. Bestraling van uien onder praktijkomstandigheden ter verbetering van de houdbaarheid. Rapport van de evaluatiecommissie voor de bestraling van uien. Issued by the Stichting Nederlandse Uien-Federatie.
- HEINS, H.G. The technological evaluation of a sea-borne trial shipment of irradiated onions from Thailand. Association EURATOM-ITAL, external report Nr. 22.
- HEINS, H.G. Praktijkafzet van bestraalde verse slachtkuikens, in samenwerking met vijf institutionele grootverbruikers. Association EURATOM-ITAL, external report Nr. 35.
- LANGERAK, D. IS, H.G. HEINS, M.H. NAQVI, G.A.A. DAMEN and R. HOVESTAD. Quality evaluation of irradiated mangoes after sea-borne trial shipment from South Africa. Association EURATOM-ITAL, external report Nr. 36.
- HEINS, H.G. Die Anwendung von Gammabestrahlung in der Praxis zur Verbesserung der Haltbarkeit von Zwiebeln. Die industrielle Obst- und Gemüseverwertung 60 (1975) 674-677.

Resultaten van het project No. 25

Hoofd van het team en wetenschappelijke medewerkers:

J.G. van Kooij, H.B. Leveling.

Titel van het project:

Coordination of research in the field of wholesomeness of irradiated food.

Beschrijving van de resultaten:

1. Reproductive function of Groot Yorkshire sows.

The study of the effects of irradiated and autoclaved feed on reproductive function were continued during 1975 on schedule. The F<sub>1</sub> sows produced their second offspring, and the F<sub>2</sub> animals produced F<sub>3a</sub>.

Preliminary performance data of both offsprings are recorded in table 1.

Table 1 - Reproductive function of sows

Feed group	Gene-ration	Number of young born		Number of piglets weaned	Mean piglet weight		Pre-weaning losses %
		Viable	Still births		at birth	at 49 days	
Auto-claved	F <sub>2b</sub>	91	3	69	1420	13451	23
	F <sub>3a</sub>	78	6	68	1501	11982	13
Irra-diated	F <sub>2b</sub>	90	5	71	1351	15430	31
	F <sub>3a</sub>	81	2	54	1306	14206	33
Contro	F <sub>2b</sub>	82	11	40	1439	15357	51
	F <sub>3a</sub>	67	4	50	1458	14324	24

The F<sub>1</sub> sows had been during the total experimental period of approx. 21 months on the respective diets. The total amount of test diets supplied to the F<sub>1</sub> animals during this period amounted to about 1300 kg per animal. From clinical observations made during the experimental period no disturbances were observed in the health of the animals of the two experimental groups, that might be attributable to the feeding of irradiated or autoclaved feed. The high percentage pre-weaning loss of the F<sub>2b</sub> litter of the Control group is due to the Aujeszky disease. The growth rates of the F<sub>2a</sub> and F<sub>2b</sub> sucklings during the various periods between two weighings are recorded in grammes per day in table 2.

Table 2 - Growth rates of F<sub>2</sub> generation

Feed group	0 thr. 5 days		6 thr. 12 days		13 thr. 21 days		22 thr. 36 days		37 thr. 49 days	
	F <sub>2a</sub>	F <sub>2b</sub>	F <sub>2a</sub>	F <sub>2b</sub>	F <sub>2a</sub>	F <sub>2b</sub>	F <sub>2a</sub>	F <sub>2b</sub>	F <sub>2a</sub>	F <sub>2b</sub>
Auto-claved	169	158	238	218	214	215	264	250	379	319
Irra-diated	160	172	237	190	238	244	235	282	478	418
Contro	175	127	232	201	223	211	261	211	404	446

The results of foregoing table clearly indicate a reduced growth rate of the piglets at the end of the lactation period for the feedgroup "autoclaved". The mean weight-increase per day during the period of 37 through 49 days is about 20% less than the values obtained for the feedgroups "Irradiated" and "Control"

2. Wholesomeness studies of radappertized ham.

This chronic toxicity study involves the feeding of ham incorporated into the standard diets of rats. Ham and lean meat was obtained from pigs fattened on control, irradiated and autoclaved feed. This toxicity test is progressing satisfactorily, and no adverse effects on the test animals of the 5 experimental groups, fed the ham incorporated diets, during the past 14 months were illustrated.

Publications - 1975

- KOOIJ, J.G. VAN. Toxicologisch onderzoek van geautoclaveerd en bestraald dieet bij varkens. Motivatie en protocol van de voedings-experimenten. External Report No. 25.
- KOOIJ, J.G. VAN. Semi-chronisch toxiciteitsonderzoek van geautoclaveerd en bestraald dieet bij varkens. Deel 1: de groei van de proefdieren. External Report No. 27.

Resultaten van het project No. 26

Hoofd van het team en wetenschappelijke medewerkers:

D. Is. Langerak, M.H. Naqvi, H.G. Heins.

Titel van het project:

Preservation of fruits and vegetables by means of ionizing radiation.

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Beschrijving van de resultaten:

### 1. Prepacked cut endive

The influence of packing ( $O_2$  and  $CO_2$  contents) and irradiation on the keeping quality has been investigated.

After processing the product was packed in perforated and non-perforated polythene bags of 0.02 and 0.065 mm thickness for varying the  $O_2$  and  $CO_2$  contents. The irradiation dose amounted to 0 and 100 krad. During various storage intervals, some of the quality parameters such as microbial count, colour changes, market quality and ascorbic acid (Vit. C) contents were studied. With a radiation dose of 100 krad the shelf-life of the packed endive in non-perforated bags was extended for 3 - 4 days.

There was some destruction of ascorbic acid immediately after irradiation, but the difference in the unirradiated and irradiated samples disappeared during the subsequent storage period. During storage a connection between the quality deterioration and the decrease of ascorbic acid contents was found. Low  $O_2$  content improved the colour (less oxidation of the polyphenols) and the market quality of the irradiated product.

### 2. Prepacked cut yellow cabbage

Prepacked yellow cabbage discolours and spoils quickly after processing (cutting). The influence of such factors as packing ( $CO_2$  and  $O_2$  content) and irradiation on the quality has been investigated. After processing the product was packed in polythene bags. The gascomposition in the bags was regulated by using: a) different foilthickness, b) various numbers of perforations, c) various diameters of the perforations.

The cabbage was irradiated with doses of 0, 50 and 100 krad gamma-rays. During storage the following parameters were studied: the quality (colour changes, desiccation, decay), the total viable count, the *Enterobacteriaceae* and the ascorbic acid contents.

The product was also tested organoleptically by a laboratory/consumer panel. These experiments showed that the colour of the non-irradiated product was better in non-perforated bags (low  $O_2$ -content) than in perforated bags (high  $O_2$  content). However, in the non-perforated and non-irradiated bags fermentation (souring) occurred caused by a too low  $O_2$  content and a too high microbial count. With the irradiated product no discolouration occurred both in perforated and non-perforated bags.

According to microbiological examinations, a dose of 50 krad reduced the total viable count with 3 - 4 decimals. A dose of 100 krad gave a reduction of 4 - 6 decimals. The *Enterobacteriaceae* were reduced with 3 decimals at 50 krad. A dose of 100 krad eliminated these organisms almost entirely ( $< \log 1.4 \text{ g}^{-1}$ ).

The influence of irradiation on the visible decay was obvious. The shelf-life was increased with 75 - 100% in comparison to the control.

Considering the influence of cutting, packing, irradiation and storage intervals on the ascorbic acid contents, the largest loss was caused by cutting. There was some destruction of ascorbic acid immediately after irradiation, but the difference with the non-irradiated samples disappeared after 2 days. During storage the ascorbic acid content in the non-irradiated samples decreased more rapidly than in the irradiated samples. This reduction of ascorbic acid runs parallel to the quality deterioration. Sensoric tests showed that an irradiation dose of 50 krad is the most appropriate.

### 3. Colour tests

Up till now the quality evaluation of prepacked vegetables has been carried out by subjective estimation, according to quality instructions. A more objective method has now been developed by means of an Agrtron-colourmeter.

The aim of this part of the project was to establish a relationship between the subjective (visual) and the objective measurements (colourmeter) and to study whether the colourmeter can distinguish between the influence of different treatments on quality.

With chicory (homogeneous product) an obvious relationship was found between subjective and objective measurements. The influence of the different treatments on the quality could also be distinguished by the colourmeter. Experiments with endive (heterogeneous product) up till now did not confirm these results.

### 4. Quality evaluation of treated mangoes

The aim of this experiment was to check the possibility to export mangoes by ship from South Africa to Europe and to evaluate the quality during storage at ambient temperature after arrival. The mangoes were harvested in an unripe state and afterwards treated as follows: a) non-irradiated, b) 75 krad gamma-rays, c) treated with a fungicide + 75 krad. The product was packed in cardboard boxes for transport (16 days at about 10°C). During storage at approx. 20°C some of the quality parameters such as appearance, texture and decay were studied. The product was also tested organoleptically by a laboratory and a consumer panel. The mangoes started to ripen only 5 days after arrival. The ripening was not only delayed by irradiation, but optimum ripening never occurred. Especially by the treatment fungicide + 75 krad - the spoilage was strongly delayed and in consequence the keeping time was increased from 6 till 12 days. The sensoric tests of the laboratory panel showed that the different treatments did not influence the odour, taste and consistence; only the flesh of the non-irradiated fruits was yellower and uniformer than those of the irradiated fruits. The consumer panel preferred the treatment fungicide + 75 krad, probably as a result of the better appearance (less decay). This experiment has shown that transport by ship of treated mangoes is possible, provided the temperature during the transport allows a complete ripening (> 10°C).

### 5. Storage silverskin onions

During storage the quality of silverskin onions deteriorates quickly by microbiological spoilage, rootformation and sprouting. The aim of this experiment was to lengthen the storability of fresh onions intended for processing by means of the combination irradiation-cooling. The silverskin onions were irradiated with 0, 10 and 250 krad and stored at 2°C and 10°C respectively.

The results showed that storage at 2°C was better than at 10°C. The storability was strongly influenced by the ventilation system in the cooling room. An irradiation dose of 10 krad prevented root formation and delayed sprouting. An irradiation dose of 250 krad unfavourably influenced the quality of the fresh and preserved product.

Storage at 10°C and an irradiation dose of 10 krad improved slightly the quality of the preserved product; but at 2°C no improvement was found as compared to the non-irradiated product.

With an optimum ventilation system silverskin onions could be stored for 35 days at 2°C and, in combination with 10 krad, for 19 days at 10°C.

#### 6. Storage of seed onions

In the past sprout inhibition was obtained by doses of 1 - 4 krad gamma-rays, when applied within four weeks after harvest. However, this period is too short for processing the whole yield in an economic way. It was shown that the dormancy could be lengthened by storage at 0 - 2°C, starting immediately after harvest. The effectiveness of gamma irradiation remained the same, even after a six weeks' period at that temperature.

#### 7. Irradiation of steam-peeled potatoes

Previous experiments showed that the shelf-life of mechanically peeled potatoes could be extended by the combination of sulphite dipping (0,5% Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>) and irradiation (50 krad). However, results with steam-peeled potatoes were less favourable due to "starch bleeding" induced by the peeling treatment. The shelf-life of non-irradiated steam-peeled potatoes was 1 - 2 days at 10°C; of the irradiated samples at most 3 days.

#### Publications - 1975.

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Resultaten van het project No. 27

Hoofd van het team en wetenschappelijke medewerkers:

C. van Heemert, A.S. Robinson and G. Zurlini.

Titel van het project:

Genetic control of the onion fly *Hylemya antiqua* (Meigen) by structural chromosome mutations.

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Beschrijving van de resultaten:

X-linked Translocations.

The complex X-linked translocation has been studied for 2 years. Numerical non-disjunction occurs at meiosis in translocation heterozygous females (TN). In this way translocation trisomic individuals (TN+X) were obtained which carried an additional sex-chromosome. Several other aneuploids were obtained but they were sterile. Since a normal X-linked translocation cannot be obtained as a homozygous stock (TT males do not exist) a sibcross was started with TN+X flies instead of TN flies. After a few generations of sibcrossing a translocation homozygous stock was obtained with two extra sex-chromosomes (see Fig. 1). The females are TT+2X and the males TT+X+Y. Although the homozygous stock has some sterility we think that this unique stock provides the opportunity for starting population studies in laboratory cages and in field cages to establish its genetic control potential.

Pericentric Inversion.

Research on the pericentric inversion In (3)2, was carried out for several reasons. Firstly, it was necessary to know the relationship between semi-sterility and unbalanced embryonic karyotypes. Semi-sterility, as found in testcrosses of inversion heterozygous (IN) females, is the consequence of crossing-over in the inversion loop. Males have no crossing-over, so there is no sterility. Many eggs (approx. 600) 10 h old were analysed cytologically. Four different types occur of which two are balanced NN and IN and two unbalanced IN and SN. Of these types only SN, which has a large deficiency, dies before hatching. The percentage of SN types (23%) is in good agreement with the percentage of sterility (26%). The IN type which carries as long duplication probably dies very early in the larval stage.

Secondly somatic chromosome pairing has been studied in eggs by analysing seven different karyotypes which occur after crossing IN x IN. The main conclusion was that besides the centromere the telomeres have an important function during mitotic prophase.

Thirdly, after interpreting the meiotic segregation data obtained after testcrosses and sibcrosses it was possible to conclude that non-random-disjunction occurs. Probably due to dragging of a long cross-over chromated with the normal or inverted chromated during anaphase I; this type of meiotic drive has been found elsewhere.

Fourthly, the isolation of a compound strain was considered starting with this inversion stock. One of the possible approaches for genetic control is crossing IN females with IN males irradiated with X-rays.

Finally we were able to locate chromosomally the white eye marker of the strain we received from Canada. By using the pericentric inversion it was established that the eye colour marker is on the long arm of chromosome 3.





Fig. 1 - Left: Normal karyotype of the onionfly ( $2n = 12$ )  
Right: Translocation homozygous karyotype of the X-linked  
translocation + 2 additional sex-chromosomes ( $2n = 14$ ).  
(Both spermatogonial metaphases, note the somatic pairing).

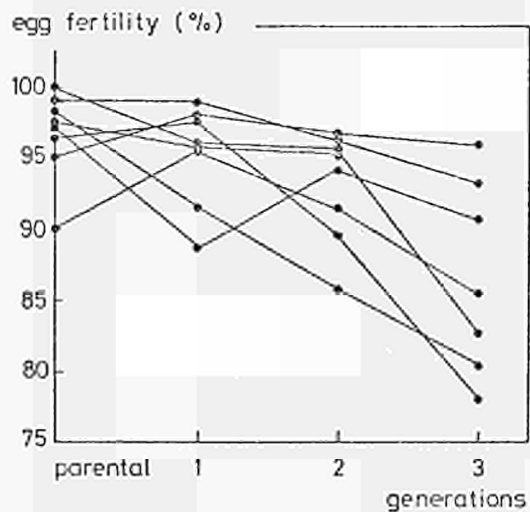


Fig. 2 - Effect of strong inbreeding on the egg fertility in  
*Hylemya antiqua*.

A Giemsa banding technique has been started in order to make possible the differentiation of different chromosomes in normal and rearranged karyotypes and to establish the breakpoint positions. The first banding has been seen and we hope to refine the technique soon.

#### New Chromosomal Rearrangement Stocks.

Following irradiation of *Hylemya antiqua* males (7 days old) F<sub>1</sub> were test-crossed and 8 semi-sterile stocks were produced (see Annual Report 1974). In 7 of these stocks new translocations have been identified and preliminary work has begun on the assessment of some of their genetical properties. Three of the stocks have been given to B. Vosselman (Dept. Genetics, Agric. University, Wageningen) who is attempting to make them homozygous. Using cytological and fertility data two of the remaining translocations have been subjected to preliminary inbreeding. Following a dose of 150 rad of fast neutrons a unique and potentially very useful rearrangement has been identified. It is a quadruple translocation in which four chromosome pairs are involved in a cyclic rearrangement. A cyclic triple translocation was already found previously. This stock is highly sterile but has enough residual fertility to be reared. Further, a paracentric inversion has now tentatively been identified in material originating from fast neutron irradiation. These preliminary results indicate that fast neutrons might be extremely useful for the induction of useful chromosomal rearrangements.

#### Induction of Dominant Lethals by Fast Neutrons.

Using identical experimental techniques as for X-rays (Annual Report 1973) the effect of fast neutrons on mature sperm has been studied. As expected fast neutrons are much more efficient in the production of dominant lethals by a factor of 3-4. The male sterilizing dose for X-rays is 3.0 krad, for fast neutrons it is 0.8 krad.

By using a standard level of dominant lethality for X-rays and fast neutrons the efficiency will be compared in the production of chromosomal rearrangements suitable for genetic control. Using the progeny descended from sub-sterilizing doses of fast neutrons a programme of test-crossing has already been started on a large scale.

#### The Effect of Strong Inbreeding on a Control Stock.

Eight lines have been studied which have been propagated by single females at each generation. The females had been mated with these ribs. The following biological parameters have been measured at each generation: 1. mating frequency; 2. fertility; 3. larval survival; 4. pupal emergence. The results of inbreeding on fertility can be seen in Fig. 2. A general trend can be discerned in that there is a gradual reduction in fertility. Also the range of the fertilities increases with the generations. The parental fertilities represent the values of the individual females used to start the 8 lines. The fertilities in the following generations represent the mean fertility of daughters descended from a single inbred female. A similar picture emerges from the other parameters measured, i.e. a gradual reduction of the value of the parameters without any major effects. It can be concluded that the inbreeding necessary for the production of homozygous rearrangements is not a serious obstacle. Further, two of the inbred lines have been given to G. Zurlini for population dynamics studies.

#### Field Experiments.

Releases of a translocation heterozygous stock and sterile insects were made into separate field cages in a ratio 1:1 with control insects. There was also a control cage. There was a significant improvement in the field cage environment, compared with 1974 (see Annual Report) and in all these cages a second generation was produced. The fertilities in the field cages at the first generation were Control - 96%; Translocation cage - 76%; Sterile insect cage - 80%.

Sampling for diapause pupae in November 1975 yielded the following results. Five samples were taken from each cage and the pupae extracted by washing. In the control cage 17 pupae were recovered and in the translocation cage 12 pupae. No pupae could be found in the sterile insect cage.

- Conclusions a) the technique of a field cage environment has now been successfully developed.  
b) the translocation can maintain itself under field conditions.

#### Homozygosing of T6.

Many homozygous T6 adult males have been identified by cytology. Difficulties in preparation prevent the same criteria being applied to females and the assumption was made that the homozygous female was also viable in the adult stage. However, following matings between homozygotes and heterozygotes the sex ratio in the progeny was severely distorted in favour of the males; in fact there were 2 x as many males as females. This might indicate that the homozygous females die before becoming adults. This was a single observation so confirmation is necessary.

#### Experiments on competition and density effect among larvae.

A relatively large mortality is observed during the larval stage of the onion fly under present laboratory rearing conditions. In preliminary experiments started this year on competition for food and density effect among larvae of *H. antiqua* (Meigen):

- the existence of a density dependent relationship was confirmed;
- survival was raised to 73% at a level of 1.5 g onion pulp available per larva, the same picture can be seen for the mean dry weight of pupae, but also quality of food seems important;
- at the lowest densities (food < 0.2 g per larva) competition for food among larvae can compensate for the changes in density caused by other disturbing factors: that is intraspecific competition can have a stabilizing effect on a population acting as a regulatory factor of density;
- the different levels of competition did not seem to affect significantly the mean development time of larvae: pupae appear 9-11 days after the larvae hatched. The larvae which remain after this time do not appear to develop to pupae.

Publications - 1975

- HEEMERT, C. VAN and K.J.A. WIJNANDS-STÄB. Radiation induced semi-sterility for genetic control purposes in the onion fly, *Hylemya antiqua* (Meigen). II. Induction, isolation and cytogenetic analysis of new chromosomal rearrangements. Theor. Appl. Genetics 45: 349-354 (1975).
- ROBINSON, A.S. and C. VAN HEEMERT. Preliminary radiobiological studies on *Hylemya antiqua* (Meigen) and data on three radiation-induced (0.5 krad) chromosomal rearrangements. "Sterility Principle for insect control 1974". IAEA/FAO Symposium, Innsbruck: 375-385 (1975).
- HEEMERT, C. VAN. Ph.D. Thesis, Agricultural University, Wageningen (1975).
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- ROBINSON, A.S. Translocations and a balanced polymorphism in a *Drosophila* population. *Heredity* in press (1976).

Resultaten van het project No. 29

Hoofd van het team en wetenschappelijke medewerkers:

A.M. Feldmann and P.W.F. de Vrijer.

Titel van het project:

Genetical and radiobiological studies on the twospotted spidermite *Tetranychus urticae* C.L. Koch, in relation to genetic control.

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Beschrijving van de resultaten:

I.1. The induction of structural chromosome mutations.

Out of 184 F<sub>1</sub>-females, derived from 1,5 krad X-ray irradiated males mated to untreated females, 71 were heterozygous for an S.C.M. The percentage of 38,6 of S.C.M.-heterozygotes is low compared to the percentage published earlier (60,8, Feldmann, 1974). Also the observed percentage of F<sub>1</sub> females heterozygous for recessive lethals (40,2 compared to 86,3), of steriles (31,6 to 19,1) of infecunds (14,4 to 23,6) and normals (25,5 to 3,4) differ significantly from earlier values. These differences can be explained by a difference in the experimental technique.

It was attempted to isolate homozygotes from 69 of the 71 S.C.M.-heterozygotes, but 42 were lost during outcrossing, before inbreeding was started. Of the remaining 27 lines only 9 could be reared in a homozygous condition. The percentage of viable homozygotes is 33, which is significantly lower ( $p < 0,001$ ) than the expectation of 80,3% based on earlier results. This expectation was based on the frequency with which recessive lethals were associated with S.C.M.'s in heterozygous conditions. It is very probable that factors such as synthetic lethality, small duplications and deficiencies finally determine the viability of an S.C.M. in a homozygous condition. The distribution of semisterile F<sub>1</sub>-females, according to the survival of their haploid- and diploid progeny, is not significantly different from the one in earlier experiments ( $G_{adj} = 1.817$ ;  $\chi^2_{0,5(1)} = 3.841$ ).

I.2. Re-irradiation of a line, homozygous for a Structural Chromosome Mutation, in order to isolate new complex rearrangements.

A stable line, homozygous for an S.C.M., induced by 200 rad X-rays in mature sperm, was chosen. Before starting the actual experiment on the induction of new S.C.M.'s in this line, preliminary experiments were set up in order to study the genetical effects of X-rays in this line (17ATT). The percentage of dominant lethals, induced in mature sperm of 17ATT, by 1,5 krad X-rays, was 23,1% + 2,4% and differed not significantly from the percentage of dominant lethals which was induced by 1,5 krad X-rays in mature sperm of wildtype males (25,7% + 7,3%). Also striking was the resemblance of the fertility pattern of the F<sub>1</sub>-females (i.e. the survival-percentages of the haploid- and diploid progenies of individual F<sub>1</sub>-females) from 1,5 krad X-rays irradiated parental 17ATT males with those of irradiated parental wildtype males. After irradiation the males were mated to virgin females of the same origin.

In order to have an idea about the height of the intersterility of new S.C.M.'s induced in 17ATT, with the wildtype material, semisterile virgin F<sub>1</sub>-females, derived from irradiated 17ATT males, were outcrossed to untreated wildtype males. The female progeny from the outcross was assumed to be either heterozygous for the 17ATT-S.C.M. or a new multiple rearrangement, associated with a high level of sterility, and wildtype. However, the sterility-levels of those females were rather homogeneous and not much different from the sterility of females heterozygous for wildtype

and the S.C.M. of 17ATT. Furthermore, about 6% of the females were normally fertile! The preliminary conclusion is that irradiation of a line, homozygous for an S.C.M. does not lead, in general to the formation of more complex rearrangements, associated with higher intersterility. The second conclusion is that reversion of the S.C.M. to the wildtype reconfiguration is induced by ionizing radiation. This reversion is much less frequent in unirradiated lines. Nevertheless, three new lines, homozygous for an S.C.M., induced by 1,5 krad X-rays in 17ATT-males, are isolated. It was observed that the level of intersterility of these new lines with either 17ATT or the wildtype was the same, with exception of one line. This line showed higher intersterility with wildtype.

#### II. Sterile- or Substerile Insect Release Technic (abbreviated resp. as S.I.R.T., S.S.I.R.T.).

Males of *Tetranychus urticae* Koch were treated with sterilizing (24 krad) or substerilizing (4 krad) doses of X-rays. Experimental methods were developed to measure the mating competitiveness (m.c.) of these males, using an albino pigment mutation as a marker.

In general the use of the albino strain in the experiments on MC proved to be very successful. The reproducibility of some experiments turned out to be remarkable high. However, there is still little information as to the extent to which the results are comparable to those obtained in experiments using the wildtype strain only.

Irradiation with 4 krad X-rays did not show any significant influence on the MC of male wildtype spidermites, compared to the 0 krad series. A dose of 24 krad X-rays demonstrated a significant decrease.

Ageing effects in 0 krad and 4 krad series were similar. The MC remained constant during the first five days, or even increased a little, after which it decreased, but on the twelfth day it was still at 2/3 of its original level.

The ratio of virgin teneral females to mature males in a population is essentially not identical with what is called in general "sex ratio". Especially when the fact is considered that MC after five days is still as high as on the first day, it is obvious that the actual "mating ratio" is rather in favour of the males.

As to the effect of mite density on MC it is demonstrated that the densities tested had no significant influence. Possibly higher densities are able to influence MC but it can be doubted if such densities are still relevant to genetic control, because when those densities occur control of spidermites has already failed.

The potexperiments have shown that, under simplified and optimum conditions, wildtype males are very well able to penetrate into the albino population. It should be stressed here that there is no evidence that the used strains showed any form of assortive mating, which is something to be prepared for when practical application is considered. Although it is too early to give a conclusive judgement on the applicability of S.I.R.T. or S.S.I.R.T. for spidermite control, both methods revealed possibly critical aspects to which more attention has to be given.

Irradiation with 24 krad X-rays causes a significant lowering of male MC, which can only be compensated by higher release ratios.

Irradiation with 4 krad X-rays has no demonstrable effect on male MC, but the fact has to be considered that the female  $F_1$ -progeny, although sterile, still causes feeding damage.

For both methods an adequate release programme has to be developed, in which the right release ratio is combined with the right release frequency in order to achieve optimal spidermite control.

Consequently the next step in this project has to be the realization of pot-experiments where different release ratios and frequencies are analysed under more practical conditions.

Publicaties - 1975.

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- FELDMANN, A.M. Contribution to the ESNA/IOBC Working group on Genetical Methods of Pest Control. The impact of repair of radiation-induced effects in sperm on the applicability of the delayed sterility method for the control of *Tetranychus urticae*.
- FELDMANN, A.M. Contribution to the Contact Group of Radioentomology of Euratom. Preliminary Experiments on the Sterile Male Method with *Tetranychus urticae*.

Resultaten van het project No. 30

Hoofd van het team en wetenschappelijke medewerkers:

S.C. van de Geijn, G.P. Mix, G. Sauer.

Titel van het project:

Measurement and localization of biological processes at tissue, cellular and subcellular level.

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Beschrijving van de resultaten:

1. Preparation of plant material for microlocalization of diffusible substance

Emphasis has been put on the procedure for embedding of tissue from tomato stems after dehydration. Epon, an epon-araldite mixture and Spurr's low viscosity resin have been tested. Epon turned out to be too viscous for a good infiltration, whereas the epon-araldite mixture used here, remains after polymerization too soft, compared to the lignified material of the tomato stem. In this respect the properties of Spurr's low viscosity resin are much better. The influence of several parameters on the quality of this resin have been tested to allow the choice of a good procedure for the processing. Propylene oxide has been rejected as an infiltration medium, as small amounts, remaining behind, may cause an excessive shrinkage. The quality of the polymerized resin was shown to be very sensitive to water. Even small amounts, taken up from the air if exposed for a short time have a rather deteriorating effect. Therefore, all manipulations have to be done in dried air, using acetone as an infiltration medium.

Room temperature dehydration produced reproducible results. For a sample size of 3 x 1 x 1 mm infiltration is good.

Dehydration at low temperature, either by freeze-drying at  $-60^{\circ}\text{C}$  or by freeze-substitution at  $-80^{\circ}\text{C}$  in acetone, apparently resulted in samples which were not comparable to those obtained by room-temperature dehydration. The difference persisted even after prolonged soaking in acetone at room temperature. Such samples kept in general floating in acetone and embedding resin for long periods. The quality of the polymerization did not allow a good sectioning at the ultramicrotome even if infiltration seemed to be good. Different procedures are tested to overcome this problem. For Spurr's low viscosity resin infiltration is improved markedly if the embedding mixture is used without accelerator, and the pressure is lowered intermittently. The accelerator is applied later as a drop to the samples which are blotted first with filterpaper.

Best results were obtained by increasing the concentration of the resin in acetone stepwise from 1:1 till 6:1, followed by a very slow evaporation of the remaining acetone, applying a slight vacuum while keeping the samples submerged continuously by a grating.

The acetone-step after dehydration at low temperature is justified by the results of tests of washing out of cadmium and phosphorus. It could be shown that washing out from dry material, obtained by freeze-drying or freeze-substitution, is negligible.

Different sample preparation techniques for micro-autoradiography of  $^{115}\text{mCd}$ ,  $^{109}\text{Cd}$  and  $^{32}\text{P}$  have been compared as to washing out. Roomtemperature dehydration, especially after fixation of the tissue by glutaraldehyde and  $\text{OsO}_4$  caused a very high loss of radioactivity (up to 70%). After freeze-drying and freeze-substitution in acetone between 95 and 99.7% of the activity was retained in the samples. Some preliminary tests were done for micro-autoradiography to obtain an estimation of the distribution of radioactivity in a transection of a tomato stem from frozen fresh sections.



X-ray and stripping film were exposed at  $-30^{\circ}\text{C}$ . The grains of the X-ray film are too coarse to reach a good resolution. Contamination of the fresh sections by the knife limits the reliability of this procedure. Plant material labelled with  $^{109}\text{Cd}$  was prepared by soaking small pieces in a labelled buffer solution. After roomtemperature dehydration the material was embedded in Spurr's low viscosity resin. A good micro-autoradiogram can be obtained in about three weeks using liquid emulsion (K5) and  $2\ \mu\text{m}$  thick sections if the activity is of the order of  $5\ \mu\text{Ci/g}$  dry weight of material.

The freeze-drying equipment has been improved essentially by the installation of an infra-red heating source. In this way differences in the temperature of the copper sample holder and the plantmaterial itself (up to  $25^{\circ}\text{C}$ ) can be avoided.

## 2. *In vivo* measurement of the distribution of $\beta$ -emitting tracers.

The methods for the *in vivo* determination of the localization and distribution of radionuclides in plant material by spectrometry of the emerging  $\beta$ -radiation described in former reports, have been adapted to the spectrum of  $^{115\text{m}}\text{Cd}$  ( $E_{\text{max}} = 1630\ \text{keV}$ ). To reduce computation time, and to allow some statistical evaluation of the results, the calculation procedure has been reconsidered and based on a "Stepwise regression" method. This approach involves the sequential insertion of the most significant contribution to the spectrum into the regression.

The method in its ultimate form has been described in more detail in a publication, and a biological application is reported in the project No. 6 of the Radiation Protection programme.

## Publications - 1975.

GEIJN, S.C. VAN DE, CH. PETIT. A non-destructive determination of the internal distribution of cadmium ( $^{115\text{m}}\text{Cd}$ ) in the stem of a tomato plant (*Lycopersicon esculentum* Mill. cv. Money maker) by the analysis of the externally measured beta-spectrum. ESNA Newsletter on the application of nuclear methods in biology and agriculture. No. 5, Dec. 1975.

GEIJN, S.C. VAN DE. A non-destructive determination of the depth distribution profile of a radionuclide by analysis of its beta-spectrum. Nucl. Instr. and Meth. submitted for publication.

Resultaten van het project No. 31

Hoofd van het team en wetenschappelijke medewerkers:

A.F. Groneman.

Titel van het project:

Recycling of liquid waste.

Beschrijving van de resultaten:

Measurements of the specific resistance to filtration indicated that irradiated sludge will drain at a considerably faster rate than untreated anaerobic digested sludge. Typical results are presented in table 1. A dose of 100 krad at an average dose-rate of 62 krad per hour had already reduced the specific resistance of the sludges from Geiselbullach and Starnberg works by 44% and 61%, respectively.

Table 1 - Effect of radiation dose on the specific resistance and compressibility of sludges from Geiselbullach and Starnberg works, irradiated at the pilot plant.

Radiation dose krad	Geiselbullach		Starnberg	
	specific* resistance sec <sup>2</sup> /g	coefficient of compressibility	specific* resistance sec <sup>2</sup> /g	coefficient of compressibility
0	22.8x10 <sup>9</sup>	0.59	18.0x10 <sup>9</sup>	0.84
100	12.8x10 <sup>9</sup>	0.80	7.0x10 <sup>9</sup>	1.34
200	15.6x10 <sup>9</sup>	0.73	6.0x10 <sup>9</sup>	1.14
300	12.5x10 <sup>9</sup>	0.79	5.6x10 <sup>9</sup>	1.20

\*Measurements of the specific resistances were performed at a pressure of 500 g/cm<sup>2</sup>.

The rate of diminution of the specific resistance decreased with increasing dose. Irradiation treatments also changed the sensitivity of the sludges to pressure. Compressibility of the sludge solids from Geiselbullach and Starnberg increased the most at a dose of 100 krad (36% and 60%, respectively). Apparently a dose of 100 krad induced changes in the structure of the flocks in the sludges resulting in better drainable and more compressible flocks.

However, it is essential to know how long the conditioning effect of the irradiation treatment is effective in practice. A conditioning effect for a short time would be of advantage for mechanical dewatering only, whereas permanent conditioning effects would also be of advantage for dewatering on drying beds. Experimental results plotted in fig. 1 indicate that the irradiation treatment had reduced the average specific resistance of 25 x 10<sup>9</sup> sec<sup>2</sup>/g to an average value of 10 x 10<sup>9</sup> sec<sup>2</sup>/g and that effect was sustained over 20 days.

Especially noteworthy is that the filtrate from the irradiated sludge was substantially higher in chemical oxygen demand (COD) than was the filtrate from untreated sludges. In the sludges from Geiselbullach and Starnberg the COD values of the filtrates increased proportionally with the radiation dose from 462 mg O<sub>2</sub>/l and 290 mg O<sub>2</sub>/l to 1305 mg O<sub>2</sub>/l and 827 mg O<sub>2</sub>/l, respectively, by a dose of 300 krad in the pilot plant. Similar increases in COD were measured in filtrates of sludges irradiated in the laboratory experiments.

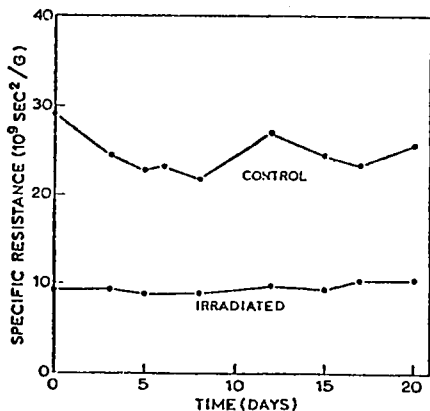


Fig. 1 - Effect of time after the irradiation treatment on the specific resistance of sludge from Geiselbullach waste water treatment plant.

Total organic carbon concentrations, however, increased to a much smaller extent, indicating that by the interaction of gamma irradiation with sludge, substances were detached from the sludge flocks. These substances were primarily oxidizable compounds and derived from organic matter. They had a significant lower initial oxygen content than the compounds normally dissolved in the sludge solution. Chemical studies indicated that irradiation of anaerobic stabilized sludge produces inorganic carbon (carbonates and bicarbonates) and  $\text{NH}_3$ , but irradiation had no consistent effect on the protein content of the sludge solution.

From the experimental evidence obtained so far one can conclude that radiolysis of some organic substance occurred during the irradiation of sludges in the laboratory as well as in the pilot plant. These radiolytic organic products have properties similar to synthetic polymers. They reduce the net negative electrostatic charge of colloidal sludge particles and also enhance the formation of larger flocks with a loose structure and thus improve the dewatering properties. Flocks with a loose structure are more compressible, which is reflected by an increase of the coefficient of compressibility. Detailed discussions of results summarized above were presented at a conference of the International Atomic Energy Agency in March 1975.

The Technical University of Munich joined the scientific cooperation programme of the Association Euratom-ITAL with the Bayerische Landesanstalt für Bodenkultur und Pflanzenbau. Research of the staff of this university confirmed the conclusions published earlier.

This led to significant technical changes in the large experimental irradiation pilot plant near Munich that is used for disinfection of sewage sludge. A new impeller pump has been installed in the pilot plant to reduce negative effects of pumping on the dewatering characteristics of sludge. Also changes in the design of the pilot plant are considered and are reported in more detail elsewhere.

Publications - 1975.

- GRONEMAN, A.F. International Symposium on the Use of High-Level Radiation in Waste Treatment - Status and Prospects. Munich, Internal Report No. 161, March 17-21, (1975).
- GRONEMAN, A.F. Consulting services in the area of nuclear treatments of waste water in Switzerland. External Report No. 32 (1975).
- GRONEMAN, A.F. Effects of gamma radiation at pilot plant level as compared to effects of pasteurization on the dewatering of sewage sludges. IAEA-SM-194/608. Proceedings of the International Symposium on the Use of High-Level Radiation In Waste Treatment - Status and Prospects. International Atomic Energy Agency (1975).
- GRONEMAN, A.F. and A. SÜSS. Technological and agricultural experiences with sewage sludge irradiated at a pilot plant. Paper presented at the VIth Annual Meeting of ESNA, Cadarache, France, September 1975.
- GRONEMAN, A.F. Nuclear methods for pollution abatement in the food industry and agriculture. Paper presented at the VIth Annual Meeting of ESNA, Cadarache, France, September 1975.
- GRONEMAN, A.F. Progress and technological experiences on dewatering properties of sewage sludge irradiated at a pilot plant. External Report (In press).
- OOSTERHEERT, W.F. and GRONEMAN, A.F. Report of the visit to the Socialistic Republic of Romania. 15-18 December 1975. External Report (In press).

Resultaten van het project No. 32

Hoofd van het team en wetenschappelijke medewerkers:

J.F. Stoutjesdijk, W.F. Pieters.

Titel van het project:

The propagation of nuclear methods in biology and agriculture.

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Beschrijving van de resultaten:

1. Courses.

.1 General Radionuclide course.

A general Radionuclide course was organized from February 24 till March 14, 1975 for 23 participants.

.2 Health Physics course.

For the second time a Health Physics course was organized in cooperation with the Health Physics Department of the Association. The course was given from April 7 till April 18, 1975 with 16 participants.

The course was concluded with an official examination on May 6, 1975 under auspices of the Ministry of Health and Environmental Protection and of the Ministry of Social Affairs.

Of the participants 15 took part in the examination and also two participants of the ITAL<sup>2</sup>course of 1974. Of these 17 candidates only two failed the examination.

.3 Liquid Scintillation courses.

Two Liquid Scintillation courses have been organized from November 24 till December 19, 1975 with 13 and 12 participants respectively. Among them were two guestworkers from Kenya and Columbia, respectively.

2. Evaluation of radiological techniques.

.1 The use of wavelength shifters in Cherenkov counting of <sup>32</sup>P.

<sup>32</sup>P often has to be determined in solutions of strong acids, obtained by destruction of the sample by those acids or by dissolving the residue of an ashed sample in acid. The use of wavelength shifters has the advantage of higher counting efficiencies, but it proved that HCl-solutions up till a normality of about 0.1 produced a strong quenching of the two investigated wavelength shifters 4-methylumbelliferone (MU) and aesculin (Ae). H<sub>2</sub>SO<sub>4</sub> gave the same result with Ae in solutions with more than 20% of H<sub>2</sub>SO<sub>4</sub>, but with MU the counting efficiency increased (see Annual report 1974). A nearly constant counting efficiency was found between 50 and 90% of H<sub>2</sub>SO<sub>4</sub>. For solutions with H<sub>2</sub>SO<sub>4</sub>-concentrations between 20 and 50% a mixture of MU and Ae might give more stable counting efficiencies; in fig. 1 the results of a 1:1 mixture of MU and Ae are shown to be intermediate between the results of the two pure wavelength shifters.

Both wavelength shifters gave stable countrates for one week in solutions up to 90% of H<sub>2</sub>SO<sub>4</sub> v/v.

.2 Quench correction methods for Cherenkov-counting <sup>32</sup>P with wavelength shifters.

Counting efficiencies in Cherenkov counting are influenced by colour quenching and if wavelengthshifters are used, also by chemical quenchers. We have investigated if corrections for these two kinds of quenchers can be applied with the sample channels ratio (SCR) method. As chemical quenchers formic and acetic acid and hydrogenperoxide were used and as colourquenchers nitric acid and methylorange. The results are given in fig. 2.

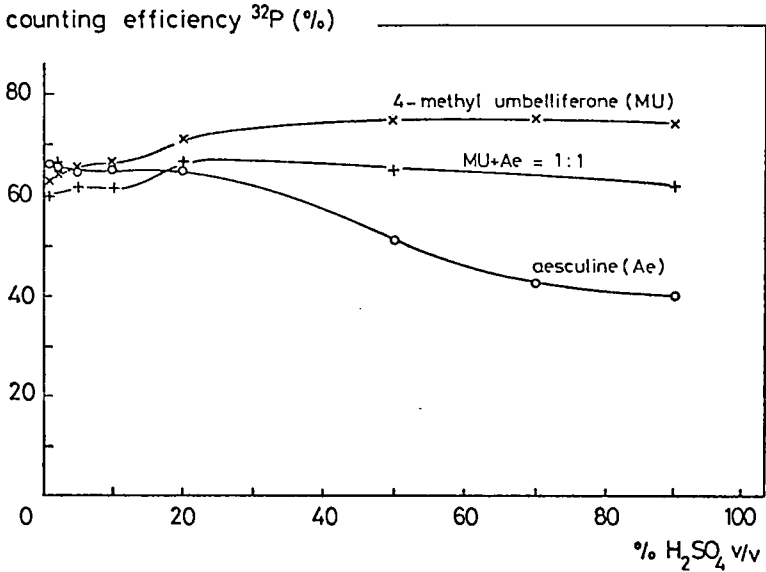


Fig. 1 - Cherenkov counting efficiency of  $^{32}\text{P}$  vs.  $\text{H}_2\text{SO}_4$  concentration in solutions with 100 mg/l of 4-methylumbelliferone and aesculin as wavelength shifters.

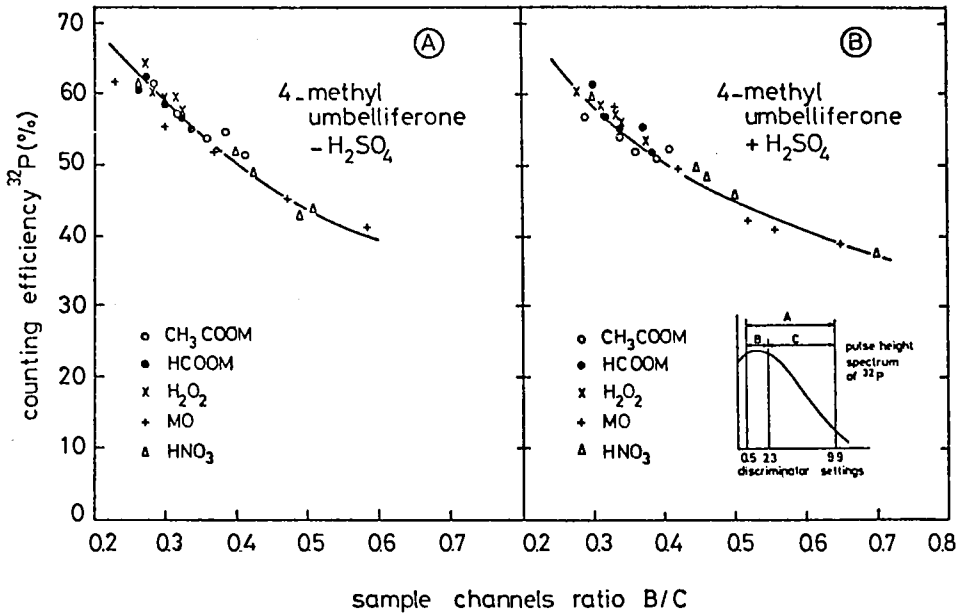


Fig. 2 - Cherenkov counting efficiency of  $^{32}\text{P}$  vs. sample channels ratio in solutions with 100 mg/l of 4-methylumbelliferone and several quenching compounds in water and in 10%  $\text{H}_2\text{SO}_4$  solutions.

The results are given in fig. 2 and this figure shows that with the SCR-method corrections for chemical and colour quenching can be applied with the help of the same calibration curve. Also for solutions with 10% of H<sub>2</sub>SO<sub>4</sub> the same curve is valid. However, with more concentrated H<sub>2</sub>SO<sub>4</sub> solutions, in HCl-solutions and also for HCl and H<sub>2</sub>SO<sub>4</sub> solutions with aesculin as wavelength shifter different calibration curves should be used.

### 3. Cooperation with other institutes.

- .1 Cooperation with mr. A. de Jong, Department of Animal Physiology, Agricultural University, Wageningen about the quantitative determination of <sup>35</sup>S in proteins with liquid scintillation counting. The wet destruction method of Mahin and Lofberg with HClO<sub>4</sub> + H<sub>2</sub>O<sub>2</sub> (1 h at 60-80°C) gave better results than total combustion or solution in alkali.
- .2 Orientating discussion with mr. W.N.J. van Gelder, Foundation for Agricultural Plant Breeding, Wageningen (SVP) about a quantitative determination of solanine in potatoes with an isotope dilution method with the help of <sup>3</sup>H-labelled compounds.
- .3 Orientating discussion with drs. K.K. van Hellemond, Foundation ILOB, Centre for Animal Nutrition Research, Wageningen, about the use of a <sup>3</sup>H-labelled anthelmintic in cows.  
As no suitable cow-house was available, it was impossible to carry out the experiments.
- .4 Orientating discussions with dr. A. Fuchs and two students, Department of Technical Biology, Technological University of Delft, about the use of tracers for studying the effect of the temperature of surface water on the toxicity of certain compounds (e.g. Hg, Cd) for algae.
- .5 Orientating discussions with Mrs. ir. M. Jansen-Jurkovicova (Department of Virology, Agricultural University of Wageningen) about the use of tracers (<sup>32</sup>P, <sup>3</sup>H, <sup>125</sup>I) in the study of virus-DNA in two different insects (*Adoxophyes orana* F.R. and *Barathra brassicae* L.).
- .6 Orientating discussions with ir. P. Doelman (National Institute for Nature Management, Arnhem) about the use of <sup>210</sup>Pb in studies about the uptake of lead by bacteria. The experiments will start in January, 1976.
- .7 Many advices about radiological measuring equipment and methods were given.

### 4. Work for committees.

- .1 Work for C.C.R.X.

For the Coordination Commission for the Measurements of Radioactivity and Xenobiotic Materials the annual report 1974 was prepared. As technical secretary for radioactivity I was a member of a commission of the Dutch Health Council to prepare safety standards for the biosphere after nuclear accidents and also of another commission of this Council to prepare an advice about the consequences for the population of the installation of nuclear reactors for energy production.

In the same function I have participated in the preparation of alarmschemes for the nuclear reactors at Dodewaard and Petten.

- .2 Work for L.A.C.

As a member of the Agricultural Advisory Board for Environmental Pollutants of the Ministry of Agriculture and Fisheries two meetings of this Board were attended.

### 5. Publication of a Newsletter.

In May the fourth issue of the Newsletter on the Application of Nuclear methods in Biology and Agriculture was published and in December the fifth issue with seven and eleven contributions respectively.

Publications - 1975.

- Newsletter on the application of nuclear methods in Biology and agriculture No. 4.
- Newsletter on the application of nuclear methods in Biology and agriculture No. 5.



Resultaten van het project No. 33

Hoofd van het team en wetenschappelijke medewerkers:

J.G. de Swart, J.F. Stoutjesdijk.

Titel van het project:

Development of nuclear techniques and related instrumentation for biological and agricultural research.

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Beschrijving van de resultaten:

The items 1 and 2 of this report are dealing with work directly related to the Association's programme. The items 3, 4, 5 and 6 are related to work carried out in cooperation with institutes outside the Association.

1. Calendar Clock module.

An experiment has been started to check the possibility of using the radio signals of the Western German radio station DCF 77 for time of the day and day of the year registration in our data collection system. All this information is in pulse-code transmitted within every distinct minute. As a result, an apparatus able to decode this information, will be self-correcting in case of disturbances like e.g. power failure. A working prototype was built and tested under conditions of practical use. In environments with a high electrical noise-level, the signal disturbance on the antenna-system was too high to guarantee reliable operation of the clock module. As a result a reliable quartz clock, especially adapted to this problem, has to be built.

2. Data collection systems.

A double data collection system is required for registration of the parameters of the growth chamber system of project No. 6.

Each data collector will register the following measurements on a magnetic cassette tape:

3 channels temperature; 1 channel air humidity; 1 channel light intensity; 2 channels air velocity; 4 channels CO<sub>2</sub> concentration; 1 channel time of the day; 1 channel day of the year.

3. Density measurements of granular material in an elevator system.

(Cooperation with Ir. D.J. van Zuilichem, Department of Food Science, Agricultural University, Wageningen).

Experiments were extended by measurements of the density of the flowing material, especially in the acceleration trajet, with the help of a  $\gamma$ -absorption technique.

As a  $\gamma$ -source 1.85 GBq (50 mCi) of <sup>137</sup>Cs has been used. The influence of several conveying variables, among which specific loading and air velocities, were studied. The total pressure drop has been split up in three components. A calculation procedure of the hold up for various transport conditions was proposed. The velocity distribution was compared with the density distribution. The oscillating behaviour of a pneumatic transport installation can now be described with a mass-vibration model. Bode and Nyquist diagrams were derived from the particle velocity distributions along the ducts, from which the intrinsic frequencies are determined.

4. Measurement of moisture transport in cattle-food pellets.

(Cooperation with Ir. D.J. van Zuilichem, Department of Food Science, Agricultural University, Wageningen).

Water redistribution, immediately after processing cattle-food pellets, is expected to be a cause for changes in their mechanical strength. This leads to the demand for a method for determining the moisture conditions in these pellets.

Because of the small dimensions (8 à 10 mm diameter and 20 mm length) a high precision detector-source combination has been realized for applying low energetic  $\gamma$ -ray absorption techniques to this problem. The preliminary tests have been carried out and the results are in good agreement with the expectations. Experimental runs are planned for the beginning of 1976.

5. Measurement on liquid/liquid displacement from packed beds via positron annihilation scanning techniques.

(Cooperation with Ir. J. Spaninks,  
Department of Food Science, Agricultural University, Wageningen).

Displacement of a liquid from a packed bed by a second liquid, gives in general an irregular liquid-liquid surface separation front. Breakthrough of the second liquid will occur under different circumstances. In order to study the influence of the various parameters on the displacement process, a tracer technique will be applied to determine the shape of the liquid-liquid surface. To obtain the required accuracy the measurement with a single collimated detector will not be satisfactory, because of the poor relation between collimation-angle and efficiency. Considerable improvement was expected by adopting the positron annihilation technique in which an (improved) localization of the tracer is performed by a two-detector combination working in coincidence.

After preliminary tests, the required amount of radioactivity proved to be in the order of some curies. For safety reasons an alternative method, using conductivity scanning will be investigated.

6. Determination of the residence time of food in the stomach of a sheep by a localization technique with a positron emitter.

(Cooperation with Ir. J. van Bruchem, Department of Animal Physiology, Agricultural University, Wageningen).

It is possible to measure the residence time of food in the abomasum of sheep after a single shot of radioactive food into this stomach, if the passage of the labelled material through the duodenum can be observed. This can be done by a localization technique with a positron emitter with two detectors in coincidence to detect the two annihilation quanta of 0.51 MeV emitted in opposite direction by the radionuclide.

An advantage of this method is the low background, as in practice it is not possible to apply heavy lead absorbers at the normally living animal. The commonly used NaI(Tl) scintillation detectors are too large for an *in vivo* detection of the labelled food in a bypass of the duodenum outside the animal and that is why the response was measured of small Geiger-Muller counting tubes (length 2 cm, diameter 0.5 cm). However, in preliminary experiments the counting efficiency of these detectors was found to be only about 2% of that of a normal scintillation detector, so it is not yet certain if these G.M.-tubes are suitable for this purpose.

7. Indexation durable goods.

In behalf of the Indexation system for durable goods at the Agricultural University of Wageningen, assistance has been given to class the nuclear installation and related instrumentation part.

8. Data-way systems - COALA.

As a result of the membership of the Commission Automatization of Laboratory Apparatus (COALA) several advices have been given concerning data collection systems and computer coupled instrumentation.

Publications - 1975.

CHALLA, H., P.A.M. HOPMANS, J.G. DE SWART. A critical study of  $\beta$ -gauging for water content variation measurement in plant leaves. Newsletter on the Application of Nuclear Methods in Biology and Agriculture No. 4, p. 9 (1975).

ZUILICHEM, D.J. VAN, H.B.G. ALLERSMA, W. STOLP, J.G. DE SWART. Local densities and instabilities in a vertical conveying system. Paper to be presented at the 3rd International Conf. on the Pneumatic Transport of Solids in Pipes, Bath (U.K.). April 1976.



- Contractant de la Commission : Université Louis Pasteur - Faculté de Médecine - Laboratoire de Biophysique des Rayonnements et de Méthodologie 11, rue Humann, 67000 Strasbourg.
  - N° du Contrat : SC 001-094-72-1 BIAN
  - Chef du groupe de recherche : R.V. RECHENMANN
  - Thème général du Contrat : Development of high-efficiency and high-resolution ionographic methods - Applications to autoradiographic problems.
- 

A study has been undertaken on the applicability of the activation treatment for electron microscopic photographic emulsions. The first experimental results let appear the possibility to enhance considerably the response of this type of recording systems.

An autoradiographic study on the incorporation of  $^{14}\text{C}$ -thymidine in isolated nerve nuclei of adult rat brains is under way. An optimal methodology has been formulated. Preliminary experiments already allowed to conclude that DNA precursors are incorporated significantly in part of the neuronal nuclei, in extra nucleolar parts of neuronal nuclei and also in glial cells.

Publications : WINTZERITH M., WITTENDORP E., ITTEL M.E., RECHENMANN R.V. and MANDEL P. Track-autoradiographic study of nucleolar DNA synthesis in adult rat liver. *Expt. Cell Res.*, 1975, 91, 279.

RECHENMANN R.V. and WITTENDORP E. Quelques applications de la photographie corpusculaire en microscopie électronique. *Jl. Microsc. Biol. Cell*, 1975, 23, 20a.

WINTZERITH M., WITTENDORP E., RECHENMANN R.V. and MANDEL P. Track-autoradiographic study on nuclear and nucleolar DNA synthesis in adult rat tissues. Fourth European Nucleolar Workshop, Varna-Bulgaria, 1975 sept. 15-20.

RECHENMANN R.V. and WITTENDORP E. Basical physical aspects of development of emulsions. *Journées d'Etudes sur les Techniques de Radioautographie*, Paris, Saclay, oct. 1975; *Jl. Microsc. Biol. Cell* (in preparation).

WINTZERITH M., WITTENDORP E., MANDEL P. and RECHENMANN R.V. Nuclear and Nucleolar DNA synthesis in adult rat brain - A track-autoradiographic study (in preparation).

RESULTATS DU PROJET N°1

- Chef du projet et collaborateurs scientifiques : R.V. RECHENMANN, E. WITTENDORP and B. SENGER.
  - Titre du projet : Development of high-efficiency and high-resolution ionographic methods - Applications to autoradiographic problems.
- 

During the year 1975, the studies on ionographic methodology and on autoradiographic applications in the Life Sciences have been continued, emphasis being led on the improvement of the electron microscope observation stage of the ionographic procedure.

A. FORMULATION OF ACTIVATION PROCEDURES FOR E.M. PHOTOGRAPHIC RECORDING SYSTEMS.

E.M. observations and subsequent photographic recordings of specimens (biological section, macromolecules, autoradiogramm, etc...) are often hampered by the fact that the electrons crossing the preparations cause radiation damage. The development of high sensitive recording systems might be one of the solutions to overcome, at least partially, this problem. We have already shown elsewhere ( 1,2 ), that in the case of nuclear emulsions the activated development procedures always resulted in a strong increase of the recording efficiency. The adaptation of these procedures to E.M. photographic layers should lead to the obtention of satisfying micrographs with good or even improved contrast for a much smaller number of electrons effectively crossing the preparations, if compared with the results obtained by means of usual E.M. procedures.

In a preliminary stage of our study we have submitted the E.M. photographic emulsions considered (Kodak Electron Image Plates, Agfa-Gevaert Scientia) to our standard procedure for nuclear emulsions ( 2 ).

The sensitive layers have been partly exposed to a  $^{14}\text{C}$  source, the mean energy of the  $\beta$  particles emitted by this isotope ( $\sim 50$  keV) laying within the energy region of the electrons applied in Electron Microscopy.

On figure 1a, b, c, d are represented the corresponding fog-corrected density development-time curves. It can be seen that for the different developers used (Kodak HRP, DI9, Amidol, Ferro-oxalate solutions), the activation procedure results effectively always in a significant enhancement of the signal as well as of the signal/noise ratio.

The increase of the fog observed on the preparations submitted to an activated treatment is partly due to the fact that the corresponding commercial plates had recorded radiation background (cosmic rays, ambient activity) which has been intensified by the activation.

It can also be noticed in the case of the Kodak E.I.P. emulsion that at the maximum of the signal/noise curve the activated development enhances the response of this detector by a factor of 10. These preliminary studies already lead to the conclusion that the so-called "nuclear" developers decrease the evolution of the general fog, if compared with the results obtained by means of the reducing solutions advised by the manufacturers.

A systematic study has been undertaken in order to formulate a specific procedure resulting not only in an enhancement of the signal/noise ratio, but also in an improvement of the electron micrographs.

#### B. AUTORADIOGRAPHY OF ISOLATED NUCLEI OF NERVE CELLS.

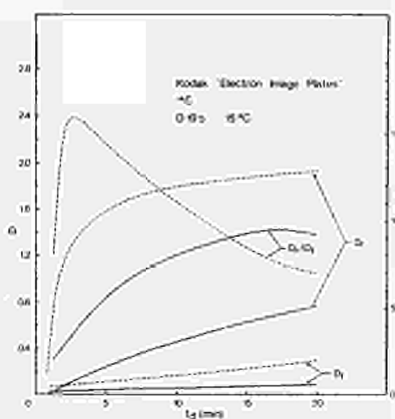
In collaboration with M. WINTZERITH and P. MANDEL (Centre de Neurochimie du CNRS - Strasbourg), the autoradiographic study on the nucleolar DNA synthesis in adult rat nerve cells has been continued.

It appeared at the light of systematic experiments that the procedure described in previous papers and reports (3) could be applied in its main lines to this study. Adult Wistar rats (200-250g) received 25  $\mu$ Ci or 50  $\mu$ Ci of methyl [ $^{14}$ C] thymidine (55.3 mCi/mM 162, CEA, France) intercisternally 4h before sacrifice. The nuclei were prepared by the classical method of Chauveau et al. in hypertonic sucrose. The isolated nuclei were smeared on mordanted gelatin coated slides and fixed in formalin. The preparations were afterwards submitted to the activated autoradiographic process.

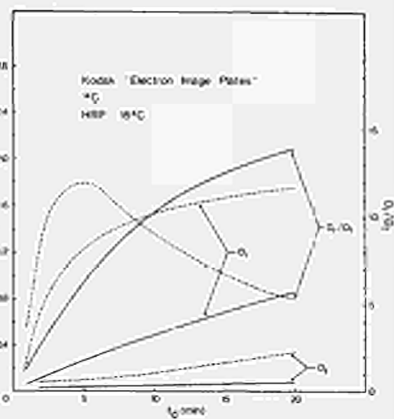
The scanning of the autoradiograms led to the following observations :  
1) A significant number of neuronal and glial nuclei showed a low  $^{14}$ C-thymidine incorporation. 2) Some of the neuronal nuclei showed unambiguous labeling in their nucleolus (Fig. 2). 3) In another fraction of the neuronal nuclei  $^{14}$ C tracks were located in the chromosomal compartment (Fig. 3). 4) Part of the glial cells were weakly labeled (Fig. 4). 5) As might be expected a small number of heavily labeled glial nuclei was counted (Fig. 5).

Quantitative determinations by track counting are under way.

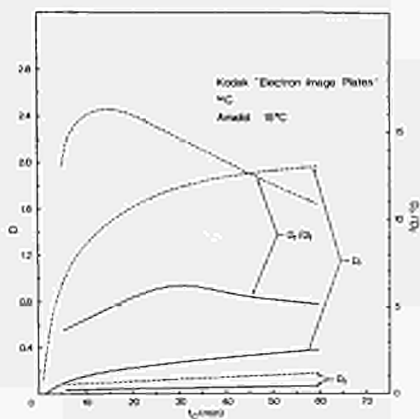
REFERENCES : 1) RECHENMANN R.V. Proc. 7<sup>th</sup> Int. Coll. Corpusc. Phot. and Visual Solid Detectors (Barcelona, 1970), 559-EUR. N°4688e, 1971. 2) RECHENMANN R.V. and WITTENDORP E. J1. of Microscopy, 1972, Vol. 96, 227. 3) WINTZERITH M., WITTENDORP E., ITTEL M.E., RECHENMANN R.V. and MANDEL P. C.R. Acad. Sc. Paris (24 sept. 1973), t. 277, Série D, 1033; RECHENMANN R.V. and WITTENDORP E. Annual Report 1973, EUR. 5138 d-e-f-i-n, 609; Annual Report Association EURATOM-Ital, EUR. N° 5226; WINTZERITH M., WITTENDORP E., ITTEL M.E., RECHENMANN R.V. and MANDEL P. Expt. Cell Res., 1975, 91, 279.



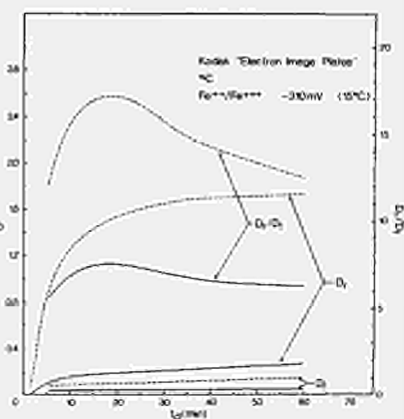
a



b



c



d

Figure 1. Fog corrected density development-time curves ( $D_F$ ) with corresponding signal/noise ratios ( $D_r/D_F$ ).

Without (—) and with (-----) gold activation.

Emulsion: Kodak "Electron Image Plates" - Source:  $^{14}\text{C}$

Developers: a) D19b b) Kodak HRP c) Amidol d) Ferro-oxalate -310 mV.

The slight increase in fog after activation may be (at least partly) due to the intensification of recorded radiation background.



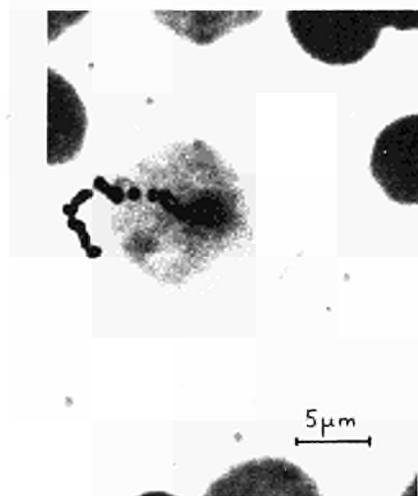


Figure 2. Activated track-autoradiogram of isolated rat brain nuclei. The tracer element is incorporated within the neuronal nucleoli at the site situated in the close vicinity of the origin of the  $\beta$  track.

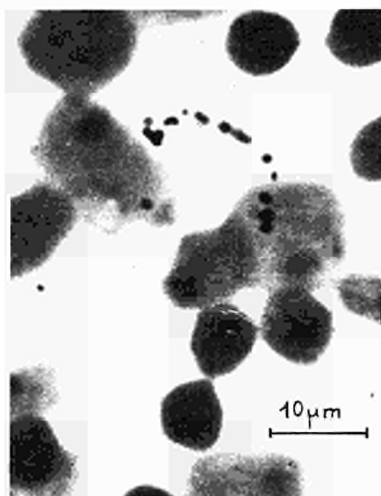


Figure 3. Autoradiogram of isolated rat brain nuclei.  $^{14}\text{C}$ -thymidine can be located within the chromosomal compartment of the neuronal nuclei.

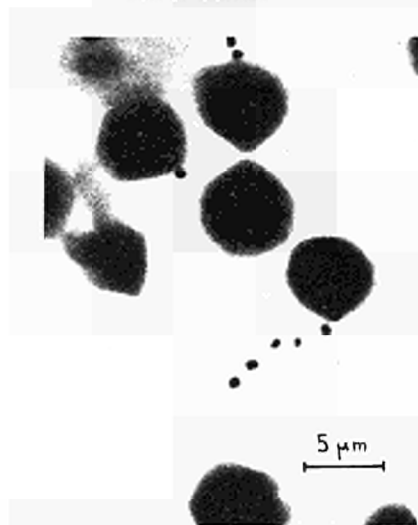


Figure 4. Electron tracks emitted by  $^{14}\text{C}$ -thymidine incorporated in isolated glial nuclei.

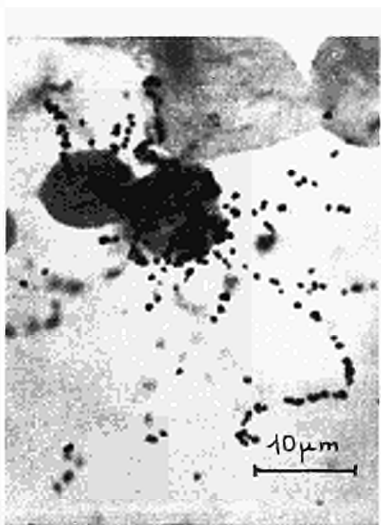


Figure 5. Strong  $^{14}\text{C}$ -thymidine incorporation in a glial nucleus.





- pollen tubes growing in the same style (Drs. L. Gilissen and student).
- VII. Activation of the ovary upon pollination (Prof.Dr. H.F. Linskens and Drs. J. Deurenberg).
- VIII. Influence of compatible and incompatible pollination on changes in enzyme activities in the ovary during progamic phase (Drs. J. Deurenberg).
- IX. Recognition of fast-moving substances in the style after pollination with radioactive labelled pollen (Drs. J. Deurenberg).
- X. Molecular biological aspects of the incompatibility reaction (Drs. J. van der Donk).
- XI. Electrophysiology of the style in relation to the incompatibility reaction (Prof.Dr. H.F. Linskens and Drs. A. Spanjers).
- XII. Relationship between pollination and flower wilting (Drs. L. Gilissen).
- XIII. Incompatibility in myxomycetes (Drs. J. Schrauwen).

Results of project no. S.C. 002 - 094 - 72 - 1 BIA N

Head of the team                      Prof.Dr. H.F. Linskens

Scientists                              Drs. J. Deurenberg  
    Drs. J. van der Donk  
    Drs. L. Gilissen  
    Drs. J. Schrauwen  
    Drs. A. Spanjers

- I. Influence of X-ray irradiation of pollen and styles on enzyme activity during progamic phase (Drs. L. Gilissen).

Changes in activity during progamic phase of the following enzymes have been tested by hand methods:

1. G.O.T. (L-aspartate 2-oxyglutarate amino-transferase) because of its intermedial function between carbohydrate and amino-acid metabolism. After pollination a decrease in specific enzyme

activity took place, which appeared to occur earlier in cross-pollinated, than in self-pollinated styles.

2. Pectinase, because of its possible role in pollen tube growth by solving the pectin in the mid-layer of the conducting tissue of the style. A hand method assay is in preparation.
3.  $\beta$ -Glucosidase, because of its function during wilting and the relation which exists between pollination and wilting, and radiation and wilting.
4. Acid phosphatase, because of the differences which could be expected on the basis of literature data in styles after cross- and self-pollination. However, no change in specific enzyme activity was observed after cross- and self-pollination.

G.O.T.- and G.P.T.-assay by auto-analysis failed in contrast to assays of paranitrophenyl-substrate splitting enzymes, like  $\alpha$ - and  $\beta$ -glucosidase,  $\beta$ -galactosidase and acid phosphatase. It appeared that a mistake was made in the hand-method assay of  $\beta$ -glucosidase. No activity was found with either the auto-analysator or the corrected hand method. Also no activity of  $\beta$ -galactosidase was obtained.

After pollination an increase and after high dose irradiation a decrease of  $\alpha$ -glucosidase activity in styles took place, while no change in acid phosphatase could be observed.

This type of experiments will be continued.

II. Influence of X-rays on the activity of glucan synthetase in Golgi-vesicles isolated from in vitro growing pollen tubes (Prof. Dr. H.F. Linskens, Drs. J. Helsper, Drs. L. Gilissen).

Isolated Golgi-vesicles were incubated with UDP-<sup>14</sup>C-glucose in a medium containing MgCl<sub>2</sub>, cellobiose and Tris (pH 8,0).

After 15' incubation at 25°C the reaction was stopped by heating at 90°C. The incubation suspension was added to hot water with cellulose powder. Insoluble material was collected by centrifugation.

The insoluble material was extracted three times with hot water, one time with chloroform/methanol (1:2), one time with methanol, two times with hot NaOH (1N) and rinsed finally with cold water. The radioactivity in the final, alkali-insoluble material is a measure for the  $\beta$ -glucan synthetase activity and exists of glucans with  $\beta$ -1,4-glucosidic and  $\beta$ -1,3-glucosidic linkages.

When a high dose of X-rays (100 kR) was applied to the Golgi-vesicles

during incubation the enzyme activity in the alkali-insoluble material was decreased with 20,0% in comparison with the non-irradiated control.

III. Effect of X-rays and different relative humidities on pollen germination including electron spin resonance (E.S.R.) measurements (Drs. L. Gilissen and student).

The hydration state of pollen grains plays a very important role in pollen germination. Different relative humidities were created in dessicators by means of saturated solutions of ammonium sulphate (80% RH) calcium nitrate (55% RH) and calcium chloride (35% RH). The dry state was created by phosphor-pentoxide and the wet state by tap water on the bottom of the dessicator.

When pollen samples were stored at room temperature at these varying relative humidities for at least one hour before germination, a linear relationship between germination capacity and the relative humidity was observed. Under dry conditions only 4% of the pollen germinated, whereas at 100% relative humidity 70% of the pollen germinated.

After irradiating pollen samples with 250 KR in air of different humidities, they were stored during one hour in a dessicator with 100% relative humidity to obtain maximum germination. It was found that germination was much more affected by irradiation under dry than under wet conditions. The germination percentages were 20 and 50 respectively.

Free radical concentrations in irradiated pollen samples have been measured with an electron spin resonance spectrometer (E.S.R.). It has been found that the biological damage must have been built up during radiation by action of the radicals formed. E.S.R. measurements on dry and wet irradiated pollen samples in relation to biological damage are in progress.

IV. Inherent biological influences on pollen tube growth in vivo (Drs. L. Gilissen).

1. Autostimulation

From experiments with fungal spores and pollen it is known that germination processes in vitro are influenced by the number of spores and pollen present in the germination medium. Fungal spores

show an auto-inhibition, which increases with increasing spore concentration. (Myrothecium verrucaria). On the other hand pollen show an autostimulation at increasing pollen concentrations.

It appeared that an autostimulatory effect also took place during pollen tube growth in the style.

It is concluded that the amount of pollen tubes growing near each other in the same part of the style is responsible for the occurrence of autostimulation.

### 2. Incompatibility phase I and phase II

In incompatible pollen tube growth two phases can clearly be distinguished: phase I in which no or little retardation (dependent on the clone used for experiments) of pollen tube growth takes place in comparison with the growth of compatible pollen tubes; phase II is recognizable by a strong retardation of pollen tube growth.

Growth rate in both phases is almost linear. The transition from phase I to phase II appears at the moment when the tips of the pollen tubes pass the style half-way down. Phase II is often partly or totally broken-down when the flowers are cut from the plants.

Autostimulation of pollen tube growth becomes more pregnant during phase II. It appeared that phase II is very sensitive for in- as well as external influences. This in contrast to phase I, which is even not to be influenced by irradiation of the style with 1000 KR (see chapter II).

In this experimental part of the research project a new topic on incompatibility is broached. Experiments to find out the cause of these two phases in pollen tube growth will therefore be extended and use of X-rays in this field of research is in progress.

### 3. Age of the flower

From literature data it is known that phase II in Nicotiana tabacum is dependent on the age of the flowers used for experiments. It appeared that the older the flowers the stronger and the sooner phase II occurred. From experiments with Petunia hybrida it became clear, that no changes in phase II took place due to changes in age of the flower. Pollination of styles in wilted flowers (five days after anthesis) caused no changes in growth rates of pollen tubes in comparison with flowers pollinated at anthesis.

- V. Effects of X-ray irradiation of pollen and styles on pollen tube growth, the incompatibility reaction, seed set and seed germination (Drs. L. Gilissen).

It appeared that seed set was strongly decreased after pollination with compatible pollen irradiated with a dose of 30 KR and higher. Seeds formed after pollination with pollen irradiated with 30 KR and more were not able to germinate. Compatible and incompatible pollen tube growth in vivo, however, was only inhibited after irradiation of pollen with 500 KR. A measurable decrease in pollen tube growth only took place after irradiation of the pollen with 200 KR or more. The incompatibility reaction (phase I) was not affected by pollen irradiation, nor by style irradiation in vitro. In case of the latter treatment (irradiation of styles) even a dose of 1000 KR given to isolated flowers caused no difference in growth rate of both compatible and incompatible pollen tubes.

Irradiation of styles 10 hours after pollination with 200 KR (compatible and incompatible pollen tubes had reached mean lengths of 6,1 and 4,3 mm respectively) had only a small effect on pollen tube growth, but no effect on the incompatibility reaction (phase I). Combining results of seed set and seed germination experiments with pollen tube growth experiments, it is concluded, that pollen tube growth and the incompatibility reaction during phase I in isolated flowers have an enzymatic base and are not governed by gene activities.

- VI. Influence of X-ray irradiated compatible pollen tubes on incompatible pollen tubes growing in the same style (Drs. L. Gilissen and student).

Styles were pollinated with a (1:1 ratio) mixture of irradiated compatible pollen and non-irradiated incompatible pollen. Irradiated (250 KR) compatible pollen germinated slowly and pollen tube growth was only retarded in the initial stage. In contrast incompatible pollen germinated fast, but pollen tube growth levels off when reaching the ovary (incompatibility phase II).

From some experiments it could be concluded that the incompatibility reaction during phase II (see chapter V-2) can be broken down by the influence of the irradiated compatible pollen tubes present in the same part of the style as the incompatible ones are.



VII. Activation of the ovary upon pollination (Prof. Dr. H.F. Linskens and Drs. J. Deurenberg).

Further studies on the protein metabolism of Petunia hybrida ovaries during the progamic phase were carried out. Differences with regard to the incorporation activity of  $^{14}\text{C}$ -leucine by isolated polysomes, which were found in clone W166K, were also found in polysomes isolated from ovaries of clone T2U after reciprocal crossings. The development of protein metabolism in ovaries of clone T2U is not exactly the same as in ovaries of clone W166K. However, in clone T2U differences in incorporation activity of  $^{14}\text{C}$ -leucine in proteins after cross- and self-pollinations were also observed. These results are in good agreement with those found in clone W166K.

In order to obtain information about the quality of the altered protein metabolism, polysomes from unpollinated, cross- and self-pollinated ovaries will be injected into egg cells of Xenopus laevis. After extraction of the synthesized plant proteins by these egg cells poly-acryl amid electrophoresis will be carried out to determine which proteins are synthesized in the ovaries at several times after pollination.

These experiments are in progress.

VIII. Influence of compatible and incompatible pollination on changes in enzyme activities in the ovary during progamic phase (Drs. J. Deurenberg).

Acid phosphatase and  $\beta$ -glucosidase activities were determined in ovaries of cross- and self-pollinated flowers at different times after pollination. Differences in enzyme activities between ovaries after cross- and after self-pollination were found between 2 and 6 hours after pollination. From these experiments conclusions can be drawn about the general metabolic activity of the ovaries, which are probably activated within a short time after pollination.

IX. Recognition of fast-moving substances in the style after pollination with radioactive labelled pollen (Drs. J. Deurenberg).

Very recently experiments were started with labelled pollen of Petunia hybrida clone W166K. Labelling of pollen was carried out as follows. Corolla and anthers of clone W166K flowers were incubated during 48 h.

in a solution of 15  $\mu\text{Ci}$   $^3\text{H}$  leucine in 5 ml  $\text{H}_2\text{O}$  at  $18^\circ\text{C}$ . After incubation pollen was sampled and stored in a freezer ( $-25^\circ\text{C}$ ) until use. Pollen grains were brought on the stigma of clone W166K styles + ovaries. The other flower parts were stripped. At several times after pollination the distribution of label over the style and ovary was determined. It appeared that within half an hour radioactive labelled compounds had reached the ovary. At successive examination times a front of labelled compounds was found to be moving downward in the style towards the ovary. Speculations can be made about the possible "signal"-function of these very fast moving compounds with respect to the observed changes in protein metabolism in the ovaries, long before pollen tubes have reached them (see chapter VII).

At present it is not clear whether these radioactive compounds are coming from either the inside of the pollen grain or from the pollen grain wall.

X. Molecular biological aspects of the incompatibility reaction (Drs. J. van der Donk).

At several stages following self- and cross-pollination the size of the pool of free nucleotides, RNA and protein synthesis were studied in styles of Petunia hybrida L. The results show:

1. the time between transcription and translation in the style is about 3 hours in average
2. recognition of the pollen being self or not-self takes place at a very early stage in the pollentube-style interaction
3. recognition probably does not occur throughout the whole progamic phase, but is restricted to 1 or maybe 3 stages

The influence of proteins and RNA from unpollinated, inter- and intra-specifically pollinated styles and from pollen on pollen tube growth in vivo was tested by their application to the stigma prior to pollination. No RNA sample nor proteins from unpollinated styles were effective, whereas pollen tubes were shortened if the S-genotypes of the pollen matched that of the style from which the proteins originated and lengthened if not so. Pollen exudate proteins seemed to have the reversed effect. Active proteins could also be obtained by injection of RNA from pollinated styles into egg cells of *Xenopus*. Fractionation by means of preparative electrophoresis and isoelectric focusing led to two groups of active polypeptides with molecular weights of about

11.000 and 6.000. Application of these polypeptides to the stigma changed the gene expression of the style. Based upon these data a model to explain the recognition part of the incompatibility reaction is proposed.

XI. Electrophysiology of the style in relation to the incompatibility reaction (Drs. A. Spanjers).

Already during the progamic phase protein synthesis in the ovary increases. This means that a signal must have been sent down from the pollentubes growing in the style towards the ovary to trigger this protein synthesis.

From experimental evidences an electric nature of this signal was supposed. With the aid of new equipment it is now possible to measure electrical phenomena from DC up to 5 kHz in styles of incompatible strains of Lilium longiflorum.

XII. Relationship between pollination and flower wilting (Drs. L. Gilissen).

Pollen tube growth in the style (Petunia ♀ x Nicotiana ♂) accelerated wilting. Pollination and germination on the stigmatic surface (Petunia ♀ x Atropa ♂) did not change the stage of flowering in comparison with unpollinated flowers. Pollination with irradiated (1.000 KR) dead pollen also causes no changes in comparison with the unpollinated situation. Pollination with incompatible pollen (Petunia W166H ♀ x W166H ♂) caused a retardation of flower wilting in comparison with flower wilting after a compatible pollination.

XIII. Incompatibility in myxomycetes (Drs. J. Schrauwen).

Preliminary experiments were carried out in order to use myxomycetes as a simple model organism for studying the incompatibility reaction.

OTHER ACTIVITIES

The research team collaborated with the working group of the Dutch foundation for biological research BION. A working meeting was held at Borger (The Netherlands) in october. All members of the research team participated the meeting of the Mutation Breeding Contact Group of EURATOM

in Cassacia (France) in september. Drs. Gilissen carried out experiments at ITAL Wageningen (The Netherlands) in december.

Drs. van der Donk gave an invited lecture in Hannover (W. Germany) in june about radiation effects on pollen.

Prof. Dr. H.F. Linskens was a member of the symposium on Gamete Composition in Plants and Animals, held at Lake Como (Italy) in august. He also participated the meeting of the Mediterranean Group of Applied Plant Physiologists at Izmir (Turkey) in november.

Prof. Dr. H.F. Linskens was elected chairman of the International Advising Committee of ITAL in place of the late Prof. Schruffelen; he was also elected in the "comité de gestion" of ITAL.

Publications in 1975

- Deurenberg, J.J.M., In vitro protein synthesis with polysomes from unpollinated, cross- and self-pollinated Petunia ovaries.  
Planta (in press)
- Donk, J.A.W.M. van der, Translation of plant messengers in egg cells of Xenopus laevis.  
Nature (London) 256, 674-675
- , Recognition and Gene Expression during the Incompatibility Reaction in Petunia hybrida L.  
Molec. gen. Genet. (in press)
- , Molecular biological aspects of the incompatibility reaction in Petunia.  
Thesis 1975
- Gilissen, L.J.W.J. and Linskens, H.F. Pollen tube growth in styles of self-incompatible Petunia pollinated with radiated pollen and with foreign pollen mixtures.  
In: Gamete competition in Plants and Animals, ed. D.L. Mulcahy, 201-205, North Holl. Publ. Comp., A'dam 1975
- Gilissen, L.J.W.J., The role of the style as a sense-organ in relation to wilting of the flower.  
Planta (in press)
- Linskens, H.F., Incompatibility in Petunia.  
Proc. Roy. Soc. (London) 188B, 299-311, 1975
- , The physiological basis of incompatibility in angiosperms.  
Biol. J. Linnean Soc. (London) 7, suppl. 1, 143-152, 1975



Contracting Research Institute : Department of Plant Breeding  
Agricultural University,  
Wageningen, The Netherlands.

Number of contract : 094-72-1 BIAN s/c 003

Head of the research team : Prof.Dr.Ir. J. Sneep

General subject of the contract: The use of mutations and mutation techniques  
in plant breeding research.

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General description of the project:

1. The population genetics of the S-alleles of a sporophytic incompatibility system (Bos, Heemstra).
2. Mutation research in potato and pelargonium (van Harten, Bouter).
3. Investigations on incompatibility in tuber-bearing Solanum-species (Hermsen).
4. Incongruity and sterility in interspecific Solanum crosses (Hermsen, Ramanna, Verdenius).
5. Mutation breeding for disease resistance. Dwarfrust in barley (Parlevliet, van Ommeren, Kuiper).

Results of the project : *Lobularia maritima* (L.) Desv.

Leader and co-worker : I. Bos, G. Heemstra.

Title of project : The population genetics of the S-alleles of a sporophytic incompatibility system.

As mentioned in the previous report it was impossible to draw clearcut conclusions about the interaction types for stigma- and pollenrelationship of the S-alleles. It was presumed that imperfect isolation of the plants from insects had caused uncontrolled pollinations. Therefore a new procedure was followed in 1975. In each of a series of about 30 small insectproof glasshouse compartments a single plant was grown. For several weeks all newly opened flowers were selfpollinated. In this way one can be sure that all the harvested seeds (in general, only a small number because of a strong degree of self-incompatibility) result from selfpollination. These seeds will be used for the analysis of the relations of the S-alleles (Haruta's method).



Results of the project : Potato (*Solanum tuberosum* L.)

Leader of the team and co-worker : A.M. van Harten, H. Bouter.

Title of the project : Mutation research in potato and pelargonium.

1. The fundamental research on radiosensitivity, mutability and diplontic selection in relation to the behaviour of the different histogenic layers and their derivatives was continued. The development of a diploid testclone with 6 marker genes in heterozygous condition could still not be completed with success because of several practical difficulties. In a trial with both dry irradiation of axillary cuttings of a possible diploid testclone and irradiation of the objects when submerged, it was shown that the submerged objects had a lower radiosensitivity. Final data on the mutability of the marker genes are not yet available.
2. The work on in vivo induction of adventitious sprouts on leaf stalks and leaflets (epidermal tissue) was stopped for the time being. In vitro methods are expected to give better results and are tried out at the I.T.A.L. with explants of areal parts of our material. So far all sprouts of a monecto-chimeric clone (EMS2) have originated from  $L_3$  tissue, and not from  $L_1$  as we had hoped they would.
3. Some of the results of the comparative field tests with irradiated c.v. Bintje material for yield and uniform resistance were less reliable owing to adverse weather conditions. However, the irradiated and re-irradiated material of c.v. Bintje corroborated the increased results of uniform resistance to leafroll. The test for resistance to  $\gamma$ -virus gave again no interpretable data and therefore this part of the project will be abandoned.
4. The possibilities of success in the project to improve the complete sterile, triploid, frostresistant cultivated South-American potato *Solanum juzepczukii* via induced mutation methods to reduce the high content of alkaloids (bitter taste) seem doubtful. A chromatographic determination of small samples of 29 clones showed hardly any glyco-alkaloids in the tuber tissue. No solanine, some solanidine and very little chaconine was found. Possibly other methods may enable us to determine other glyco-alkaloids as e.g. demicine and tomatine.

5. The second year of the Pelargonium zonale programme has still one of orientation. To explain the results of the primary trials of 1974 it proved to be necessary to examine the generative progenies for inheritance of several characters. Special attention was paid to the flowercolour using chromatography to analyze the inheritance of the anthocyanins. We started investigations of the meso-chimeric clones B 55, which shows leaf-variegation (green, white, green). The aim is to study the occurrence of the histogenic effects in the apices after X-ray treatment by morphological and microscopic observations.
6. Prospects for 1976.
- In the following years no fundamental changes from the present approach are expected.
- Moreover since april 1975 scientific guidance has been given to a large-scale practical experiment of a cooperative breeding company, which has been interested in the possibilities of creating new potato varieties via mutation breeding.

Publications:

- van Harten, A.M., 1975. Report Meeting Euratom Mutation Breeding Contact Group, Wageningen 1974 : 23-26.
- van Harten, A.M. and H. Bouter, 1975. Association Euratom-ITAL Annual Report 1974 : 27.28.
- van Harten, A.M. and H. Bouter, 1975. Annual Report Biologic Programme Euratom 1974 : 656-658.
- Broertjes, C. and A.M. van Harten (in preparation). Mutation breeding in vegetatively propagated plants. A handbook. To be published in 1976/1977.
- van Harten, A.M. (in preparation). Ph.D. thesis on mutation breeding in potato. To be published in 1976/1977.
- Bouter, H. (in preparation). Increased uniform resistance against potato leafroll (Solanum virus 14) by means of induced mutations.

Title of the project : Investigations on incompatibility in tuber-bearing  
Solanum-species.

Leader : Dr.Ir. J.G.Th. Hermesen with the cooperation of graduate  
students.

Self-compatibility in some dihaploids from S. tuberosum L. cv. Gineke could be ascribed to the occurrence of a duplication of the S-locus carrying  $S_1$  with a stylar part mutation. On the basis of segregation ratios  $sc : si$  (see report 1974) it was assumed that duplication homozygotes are lethal. A crucial experiment was carried out in 1975. Self-compatible  $F_1$ -plants from  $sc \times sc$  crosses should consist of 55% duplication-heterozygotes and 45% duplication-homozygotes, if the latter group is not lethal. Crossing the self-compatible  $F_1$ -plants with a self-incompatible clone (i.c. G 609 =  $S_1S_2$ ), the progeny will segregate 1 s.i. : 1 s.c. if duplication heterozygotes are involved, whereas only self-compatible progeny is expected in case of duplication homozygotes.

In a previous experiment only 1 : 1 ratios were found in such progenies, but in this case only 6 self-compatible  $F_1$ -plants were tested. In 1975, 28 self-compatible  $F_1$ -plants were tested for hetero- and homozygosity of the duplication. From the results it appeared that two out of these 28 plants (7%) were non-lethal duplication homozygotes. Therefore it is apparent, that homozygosity for the duplication mostly, but not always leads to lethality.

An incompatibility experiment involving a set of reciprocal  $F_1$ 's of S. bulbocastanum DUN., each  $F_1$  consisting of 16 plants, showed the occurrence of more than four incompatibility groups. Therefore more than one locus is involved in the gametophytic incompatibility system of S. bulbocastanum. A female incompatible group, which Pandey (1962) found in S. pinnatisectum DUN. did not show up in this experiment, although reciprocal differences were detected.

Title of the project : Incongruity and sterility in interspecific *Solanum* crosses

Leader : Dr.Ir. J.G.Th. Hermsen. Co-workers: Dr. M.S. Ramanna, Ing. J. Verdeniu

This project comprises fundamental research on interspecific barriers (incongruity sensu lato) in the genus Solanum and on ways to overcome these barriers. More specifically the research is concentrated on the Mexican wild species which are the best sources of resistance to Phytophthora infestans, the potato disease.

Incongruity sensu lato indicates all interspecific barriers, whereas incongruity sensu stricto points to those barriers which occur in the pistil between pollination and fertilization. Incongruity relations of five species were studied and are summarized in the following scheme.

♀ \ ♂	Solanum				
	<u>bulbocastanum</u>	<u>demissum</u>	<u>verrucosum</u>	<u>2x-tuberosum</u>	<u>pinnatisectum</u>
<u>S. bulbocastanum</u> DUN.	+	-	-	-	-
<u>S. demissum</u> LINDL.	-	+	-	+	
<u>S. verrucosum</u> SCHLECHTD	± s	± s	+	+s	± s
2x- <u>S. tuberosum</u> L.	-	+	+ A -	+	
<u>S. pinnatisectum</u> DUN.	+		-		+

+ successful crosses

- not-successful crosses

± difficult crosses

s = F<sub>1</sub> plants sterile

A = rare clones of S. tuberosum, accepting pollen of S. verrucosum.

A small scale experiment was carried out in 1975, aimed at studying the possibilities of improving interspecific crossability through chronic gamma-irradiation. From the beginning of anthesis ten seedlings of each of five introductions of both S. verrucosum and S. bulbocastanum and eleven selected dihaploid genotypes of S. tuberosum were treated 20-21 hrs per day from June 16 to August 6.

Gamma source:  $^{137}\text{Cs}$  (Cecile), daylength: 16 hrs, temperature:  $23^{\circ}\text{C}$  day,  $16^{\circ}\text{C}$  night. The plants of S. verrucosum and S. bulbocastanum were placed at ten distances from the source (daily dose from 12-933 rad), dihaploid S. tuberosum at two distances (daily dose 80 and 143 rad). The dose 933 rad/day suppressed flowering completely, while a negative effect on flowering was also observed at doses of 300 and 143 rad/day. The following crosses (on an average 214 flowers per cross) were made: S. verrucosum (irr. and non-irr.)  $\times$  S. bulbocastanum (irr. and non-irr.); S. tuberosum (irr. and non-irr.)  $\times$  S. bulbocastanum (irr. and non-irr.). Chronic irradiation did not improve crossability of the species in this experiment.

In 1976 the method of using "mentor" pollen will be attempted. This means pollen of the female parent is inactivated by irradiation and mixed with pollen of the male parent before pollination. Furthermore it will be tried to induce mutations of genes for non-crossability, which as a rule are dominant. In order to prevent cytoplasmic male sterility when using S. verrucosum as a female parent, the cytoplasm of this species will be substituted for S. tuberosum-cytoplasm by crossing an acceptor clone of S. tuberosum ♀ with S. verrucosum ♂ followed by a number of backcrosses with the latter species ♂. In 1976 the second backcross will be made.

#### Publication

Hermesen, J.G.Th. and M.S. Ramanna, 1976. Barriers to hybridization of Solanum bulbocastanum Dun and S. verrucosum Schlecht. and structural hybridity in their  $F_1$  plants. Euphytica 25. In press.

Title of the project : Mutation breeding for disease resistance. Dwarffruit in barley.

Leader of the team and co-workers: J.E. Parlevliet, A. van Ommeren and H.J. Kuiper.

The cultivar Minerva was used in our mutation experiment as it had already a fair level of partial resistance and is a modern, well adapted barley cultivar. In order to know something of the genetics of the partial resistance of this cultivar a number of crosses were made with three other cultivars, L92 and L94, both very susceptible and having a short latent period and with Vada, like Minerva being partially resistant and having a long latent period. From the  $F_1$ ,  $F_2$  and  $F_3$  studies it appeared that the long latent period of Minerva is determined by a recessive gene with a fairly large effect and some 4 to 5 minor genes with small, additive effects. All genes show cumulative effects without interactions.

Also a preliminary trial was done to investigate some other quantitative characteristics like stemlength, number of tillers, number of kernels/ear and 1000 kernelweight. From the  $F_2$  and the  $F_3$ 's of the controls and mutagenic treatments 140 normal looking seedlings were planted out in a greenhouse in 14 replicates. Per plot one row of 10 plants were planted at a row distance of 0.20 m and a distance within the row of 0.10 m.

The mean value of all traits decreased in  $M_2$  and  $M_3$  after mutagenic treatment. The decrease in population mean was often more pronounced in the  $M_3$  than in the  $M_2$ , which is contrary to many other observations. Also the coefficient of variation of the  $F_3$  was as large as or even larger than the one of the  $F_2$ . Among the mutagenically treated populations some to many male-sterile plants were found. The variation (cv) among the male sterile plants, measured on number of tillers and height of plants was far larger than those among the non-male-sterile plants. This might suggest clustering of mutations.

Table 1. Mean values and Coefficients of variation (CV) of 4 characteristics in  $M_2$  and  $M_3$  of some mutagenic treatments.

Treatment	Generation	mean				CV			
		Number of tillers	Plant length (cm)	Number of kernels per ear	1000 kernel weight	Number of tillers	Plant length	Number of kernels ear	1000 kernel weight
0 Kr	$M_2$	11.4	132.2	14.9	42.7	20.4	3.1	30.3	14.7
	$M_3$	10.4	131.5	15.8	43.0	21.7	3.2	24.2	14.3
15 Kr	$M_2$	10.0	128.4	14.0	42.0	24.9	6.6	28.8	15.3
	$M_3$	10.2	127.9	14.6	42.5	19.8	5.6	33.0	16.7
0 EMS	$M_2$	11.0	133.1	15.8	43.7	20.6	3.3	27.7	15.1
	$M_3$	10.0	130.8	15.8	42.7	18.0	3.3	25.7	16.1
25 EMS	$M_2$	10.1	127.1	13.0	42.8	32.3	8.0	37.2	17.8
	$M_3$	8.0	125.3	13.5	40.2	37.3	7.7	39.6	20.6
50 EMS	$M_2$	8.2	115.9	10.7	40.2	44.2	16.4	43.1	17.5
	$M_3$	6.7	108.2	9.4	39.5	50.4	15.2	49.1	16.6





Associato della Commissione: Comitato Nazionale  
per l'Energia Nucleare, Laboratorio per le  
Applicazioni in Agricoltura

N° del contratto: S/C 004-094-72-1 BIAN

Capo del gruppo di ricerca: Tommaso Cervigni

Tema generale del contratto: Application of mutagenesis for  
the improvement of some economically interesting  
characteristics in higher plants.

Fundamental and applied research with the gametophytic  
system of incompatibility in higher plants.

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The researches carried out in 1975 were principally oriented  
in three directions:

- Spontaneous and induced mutations at the S-locus:  
a comparative analysis on the nature and origin of  
constructive (generation of new alleles) and negative  
(genetic losses) mutations.
- Establishment of linkage relationships with the S-locus  
of self-incompatible plants and identification of the  
S- bearing chromosome.
- Selection and analysis of spontaneous and induced mutations  
for disease resistance in cultivated species.

Risultato del progetto n. 1: see report of the  
Association Euratom-Ital.

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Risultato del progetto n. 2

Capo del progetto e collaboratori scientifici:

M.Devreux, K.Sree Ramulu, U.Laneri, B.Donini (Casaccia),  
A.J.G.van Gastel, G.Bredemeyer (Wageningen).

Titolo del progetto: The spectrum of spontaneous and  
induced mutations at the S-locus: a comparative ana-  
lysis of the origin and nature of constructive (gene-  
ration of new alleles) and negative (genetic losses) mu-  
tations.

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2.1. Analysis of mutation spectra at the S-locus of  
N.Alata: see report of the Association Euratom-Ital.

2.2. The detection and origin of spontaneous mutations  
at the S-locus of inbred plants of *L.peruvianum*.

In continuation of the work completed last year (see  
annual report of 1974), researches were made to understand  
the mechanism by which new self-incompatibility alleles  
arise in the inbred plants of *Lycopersicum peruvianum*.  
The inbred progenies obtained from selfed  $S_1S_2$  stocks bred  
in different genetic backgrounds were crossed reciprocally  
with the original mother clone 006- $S_1S_2$  to find out whe-  
ther there are any new S-specificities are induced. The  
results showed that half of the population was S-homozy-  
gous and the remaining half was of  $S_1S_2$  constitution. To  
ascertain if any chimeric condition exists in the styles,  
since half of the population was cross-compatible as pis-  
tillate parents, crosses were made with various tester  
-stocks. The results showed that they are S-homozygous but  
not chimeric.

2.3. Studies on the genetic analysis of in vitro regenerated plants in different self-incompatible genotypes of *L. peruvianum*.

In  $S_{12}S_{13}$ , the self-pollination tests on 10 tetraploids, 3 diploids and 3 cytochimeras, and their reciprocal crosses with the mother clone, revealed the occurrence of 1 tetraploid which showed self- and cross-incompatibility and 1 self-incompatible diploid giving fruit and seed-set when crossed as staminate parent with the mother clone. When the progenies of the diploid were backcrossed there was fruit and seed-set indicating probable presence of some new S-allele.

In  $S_1S_3$ , self-pollination tests on 34 diploids and 3 cytochimeras, and their bilateral crosses with the original mother clone indicated the occurrence of some changes or other at the S-locus in at least 22 plants. Among them 16 were self-incompatible, but they gave fruit and seed-set when crossed as staminate parents with the mother clone. Four plants showed fruit and seed-set after self-pollination, but gave no fruit when crossed with the mother clone. The remaining two plants were self-compatible and also gave fruits and seeds after crossing as staminate parents with the mother clone. Progeny tests completed so far indicate probable presence of new S-specificity in these plants. The analysis of cytochimera with  $2n-4n-4n$  in  $L_1$ ,  $L_2$  and  $L_3$  gave results similar to that of an autotetraploid. Of the remaining two cytochimeras with  $4n-2n-2n$ , one was self-incompatible, but gave fruits and seeds after crossing in two ways with the mother clone, and the other showed fruit and seed-set upon self-pollination but no fruit when crossed bilaterally with the mother clone

2.4. The induction of self-compatibility in allogamous species.

Through radiation treatment, desirable morphological mutants such as spur types have been induced in cherries and olives, and attempts were made to isolate self-compatible types if any among them. Experiments were also aimed at inducing through ionizing radiations, self-compatible types. Studies were made to find out the exact system of self-incompatibility and also on the ultrastructural features of self-incompatibility.

### Risultati del progetto n. 3

Capo del progetto e collaboratori scientifici:

M.Devreux, K.Sree Ramulu, F.Carluccio (Casaccia),  
A.J.G. van Gastel (Wageningen).

Titolo del progetto: Establishment of linkage relationships with the S-locus of self-incompatible plants and identification of S-bearing chromosome.

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As in 1974, this problem has been tackled at the cytological and genetical levels in collaboration with the Wageningen laboratory of the Euratom-Ital Association.

The work carried out at the Casaccia can be subdivided into the two following parts:

3.1. Analysis of the mechanism leading to the formation of self-compatibility mutation in *Nicotiana glauca*.

Identification of S-bearing chromosome was concluded after a series of karyotype analyses in the aneuploids of *N.glauca*. It was found that the S-bearing chromosome was the sub-terminal chromosome 1 (ST.1). These researches were carried out in collaboration with Euratom-Ital Association from where the fixed root meristems of aneuploids were obtained. (see also the report of the Euratom-Ital Association).

3.2. Identification of the S-bearing chromosome by means of trisomic method in *Lycopersicon peruvianum*.

Out of 22 trisomics ( $2n=25$ ) analysed, only six gave seed-set. In 3 trisomics, the average number of seeds per selfed flower ranged from 3 to 20, whereas it was below 1 in the remaining trisomics. Among 71 diploids, 4 showed after selfing some fruits and seeds; 1 gave 3 seeds per flower and the other 3 had an average of 1 or below 1 seed per flower. The plants with 26 or 27

or 28 chromosomes showed no fruit development after self pollination. The plant phenotype, leaf, flower, fruit and seed characters and percentage of pollen fertility varied to a great extent among the aneuploids and the diploid plants. Karyotype analysis in root meristems of one trisomic plant revealed that the extra chromosome is the chromosome number 1, the longest of the complement. In the other trisomics, it was found that the extra chromosome is the number 3. This was also ascertained with pachytene analysis. The meiotic studies have revealed that these three trisomics were of primary type. There appeared some differences in their meiotic behaviour. Genetic analyses were carried out by crossing the self-compatible plants as pistillate parents with various tester-stocks to ascertain the presence of three S-specificities in the style. It was, however found that the self-compatible plants gave complete fruit and seed-set with all the tester-stocks revealing that there are no three specificities in the style, but probable presence of some new S-allele; progeny tests are being carried out to ascertain the situation.

Self-compatible disomic and trisomic plants of L.peruvianum were utilized in crosses with the cultivated species of tomato (L.esculentum) and with the hybrid between L.esculentum and L.peruvianum in an effort to obtain fruit and seed-set. When L.esculentum and the hybrid were used as pistillate parents, there was complete fruit-set but no seed development. The crosses using self-compatible plants of L.peruvianum as pistillate parents gave no fruit development. Nevertheless, very few viable seeds were obtained when the hybrid was used as pistillate parent.

Complement to projects n. 2 and n. 3 :

Studies on the ultrastructural features of pollen tubes blocked in the cross-pollinated styles following gamma irradiation in *L.peruvianum*.

In continuation of the work completed last year (see annual report of 1974), attempts were made to determine the particular dose of gamma rays which can stop the pollen tube growth at one third or half length of the style after cross-compatible pollination in *L.peruvianum* so as to make a comparative study on the ultrastructural features of this with that observed after self-pollination. After a series of experiments, finally 200 kR gamma rays delivered at 60 kR per hour was found to block the pollen tube growth at one third length of the style as observed with fluorescent microscopy. Analyses with electron microscopy are being carried out.

### Publications

- M.Devreux, U.Laneri, E.Magnien and M.R.Celestre - Biological screening method for mutated pollen at the S-locus by in vitro culture of pollinated pistil. Incompatibility Newsletter, 5: 17-13, 1975.
- K.Sree Ramulu, F.Carluccio and D. de Nettancourt - Identification of the S-bearing chromosome by means of trisomic method in wild tomato species, Lycopersicum peruvianum Mill. Incompatibility Newsletter, 5: 22-23, 1975.
- K.Sree Ramulu, M.Devreux and U.Laneri - Detection and analysis of cytochimerism in Lycopersicum peruvianum plants regenerated from in vitro culture of anthers and stem internodes. Incompatibility Newsletter, 5: 19-21, 1975.
- K.Sree Ramulu, M.Devreux, G.Ancora and U.Laneri - In vitro culture conditions, regeneration of plants with different ploidies and chimerism, and cytophotometric analyses in Lycopersicum peruvianum. Incompatibility Newsletter, (in press).
- K.Sree Ramulu, M.Devreux and P.de Martinis - Ontogenetic origin of in vitro regenerated plants in Lycopersicum peruvianum. Incompatibility Newsletter, (in press).
- K.Sree Ramulu, D. de Nettancourt and M.Devreux - Genetic analyses of plants regenerated from in vitro culture of anthers and stem internodes in Lycopersicum peruvianum. Incompatibility Newsletter, (in press).
- K.Sree Ramulu, F.Carluccio, D.de Nettancourt and M.Devreux - Cytological and genetic analysis of self-compatible plants obtained from crosses between triploid and diploid S-genotypes in Lycopersicum peruvianum. Incompatibility Newsletter, (in press).
- K.Sree Ramulu, M.Devreux, G.Ancora and U.Laneri - Chimerism in Lycopersicum peruvianum plants regenerated from in vitro culture of anthers and stem internodes. Z. Pflanzenzüchtung, (in press).
- D. de Nettancourt and M.Devreux - Incompatibility and in vitro culture. In: J.Reinert and Y.P.S.Bajaj (éd.) Basic and Applied Research in Plant Tissue Culture, (in press).
- D. de Nettancourt, M.Devreux, F.Carluccio, U.Laneri, M.Cresti, E.Pacini, G.Sarfatti and A.J.G. van Gastel - Facts and hypotheses on the origin of S mutations and on the function of the S gene in Nicotiana alata and Lycopersicum peruvianum. Proc.R.Soc.Lond., B, 188: 345-360, 1975.



Risultati del progetto n. 4

Capo del progetto e collaboratori scientifici:

L.M. Monti, F. Saccardo, A. Bozzini, L. Tomarchio

Titolo del progetto: Selection and analysis of spontaneous and induced mutations for disease resistance in cultivated species .

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#### 4.1. Tomato

A programme for the selection and analysis of spontaneous and induced mutations for resistance to the most important diseases is going on in tomato.

As no resistance is so far available against Verticillium dahliae (race 2) and Phytophthora infestans (race T<sub>1</sub>), a mutagenic programme is in course to induce ad isolate resistant plants.

About 30.000 M<sub>2</sub> seedlings coming from several mutagenic treatments on gametes and on seeds of different tomato varieties were scored against V.dahliae (race 2) by the dip root technique; about 16.000 M<sub>2</sub> plants were analyzed for resistance to P.infestans (race T<sub>1</sub>), by spraiyng the sporangia on the seedlings.

All the M<sub>2</sub> escaperswere again infected. Some M<sub>2</sub> plants escaped also to the 2nd inoculation; the progenies of such plants will be analyzed next year.

The partial resistance we found last year in L.hirsutum against P.infestans (race T<sub>1</sub>) will be evaluated in the F<sub>2</sub>

coming from an interspecific cross with cv. S. Marzano.

A back cross programme to transfer spontaneous mutations already present in the germoplasm of this species in Italian tomato varieties is in course; material in advanced generations has been checked against Fusarium oxysporum f.sp. lycopersici (race 1 and race 2), V. dahliae (race 1), Corynebacterium michiganense, Tobacco Mosaic Virus (T.M.V.) and Meloidogyne incognita.

#### 4.2. Pepper

A program started in order to induce in pepper mutations for resistance to Cucumber Mosaic Virus (C.M.V.) and to V. dahliae, against which no resistance is available in the germoplasm of such species.

A technique for a mass screening of pepper seedlings in greenhouse against C.M.V. has been set up by a pressure spraying method, in collaboration with the Laboratorio di Fitovirologia Applicata of C.N.R. in Torino (Italy).

M<sub>1</sub> plant coming from gamete and seed treatments were grown and their M<sub>2</sub> seedlings will be analyzed next year for resistance to C.M.V. and to V. dahliae.

#### 4.3. Wheat

Analysis has been continued of lines and varieties of durum wheat subjected to bunt (Tilletia triticoides) infection. It is confirming the existence, among mutant lines, of a wider range of variability than mother lines, as far as the reaction to the fungus is considered, independent from any given morphological mutated character. The more resistant lines have been further submitted to the fungus attack in the attempt of evaluating more closely the actual degree of tolerance. This further evaluation is still underway.

Publications

F. Saccardo, L. Tomarchio e L.M. Monti - Lycopersicum hirsutum come fonte di resistenza alla Phytophthora infestans in pomodoro. *Genetica Agraria*, XXIX, 215-222 (1975).

F. Saccardo - Fonti di resistenza al C.M.V. (Cucumber Mosaic Virus) nel genere Capsicum . *Genetica Agraria* 1, 97-104 (1974).

M. Conti and F. Saccardo - Testing some american lines of pepper for resistance to Cucumber Mosaic Virus italian isolates. IIInd Int. Conf. Progress and Problems in Vegetable Virus Research. ISHS Working Group on Vegetable Viruses and EUCARPIA Vegetable Section: Montfavet-Avignon (France) (1975).

L.M. Monti e F. Saccardo - Miglioramento genetico per resistenza a malattie in pomodoro. Congresso della Società Italiana Genetica Agraria, Milano, Ottobre 1975.

L.M. Monti - Miglioramento genetico per resistenza a malattie in pisello. Congresso della Società Italiana Genetica Agraria, Milano, Ottobre 1975.

F. Saccardo e M. Conti - Miglioramento genetico per resistenza a malattie in peperone. Congresso della Società Italiana Genetica Agraria, Milano, Ottobre 1975.



Associato della Commissione: Istituto Sperimentale per la  
Cerealicoltura,  
Via Cassia 176 - Roma

No. del contratto: sc 005/094-72-1 BIAN

Capo del gruppo di ricerca: Prof. Angelo Bianchi

Tema generale del contratto: Induction of variability for protein  
quantity and quality in opaque-2  
maize (Zea Mays L.)

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The experiment, as previously reported, started in 1973 with X-ray treatment of the inbred line B37 (e2 and normal). In 1974 the treated and untreated progenies were screened for the presence of macromutations (defective endosperm and seedling mutations) and micromutations affecting quantity or quality of the endosperm proteins. The 1974 results demonstrated that the X-rays were quite effective in inducing macromutations (the 8000r mutation frequency was the highest; however the 4000r dose is to be preferred in radiogenetical experiments with maize because of less drastic effect on the seed germination and plant fertility); moreover, when compared to the control the 4000r and 8000r derived X2 progenies showed a greater coefficient of variation for total protein, dye binding capacity (DBC) and quality index (QI). The last index was calculated as the percent of the observed DBC value on the one expected on the basis of the protein content.

In 1975 we have: (A) studied the protein quality of the endosperm macromutations; and (B) evaluated in the e2 progenies derived from the 4000r dose the effect of selection in favor and against total protein, DBC, QI.

Descrizione dei risultati

INDUCTION OF VARIABILITY FOR PROTEIN QUANTITY  
AND QUALITY IN OPAQUE-2 MAIZE (Zea Mays L.)

A) Protein studies on the induced endospermic macromutations

Two classes of endospermic macromutations segregating with Mendelian ratios were followed:

Class 1) - Vital mutations effecting the endosperm texture

Class 2) - Vital or lethal mutations effecting the endosperm size (defective endosperm mutations).

To the class 1 mutations belongs the mutant de-4Kr 189, isolated in the e2 version of the inbred under study, and whose phenetic effect is an increase of the specific weight of the e2 kernel (tab.1). The protein fractionation, based on solubility, showed that the increased kernel density is not matched by a decreased quality of the protein. On the contrary the variation of the various protein fractions is in agreement with an increase of the protein quality; this is confirmed by the tryptophan content that is higher in the double mutant kernels than in opaque-2 ones. The mutant de-4Kr 189 may be a solution for practical problems associated with the low density of the opaque-2 kernels.

Eighteen defective endosperm mutants, isolated in the e2 version of the inbred B37 belonging to the class 2, were chemically studied. A preliminary analysis revealed that the tryptophan contents of 14 of them were similar to the content of the inbred B37 e2 from which they originated. These 14 mutants have been disregarded. The protein fractionation results for the remaining four mutants (de-4Kr 176, de-4Kr 211, de-4Kr 217, de-4Kr 220) are shown in table 2. The

TABLE 1 Specific weight, total proteins, tryptophan and protein fraction percentages in the endosperm of normal, o2o2 and o2o2 de-4Kr189 de-4Kr189 versions of the inbred line B37

Version	Specific weight	Tryptophan content (%)	Total protein (%)	Percent of total proteins			
				Albumins and globulins	Zein 1 (1)	Zein 2 (2)	Glutelins
normal	1.312 A	0.57 C	9.35 A	4.63 B	44.40 A	11.11 a	39.83 B
<u>opaque-2</u>	1.137 C	0.84 B	6.72 B	15.55 A	27.68 B	9.82 b	46.91 Ab
<u>opaque-2 de-4Kr189</u>	1.265 B	1.04 A	6.11 B	14.14 A	25.11 B	7.67 C	53.05 Aa

(1) Soluble in 70% EtOH

(2) Soluble in 70% EtOH plus 2-mercaptoethanol

Within a column the values followed by different letters are significantly different

(F test; capital letters, P=0.01; small letters, P=0.05)

TABLE 2 Total proteins, tryptophan and protein fraction percentages in the endosperm of B 37 o2 and of four high albumin and globulin mutants

Mutant	Tryptophan content (%)	Protein content (%)	Percent of total proteins			
			Albumins and globulins	Z1 (1)	Z2 (2)	Glutelins
B37 <u>opaque-2</u>	0.80 B	7.02 b	12.95 Cd	24.74 A	10.67 b	50.45 A
<u>o2, de-4Kr176</u>	0.91 A	6.76 b	57.32 A	8.52 D	7.92 c	27.23 C
<u>o2, de-4Kr211</u>	0.89 A	8.15 a	16.04 Cc	20.47 B	11.04 b	52.44 A
<u>o2, de-4Kr217</u>	0.81 B	6.78 b	39.76 B	12.50 C	13.16 a	34.58 B
<u>o2, de-4Kr220</u>	0.94 A	6.25 b	32.70 B	7.35 D	7.63 c	52.27 A

(1) Soluble in 70% EtOH

(2) Soluble in 70% EtOH plus 2-mercaptoethanol

Within a column the values followed by different letters are significantly different

(Duncan test; capital letters, P=0.01; small letters, P=0.05)

endosperm proteins of the four mutants appear particularly endowed of the saline soluble fraction. On the contrary the synthesis of Z1 is strongly reduced; Z2 seems to be less affected. A reduction in the glutelin fraction is also evident in the mutants de-4Kr 217 and de-4Kr 176. The mechanism of action of these four mutations may be simply related with the shut-down of the development of the endosperm at a time when the zein synthesis is not predominant over the other classes of storage proteins. This type of mutants may be of particular utility in studying the regulation of the protein synthesis during development of the endosperm.

B) Effect of 1 cycle of selection in favor and against total proteins, DBC and QI

The effect of selection for total proteins, DBC and QI has been studied in the B37 a2 progenies derived from the 4000r dose. For each criterion of selection the best and the worst 16 X2 progenies were chosen. The X3 generation obtained from the selected X2 progenies was grown in 1975 in a randomized block with three replications. In table 3 are reported the total protein, DBC and QI means for the six groups of progenies. The selection resulted effective for each character considered, as revealed by the significant differences between the groups selected in favor and against (table 3).

The three protein characters were to some extent correlated one another and with seed weight. As a result, the selection applied for a character generates correlated responses in the others traits. The data of table 4 underline this mutual interdependence of the three protein characteristics. Moreover a positive response to the selection for protein, DBC and QI is always associated with a reduction in seed weight.

The experiment will be continued by a recurrent treatment of the selected progenies with Xray, followed by selection in X2. For this purpose the 16 progenies belonging to each of the six groups have been intercrossed in 1975 as to prepare the seeds to be irradiated in 1976.



TABLE 3 Effect of selection on the 4000r X2 progenies of B37 o2

Character selected	X2 progenies		Type of selection	Mean of the selected X2 progenies (1)	X3 progenies	
	number	mean			mean	mean square (FAVOR vs AGAINST)
Percent of proteins	655	9.15	FAVOR	10.97	10.22	
			AGAINST	7.63	9.60	8.34 **
D B C	655	53.02	FAVOR	70.5	77.52	
			AGAINST	38.9	69.32	10,297.04 **
Q I	655	100	FAVOR	124.10	101.10	
			AGAINST	83.5	96.10	416.78 **

(1) 16 progenies were selected each character

\*\* Significant at the 1% level

TABLE 4 Correlated responses to the selection for the traits under study

Character selected	Type of selection	Percent of proteins		D B C		Q I		Seed weight	
		mean	mean square (FAVOR vs AGAINST)	mean	mean square (FAVOR vs AGAINST)	mean	mean square (FAVOR vs AGAINST)	mean	mean square (FAVOR vs AGAINST)
Percent of proteins	FAVOR			71.29		98.05		4.86	
	AGAINST	-		68.54	165.16 **	95.60	124.85 **	5.08	0.95 **
D B C	FAVOR	9.53				108.83		4.73	
	AGAINST	9.35	0.88 **	-		97.48	2958.01 **	5.23	5.59 **
	FAVOR	10.09		73.26				4.90	
	AGAINST	9.40	10.39 **	68.52	459.80 **	-		5.20	1.91 **

\*\* Significant at the 1% level



Associato della Commissione: Università degli studi di Bari

N° del contratto: 006 - I/094 - 72 - I BIAN

Capo del gruppo di ricerca: Prof. G.T. Scarascia Mugnozza

Tema generale del contratto: Induction of mutations for disease resistance  
in Pisum sativum L.

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In the third year of the project, analysis of the progenies of the "Freezer-69" and "Sprinter" pea cultivars derived from the treated seeds have been carried out both in the experimental fields and in greenhouse.

In the experimental fields 2,360  $M_2$  plant-progenies, coming from  $M_2$  mutant plants (or suspected to be mutants) have been sown, and a total of about 35,000  $M_3$  plants have been analyzed in different growth stages. 345 of the examined  $M_2$ -plant-progenies have been ascertained to carry on a mutation. The morphological and physiological mutations so isolated are now under study and classification. All these progenies have been submitted to heavy infection of powdery mildew, but not a single case of tolerance or resistance, neither of vertical nor of the horizontal type, has been found.

Equally, none of the 70,000  $M_3$  plants of the bulk formed from seeds of 2 pods per each normal-looking plant (regardless of its degree of fertility) from each of the  $M_2$  progenies, showed any degree of resistance or tolerance to powdery mildew.

Greenhouse tests have been carried in order to accomplish the screening for mildew resistance of the  $M_1$ -plant-progenies. A total number of 2,520 progenies including 41,876  $M_2$  plants taken <sup>from</sup> seeds of the main stem and the lateral branches B, C and D were screened for resistance. None of them showed any vertical resistance to powdery mildew.

Special attention has been given to the offspring of the  $M_2$  plant SC2-468 B-7, isolated in the  $M_1$  progeny 468 B coming from a plant of the variety Sprinter treated with DES 1,5%, which - grown in open field - showed a certain degree of resistance to Erysiphe polygoni. Inoculation tests on  $M_4$  plants, grown both in greenhouse and in the open field, are now in progress. Moreover, screening for mildew resistance has been carried out on the offspring of the  $M_2$  sister plants which belong to the same progeny (468 B) of the suspected resistant plant. No case of resistance or tolerance was observed.

At the beginning of 1976 the program is going to a) ascertain, through greenhouse and field tests, the induction of a factor for mildew-resistance in the progeny of the SC2-468 B-7 plant; b) study from a genetical and radio-genetical point of view the induced and isolated mutations; c) evaluate from the agrobiological viewpoint the potential value and usefulness of the induced mutations.

Risultati del progetto n. 006-I/094-72-1 BIAN

Capo del progetto e collaboratori scientifici:

G.T. Scarascia-Mugnozza and A. Ciccarone, A. Graniti, M. Cirulli, G. Pacucci, V. Dellacecca, C. De Pace, A. Blanco, A. Filippetti, A. Montemurro.

Titolo del progetto:

Induction of mutations for resistance to Erysiphe polygoni in commercial varieties of Pisum sativum L.

In the third year of the project, analyses of the progenies of the "Freezer-69" and "Sprinter" pea cultivars derived from the treated seeds have been carried out both in the experimental fields and in greenhouse.

In the experimental fields 2,360  $M_2$  plant-progenies, coming from  $M_2$  mutant plants (or suspected to be mutants) have been sown, and a total of about 35,000  $M_3$  plants have been analyzed in different growth stages (Tab. I.). 345 of the examined  $M_2$ -plant progenies have been ascertained to carry on a mutation. At harvest, seeds of each plant of each mutated progeny have been collected separately. The experimental material thus accumulated is shown in Tab. II.

The morphological and physiological mutations so isolated are now under study and classification. At the present stage of study, they can be tentatively classified as: chlorophyll-mutations (xantha, chlorina, chlorotica, variegata); leaf-mutations (shape and size); pod-mutations; seed-mutations (colour and size); mutations affecting the plant habit (height, waxiness, fasciation, length of internodes, absence of stipules, etc.); mutations affecting the life cycle (lateness, earliness).

Obviously, all the above mentioned progenies have been submitted to heavy infection of powdery mildew, but not a single case of tolerance or resistance, neither of vertical nor of the horizontal type, has been found. Equally, none of the 70,000  $M_3$  plants of the bulk formed from seeds of 2 pods per each normal-looking plant (regardless of its degree of fertility) from each of the  $M_2$  progenies, showed any degree of resistance or tolerance to powdery mildew.

Greenhouse tests have been carried in order to accomplish the screening for mildew resistance of the  $M_1$ -plant-progenies.

Using the large-scale screening method devised in the 1973,  $M_2$  progenies of the "Freezer-69" and "Sprinter" pea cultivars were tested in greenhouse for resistance to the local populations of Erysiphe polygoni. Seedlings at the 6th leaf stage were inoculated with freshly harvested conidia of the fungus and the final disease ratings were taken 6 to 12 days after inoculation.

A total number of 2,520 progenies including 41,876  $M_2$  plants taken from seeds of the main stem and the lateral branches B, C and D were

Table I.  
Number of  $M_2$ -plant-progenies and  $M_3$  plants  
grown in open fields in 1974-75

Treatment	No. of $M_2$ plant-progenies	No. of $M_3$ plants
FC <sub>1</sub>	301	4,414
FC <sub>2</sub>	260	3,795
FR <sub>1</sub>	224	3,027
FR <sub>2</sub>	372	4,673
SC <sub>1</sub>	266	3,272
SC <sub>2</sub>	555	7,902
SR <sub>1</sub>	241	3,169
SR <sub>2</sub>	141	1,707
S <sub>test</sub>	-	1,200
F <sub>test</sub>	-	1,200
TOTAL	2,360	34,359

S = Sprinter; F = Freezer 69; R = X-ray; C = DES

Table II.  
Number of mutated progenies of  $M_3$  plants  
harvested and of  $M_4$  seeds collected in 1975

Treatment	No. of $M_2$ progenies	No. of $M_3$ plants	No. of $M_4$ seeds
FC <sub>1</sub>	79	220	15,049
FC <sub>2</sub>	43	143	8,823
FR <sub>1</sub>	18	30	2,480
FR <sub>2</sub>	23	73	6,011
SC <sub>1</sub>	62	135	9,442
SC <sub>2</sub>	89	235	12,008
SR <sub>1</sub>	8	33	1,925
SR <sub>2</sub>	23	32	2,877
TOTAL	345	901	58,615

Table III.

Number of  $M_3$  progenies and  $M_4$  seeds sown in  
november 1975.

Treatment	No. of M3 progenies	No. of M4 seeds
FC <sub>1</sub>	220	6,042
FC <sub>2</sub>	143	4,002
FR <sub>1</sub>	30	825
FR <sub>2</sub>	73	2,200
SC <sub>1</sub>	135	3,631
SC <sub>2</sub>	235	5,908
SR <sub>1</sub>	33	860
SR <sub>2</sub>	32	950
F test	-	1,000
S test	-	1,000
TOTAL	901	26,418

screened for resistance. None of them showed any vertical resistance to powdery mildew.

Observations on the offspring of the suspected "resistant" M<sub>2</sub>-plant. Special attention has been given to the offspring of the M<sub>2</sub> plant SC2-468 B-7, isolated in the M<sub>1</sub> progeny 468 B coming from a plant of the variety Sprinter treated with DES 1,5%, which - grown in open field - showed a certain degree of resistance to Erysiphe polygoni.

In the progeny of this plant segregation occurred for normal looking plants (42.4%), dwarf and semi-sterile plants (7.6%), semi-sterile and chlorophyll-mutants (50%, belonging to the viridis and variegata types). It might be assumed that a structural aberration was also induced in the chromosome set of this plant.

Since fertile surviving plants were also used as male parents in crosses with the mother variety, a total of 480 M<sub>4</sub> seeds have been harvested, 364 of them after selfing.

Inoculation tests on M<sub>4</sub> plants, grown both in greenhouse and in the open field, are now in progress. Moreover, screening for mildew resistance has been carried out on the offsprings of the M<sub>2</sub>-sister plants which belong to the same progeny (468 B) of the suspected resistant plant. No case of resistance or tolerance was observed.

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At the beginning of 1976 the program is going to a) ascertain, through greenhouse and field tests, the induction of a factor for mildew resistance in the progeny of the SC2-468 B-7 plant, b) study from a genetical and radiogenetical point of view the induced and isolated mutations, and c) evaluate from the agrobiological viewpoint the potential value and usefulness of the induced mutations. Table III shows the number of M<sub>3</sub> progenies and M<sub>4</sub> seeds sown in experimental plots during November 1975.





Contract partner  
of the Commission: Prof. Dr. Werner Gottschalk  
No. of the contract: SC O7/94-72-BIAN  
Head of the research group: Prof. Dr. Werner Gottschalk

General theme of the contract:

"Gene physiological and biochemical investigations on radiation induced mutants of Pisum sativum"

General report

During 1975, problems of basic research in mutation breeding, of gene ecology and of the genetics of seed protein production and composition on our pea mutants and recombinants were carried out. Moreover, a voluminous cytogenetic programme was finished. The studies on the reaction of some useful genotypes of our collection to different climatic conditions were intensified by establishing a close co-operation with colleagues of four Indian research institutes.

Results of project no. 1

Head of the project and scientific co-workers:

- Prof. Dr. Werner Gottschalk
- Prof. Dr. Hermann Müller

Title of the project:

Basic research in mutation breeding in the field of protein genetics

In the field of mutation breeding, we have concentrated our work in producing a great number of recombination lines homozygous for several mutant genes controlling characters of economic interest. At present, more than 60 different recombinants are available in our institute. Most of these strains are used for studying the co-operation of the respective genes and for discovering positive or negative interactions between them.

The promising recombinants are included in our protein programme together with their parental mutants. Some fasciated recombinants are available which surpass the fasciated parental mutants with regard to

- earliness,
- dwarfness,
- seed production
- seed size,
- protein production.

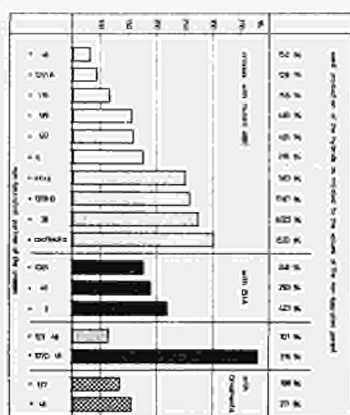
Moreover, some neutron-induced mutants have been selected showing very good yielding properties. Unfortunately, they are tall and late, but their seed production is 20 - 65% higher than that of the initial line considering five generations. The protein production per plant is about 30% higher than that of the initial line. Two new fasciated mutants were

found to show excellent yielding properties in combination with other favourable traits such as relative earliness and big seeds.

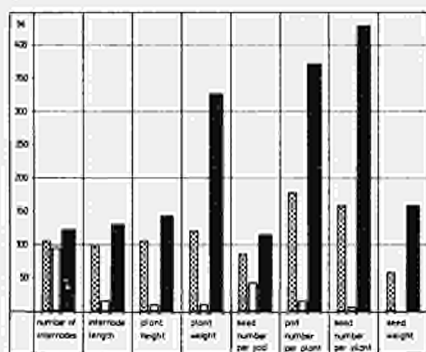
Nearly all our fasciated genotypes show a pronounced heterosis effect when crossed with non-fasciated mutants or with the initial line. In the  $F_1$ -hybrids, there is a considerable increase in

- plant height and weight,
- number of internodes,
- number of seeds per plant,
- seed size.

Details are given in figures 1 and 2. It is not yet clear, whether this phenomenon is a monohybrid heterosis due to the different fasciata genes or alleles of our collection, or whether it is due to heterozygosity of a small group of mutant genes.



**Figure 1:** Heterosis in F<sub>1</sub>-plants of crosses, in which fasciated pea mutants are used as one of the parents. The mean values for the character "number of seeds per plant" (columns) are related to the corresponding means of the fasciated parent. At the top of the figure, the same values are related to the non-fasciated parent.



**Figure 2:** Heterosis in F<sub>1</sub>-plants from the cross of mutants 489C (stem fasciation) x 26 (dwarfness) with regard to different criteria. All the mean values are related to the corresponding means of the initial line = 100%.

Left column: mutant 489C  
 middle column: mutant 26  
 right column: F<sub>1</sub> 489C x 26

Results of project no. 2

Head of the project and scientific co-workers:

- Prof. Dr. Werner Gottschalk

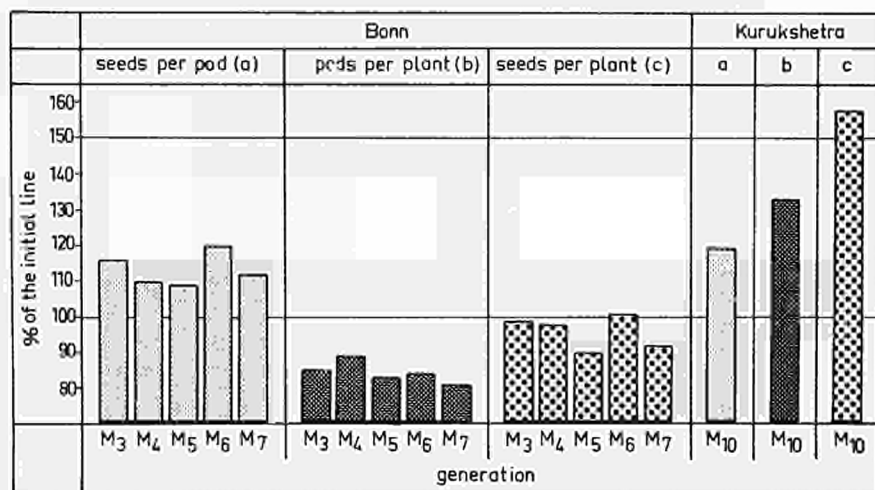
Title of the project:

Gene ecological investigations

Our co-operation with Indian universities has been intensified. Groups of our mutants are grown now at four universities of the country. We are still in a very early stage of studying gene-ecological problems. What we are doing at present is to select mutants and recombinants, which show a specifically diverging response to the Indian climatic conditions as related to the initial line, i.e. which have an altered climatic adaptability. These alterations include quantitative as well as qualitative characters. Some of the results hitherto obtained are as follows:

- Some fasciated mutants and recombinants, which show an excellent seed production in Germany, do not flower at all in India and cannot be used for breeding purposes.
- Some other fasciated genotypes are richly flowering in India. They form fully fertile pollen grains, but they are completely seed sterile.
- The pleiotropic spectrum of one of our promising mutants is composed of a positive and a negative component influencing the breeding value negatively. In North-India, the negative component of the spectrum is altered into a positive one resulting in a considerable improvement of the yield (figure 3).
- One of our recombinants shows a pronounced stem fasciation in Germany. At two Indian locations, the plants were non-fasciated when sown during the normal sowing time.

Sown four weeks later, all the plants were fasciated. The manifestation of the gene action depends in this case obviously on the photoperiodic conditions existing during specific stages of ontogenetic development.



**Figure 3:** Comparison of some yield components of mutant 68C grown in Bonn/Germany and Kurukshetra/India. All the values are related to the control values of the initial line = 100%. The yielding properties of the mutant are in India essentially better than in Germany.

Results of project no. 3:

Head of the project and scientific co-workers:

- Prof. Dr. Werner Gottschalk
- Dr. Dagmar Müller

Title of the project:

Basic research in the field of mutagenesis

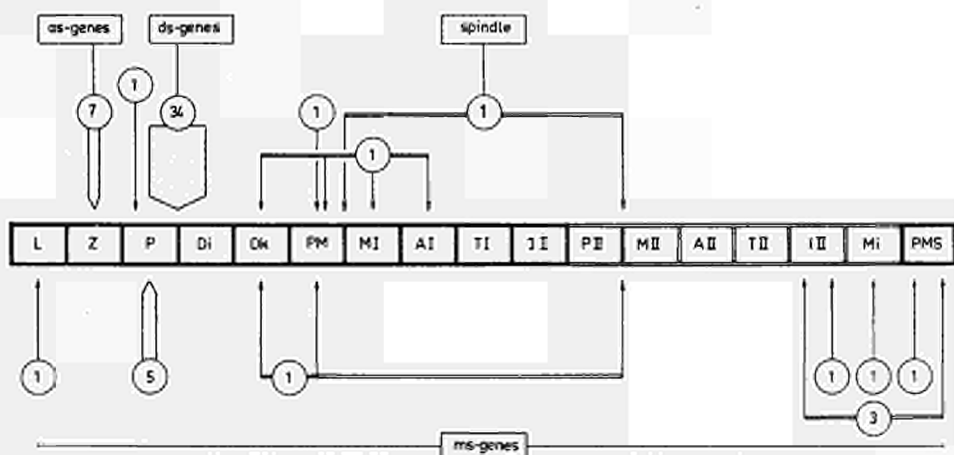
1) The genetic control of meiosis

This problem has been finished now. We have discussed it several times during the past meetings; therefore, it is not necessary to go into the details. Let me only mention the final results obtained during ten years of cytogenetic work.

56 genes of our collection of *Pisum* mutants were found to have control functions over specific meiotic processes (figure 4).

- 5 genes control the pairing behaviour of the homologous chromosomes.
- 34 genes control chiasma formation and chiasma frequency.
- 13 genes cause complete breakdown of microsporogenesis during a specific meiotic stage characteristic for each gene of this group. Macrosporogenesis is not influenced by these genes. Thus, they cause male sterility.
- 4 genes are responsible for the control of other meiotic processes such as spindle activity and the persistence of the nucleolus among others.

This programme was carried out in co-operation with Dr. Klein and with some foreign cytologists working on a fellowship-basis at our institute. Moreover, strains homozygous for reciprocal translocations were isolated for further evaluation.



**Figure 4:** Genes of the Pisum genome controlling different processes of the course of meiosis.

Middle: meiotic stages.

The arrows show the stage, in which the mutant genes become effective. The number of genes belonging to specific groups are given in the circles.

as-genes: mutant genes causing asynapsis.  
 ds-genes: mutant genes causing desynapsis.  
 ms-genes: mutant genes causing male sterility.



2) The genetic basis of stem fasciation in peas

With regard to the genetic basis of stem fasciation in *Pisum*, the following results have been obtained:

- All our fasciated mutants contain at least 10 different mutated genes, which have been changed  $\pm$  simultaneously during the irradiation experiments. They are obviously distributed over the genome. Because of this complex situation, very complicated segregations are obtained, when the fasciated mutants are used in crosses.
- Only a few genes of the mutated group just mentioned become directly discernible in the fasciated mutants. The other ones can be evidenced after having crossed the fasciated mutants with non-fasciated genotypes. The mutual relations between the genes of the group are regulated by a hypo- and epistatic system which is not yet clarified in detail.
- The phenomenon of stem fasciation exists in the pea at least in the form of three different types of fasciation. They are partly due to different alleles of a multiple series, partly to non-allelic genes of the genome, which show obviously likewise hypo- and epistatic relations between each other.
- The genes or alleles for stem fasciation cause in crossings a marked heterosis effect the intensity of which is influenced to some extent by the second partner.

Results of project no. 4:

Head of the project and scientific co-workers:

- Prof. Dr. Werner Gottschalk
- Prof. Dr. Hermann Müller

Title of the project:

Biochemical investigations of the seed proteins of pea mutants and recombinants

A part of the proteins present in the seeds are enzymes having specific functions in plant metabolism. These enzymes can be separated gelelectrophoretically into different sub-fractions called isozymes. They are the primary gene products. It can be expected, that the analysis of these isozymes can give us certain information concerning the action of the mutant genes.

In dry Pisum seeds, three enzymes were already known to be biochemically detectable. Two more were found in our investigations:

- LDH (lactate-dehydrogenase) and
- MDH (malate-dehydrogenase).

The isozyme patterns of these 5 enzymes of three genotypes are illustrated in figure 5. Besides the initial line, mutants 17A and 488 of our collection were analysed, which are not homozygous for a gene mutation but for a reciprocal translocation. Three out of the five enzymes analysed have the same number of isozymes in all the three genotypes. The LAP, however, differs in the three genotypes qualitatively. A similar situation is valid for MDH: one isozyme of this enzyme is lacking in mutant 17A. Besides the qualitative differences, also quantitative differences between the isozymes of the genotypes studied were found.

During our experiments, we have got the impression, that the behaviour of the isozymes could be influenced by distinct environmental factors. Therefore, mutant 488 was comparatively grown in the field and the greenhouse. The

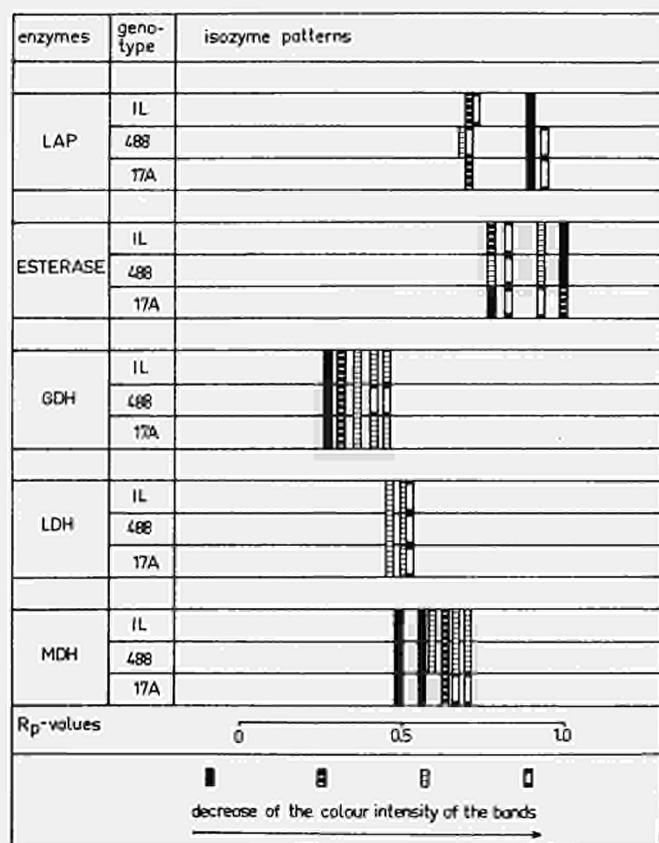


Figure 5: The isozyme patterns of 5 enzymes of the initial line and mutants 17A and 488 homozygous for reciprocal translocations.

seeds were separately harvested and analysed. The results are given in figure 6. With regard to the esterase, GDH and LDH, no differences were found between field and greenhouse material. The LAP, however, has only two isozymes in the greenhouse material, while four isozymes are present in the field material. With regard to MDH, the reverse situation was found: the greenhouse material has 8, the field material only 6 isozymes. The total protein content of the seed meal of the mutant is not altered; it is equivalent to that of the initial line.

The seeds of both plant groups were harvested in a fully ripened stage. But it is possible, that certain differences in the physiological ripening state of the greenhouse and field material are responsible for the diverging biochemical situation found in this material. But in any case, these differences are due to the genetic constitution of mutant 488.

In the same mutant (488), not only the isozyme patterns of two enzymes, but also the pattern of the protein fractions are altered under the influence of environmental factors. This can be seen from material again grown in the field and in the greenhouse. The soluble seed proteins were denatured and electrophoretically separated into 12 sub-fractions. The stained sub-fractions were densitometrically evaluated.

In the initial line, only small quantitative differences between field and greenhouse material were found. In mutant 488, however, there is a striking qualitative difference. In the field material, all the 12 sub-fractions of the seed proteins are present. In the greenhouse material, however, fraction no. 3 is lacking. That means, that the composition of the seed proteins of mutant 488 is specifically altered under the influence of the greenhouse conditions.

We are still in the very beginning of the study of this problem. Therefore, it is too early to speculate on the relations between

- the heterogeneity of the isozyme patterns,
- the genetic variation and
- the regulating mechanisms controlling seed protein synthesis.

The findings obtained in the translocation-homozygous strains encourage us to carry out similar investigations in mutants homozygous for recessive genes. In this material, it may be easier to interpret and to understand the biochemical alterations found.

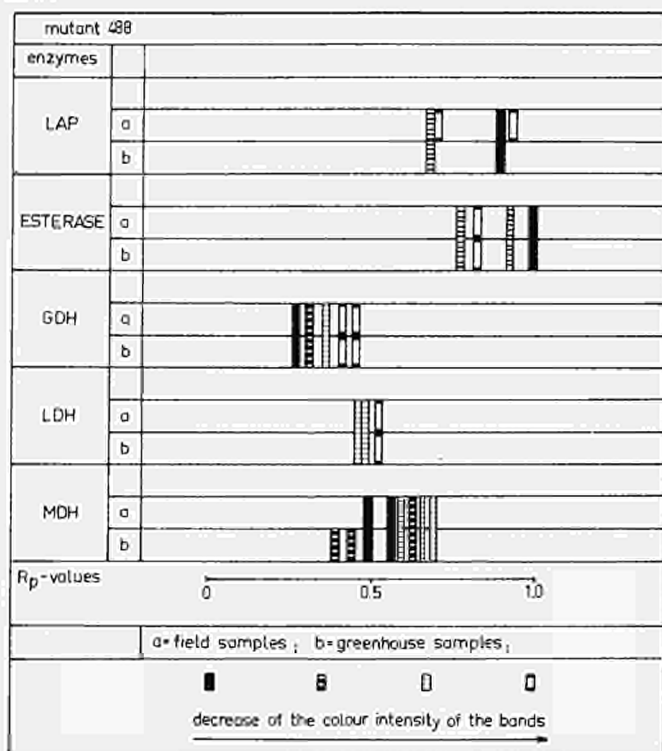


Figure 6: The isozyme patterns of 5 enzymes of mutant 488 comparatively grown on the field and in the greenhouse.

Activities during 1975:

The leader of the research programme, Professor Gottschalk, evaluated his material grown in India and gave lectures at the Universities of Kurukshetra, Simla, Varanasi, Calcutta, Bhubaneshwar, Gauhati, Jorhat and the Indian Agricultural Research Institute in Delhi; moreover at the Universities of Cairo and Alexandria. Furthermore, he presented papers at the International Botanical Congress in Leningrad and at two symposia of the International Atomic Energy Agency in Hahnenklee and Vienna. Professor Müller gave a lecture at the University of Marburg.

The following papers were published or given to press:

Gottschalk, W.:

1. Zwei hochpleiotrope, polymere Gene von *Pisum sativum*. *Decheniana* 127, 91-104 (1974)
2. Investigations on the co-operation of mutated genes. I. Polymeric genes. *Egypt.J.Genet.* 4, 336-344 (1b75)
3. The response of a recombinant to virus attack. *Pisum Newsletter* 7, 9 (1975)
4. The genetic control of chiasma formation and chiasma frequency. *Pisum Newsletter* 7, 10 (1975)
5. Fasciated mutants and recombinants in *Pisum*. *Pisum Newsletter* 7, 8 (1975)

Gottschalk, W. and H.A.S. Hussein:

6. The productivity of fasciated pea recombinants and the interaction of the mutant genes involved. *Z.Pflanzenzüchtung* 74, 265-278 (1975)

Gottschalk, W. and M.L.H. Kaul:

7. The genetic control of microsporogenesis in higher plants. *Nucleus* 17, 133-166 (1974)
8. Gene-ecological investigations in *Pisum* mutants. I. The influence of climatic factors upon quantitative and qualitative characters. *Z.Pflanzenzüchtung* 75, 182-191 (1975)

Gottschalk, W. and O. Konvicka:

9. Die Meiosis einer partiell asynaptischen Mutante von *Pisum sativum*.  
*Cytobiologie* 10, 458-467 (1975)

Gottschalk, W., Müller, H.P. and G. Wolff:

10. Relations between protein production, protein quality and environmental factors in *Pisum* mutants.  
Breeding for Seed Protein Improvement using Nuclear Techniques. IAEA Vienna; 105-123 (1975)
11. Further investigations on the genetic control of the seed protein production in *Pisum* mutants.  
Symp. GSF/IAEA; in press

Müller, H.P.:

12. Relations between seed production and the number of root nodules in pea mutants.  
*Pisum Newsletter* 7, 37 (1975)
13. The reaction of two pea genotypes to different climatic conditions.  
*Pisum Newsletter* 7, 38 (1975)
14. Incorporation of different protein fractions in pea seeds during seed ripening.  
*Pisum Newsletter* 7, 39 (1975)
15. Changes in protein patterns of the albumin fraction in seeds during ripening.  
*Pisum Newsletter* 7, 40 (1975)





Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung mbH, München

Nummer des Vertrages: SC 08/94-72-1 BIAN

Leiter der Forschungsgruppe:

Prof. Dr. H. Gaul, Leiter der Abteilung für Pflanzengenetik

Allgemeines Thema des Vertrages:

Production and selection of barleys with increased protein quantity and improved protein quality

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To select improved protein mutants from a large number of induces mutants in segregating generations, we applied during a two years trial a selection model, based on three selection criteria. For protein quantity, selection was performed with the following criteria:

1. grain yield in  $\text{g/m}^2$
2. protein content in % protein in dry matter (i.d.m.)
3. protein yield in  $\text{g protein/m}^2$

Any genetic improvement in protein quantity should result a higher amount of  $\text{protein/m}^2$  and thus a higher protein yield. This could be achieved either by substituting starch for protein or by increasing protein and starch at different ratios.

A selection model should also enable the breeder to differentiate between genetically determined and arbitrarily improved protein mutants. A none-genetic improvement in protein is often found in mutants which increase the protein content by decreasing the starch yield. This is quite well supported by a negative relation between protein content and grain yield.

In a bivariate selection model, established for the purpose of reliable selection of protein-improved mutants, both assumptions were considered. According to this model, selection was performed in diploid and tetraploid barley mutant groups.

Ergebnisse des Projektes Nr. 1

Leiter des Projektes und wissenschaftliche Mitarbeiter:  
Dr. H. Walther

Titel des Projektes:

Utilization of natural and mutagen-induced protein variability in diploid barley.

From a bivariate selection model, using grain yield and protein content as selection criteria, we calculated protein yield values for all parental lines and their derived mutants. The fields of covariation in this model can be used to discriminate arbitrarily improved mutants from genetically improved mutants by using iso-protein-yield-lines. As selection limit for positive mutants the mean protein yield of the parents ( $\bar{x}_p$ ) plus a 5 % least significant difference level (LSD 5 %) was used.

Mutants outside the range of covariation (% protein content i.d.m., grain yield  $g/m^2$ ) of the parents and above the limit of  $\bar{x}_p + \text{LSD } 5 \%$  for protein yield are mutants showing a possible genetic protein improvement.

Strong environmental influences are reduced by using mean values over replications and environments (locations, years). The evaluation of significant mutants is therefore aided by means of an analysis of variance giving genotypic, environmental and experimental error components.

This selection model was applied to two groups of diploid spring barley mutants:

### 1. Micromutants

40 mutants out of 1103 deviated significantly in protein yield from their parents, however at a low level of protein content, but at a high level of grain yield. Since these mutants show only minor changes in all other plant characters, this improvement in protein yield, here about  $8.5 \text{ g/m}^2$ , opens the possibility of direct selection in small steps.

### 2. Macromutants

A broad variation was found in all three selection criteria within this group of mutants. This is partially due to drastic changes in other plant characters like culm length and kernel size. Comparing the mean of the parents with the mean of the derived mutants, we found large differences to the negative side of the variation among the derived mutants. This indicates that most of the increase in protein content was due to none-genetic components like a decrease in starch.

Only two mutants out of 684 were found to be significantly improved at a level of LSD 5 %. Most of these morphological macro-mutants high in protein content therefore are not worthwhile to be used as cross-parents, since their high protein content results from an indirect increase depending on kernel size and starch content.

Ergebnisse des Projektes Nr. 2

Leiter des Projektes und wissenschaftliche Mitarbeiter:

Dr. H. Walther

Dipl.-Landwirt W. Friedt

Titel des Projektes:

Utilization of the protein variability of diploidized tetraploid barley.

The same selection model as was applied to diploid mutants was used also for the group of tetraploid strains. Here we found 10 mutants out of 186 to be significantly improved in protein yield and thus better than their parents.

This is of special interest, since the high level of protein content and protein yield seem to be characteristic for this ploidy-level.

The preference of using protein yield as measure instead of protein content is supported by three further results.

1. A strong positive correlation was found between protein yield and grain yield with  $r = 0.85 \dots 0.9^{**}$ .
2. The protein yield measure includes both genetical steps of protein improvement; the substitution of starch for protein and the addition of protein and starch at different ratios, without an arbitrary increase in protein content.
3. High protein yield and high lysine yield are increasing in the same direction.

To make use of the natural and induced variation in protein quantity, we follow a three-step strategy:

1. We increase protein yield by increasing grain yield at a constant protein content level.
2. We increase protein yield by increasing the protein content at a high grain yield level.
3. We increase the biological value of the protein by introducing high lysine mutants into material already high in protein yield.

Among tetraploid mutants as well as among diploid mutants the increase in protein yield was found to occur only in small quantitative steps. These mutants are therefore defined as protein-micromutants.

As regards quantitative protein characters, no drastic genetic improvement is to be expected within the group of mutants, derived from one parent. However in all mutant groups tested, a number of mutants proved to be significantly better than their parents.

In this experiment selection for high lysine mutants was not as successful as for protein yield, except for one mutant with 4.1 % lysine in protein.

Publications 1975

Gaul, H., Walther, H., Brunner, H., Mikaelson, K., Seibold, K.H.:  
Estimates of selection parameters in protein mutants of spring barley.  
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Ulonska, E., Gaul, H., Baumer, M.: Investigations of selection methods  
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Techniques, 61-77 (1975)

Walther, H.: Schätzwerte für den Selektionsfortschritt bei der Auslese  
von induzierten Protein-Sommergerste-Mutanten. GSF, Neuherberg:  
B 626, 63-64 (1975)

Walther, H.: Selection of improved protein and lysine mutants induced  
in spring barley by EMS and X-ray treatment. GSF, Abt. Pflanzengenetik,  
Grünbach: Barley Genetics III (in press)

Walther, H.: A bivariate selection model applied for screening of  
improved protein mutants in spring barley. Association EURATOM-ITAL,  
External Report Meeting Mutation Breeding Contact Group, Cadarache 1975  
(in press)

Walther, H., Gaul, H., Ulonska, E., Seibold, K.H.: Variation and  
selection of protein and lysine mutants in spring barley. IAEA,  
Vienna: Breeding for Seed Protein Improvement Using Nuclear Techniques,  
79-89 (1975)

Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung mbH., München

Nummer des Vertrages: SC 08/94 - 72 - 1 BIAN

Leiter der Forschungsgruppe:

Prof. Dr. H. Gaul, Leiter der Abteilung für Pflanzengenetik

Allgemeines Thema des Vertrages:

Production and selection of barleys with vertical and horizontal resistance against mildew (Erysiphe graminis)

In project 1 seeds of three barley varieties have been treated with X-rays and EMS. In the M<sub>2</sub> two mildew resistant mutants were selected, resistant to the mildew population of Grünbach. The experiments will provide information on the frequency of resistant mutants and on the efficiency of the mutagenic treatments used for the induction of these mutants.

In project 2 mildew resistant mutants have been subjected to genetic analysis. Different crosses revealed that they carry the ml-o gene on chromosome 4. Comparison of their phenotypes suggests the existence of different sites at the ml-o locus or the simultaneous mutation of other genes. Further experiments have been set up in an attempt to remove the chlorotic flecking of ml-o mutants and to make their use in plant breeding programs a practical proposition.

Ergebnisse des Projekts Nr. 1

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Dipl.-Ing.agr. V. Lind  
Prof. Dr. H. Gaul

Titel des Projekts:

Selection of mildew resistant barley mutants following  
mutation induction

Darstellung der Ergebnisse:

Seeds of the spring barley varieties Villa, Carina and Bido were treated with EMS and X-rays. The treatment used was the same as reported previously. The aim is to obtain information concerning the efficiency of different mutagenic treatments in the induction of mildew resistance.

The  $M_2$  had been tested for resistance in the greenhouse using the Grünbach mildew population. The X-ray treated material was grown as a bulk population. The  $M_2$  seed of the EMS treated barley was harvested in the form of spike progenies, and the unthreshed spikes sown out. Among the  $M_2$  plants derived from the EMS-treated material plants of the variety Villa, two plants were selected showing resistance. The first of these plants was selected from 130 000  $M_2$  plants in 1974 and the second from 25 000  $M_2$  plants in 1975. They have different infection types. No mildew pustules develop on one of the plants, this has been designated as infection type 0. On the leaves of the other plant designated 0-1 mildew development is very poor but necroses do appear. Some of the plants selected in 1974 did not yield any kernels.



## Ergebnisse des Projekts Nr. 2

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Prof. Dr. H. Gaul  
Dipl.-Ing.agr. V. Lind

Titel des Projekts:

Influence of the genetic background on the expression  
of mildew resistance

Darstellung der Ergebnisse:

Backcrosses between 19 mildew resistant mutants and their mother varieties Gerda, Matura and Villa revealed a recessive mode of inheritance of resistance.

In the  $F_1$  as well as the  $F_2$  from the diallel crosses between these mutants no susceptible plants appeared. Thus we concluded that all mutations were induced in the same locus.

Six of the mutants were crossed with M.C. 20, a barley mutant carrying the  $m1-o$  gene on chromosome 4. All plants in the  $F_1$  and  $F_2$  proved to be resistant. Hence it appears that the mutants have the  $m1-o$  gene. To confirm these results  $F_2$  plants will be grown in further generations and some more mutants will be included in crosses with M.C. 20.

There are differences in leaf spotting between the mutants, these being dependent on the genetic background of the mother varieties. The leaves of the mutants derived from Gerda and Volla exhibit large chlorotic areas which originate from the leaf tip. Among the mutants derived from Matura there is also variation in leaf flecking, particularly in the number of necrotic and chlorotic spots. The phenotypic diversity of the Matura-mutants may be regarded as suggestive

of the existence of different sites at the ml-o Locus or the simultaneous mutation of other genes. The latter assumption may be supported by the appearance of several phenotypic classes of chlorosis in some F<sub>2</sub> generations.

Crosses have been initiated to unite the ml-o gene (oo) with other dominant genes (MM) located at the M1-a locus. To select the MMoo-genotype we have adapted a special crossing procedure. It will require five generations after crossing to obtain the derived plants. By backcrossing, the MMoo-genotypes can be incorporated in a commercial variety.

Another program is concerned with the identification of resistance genes in mutants of various origin. Test crosses have been made between mutant 501 ( induced in Friedrichswerther Berg ), M.C. 25 ( induced in Maltera Heda ) and a translocation tester set. The cytogenetic method employed for the localization of the genes was as developed by FAVRET.

#### Publications 1975

LIND, V., FAVRET, E.A. and GAUL, H., Investigations of mutagen-induced mildew resistance in spring barley. Meeting of the Mutation Breeding Contact Group, Cadarache, September 9-11, 1975 ( in press ).

FAVRET, E.A., Breeding for disease resistance. FAO/IAEA Advisory Group Meeting on Induced Mutations in Cross Breeding, Vienna, October 13-17, 1975 ( in press ).

Vertragspartner der Kommission: Gesellschaft für Strahlen- und  
Umweltforschung mbH. München  
8042 Neuherberg, Ingolstädter  
Landstr. 1

Nr. des Vertrags: SC 009/094-72-1 BIAN

Leiter der Forschungsgruppe: Priv. Doz. Dr. W. Kühn

Allgemeines Thema des Vertrags: "Strahlenanalyse im Landbau"

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Das entwickelte Verfahren zur Messung eines Pflanzenbestandes durch Absorption von Gammastrahlung ist 1975 erstmals zur Bestimmung der Pflanzenmasse eines natürlich wachsenden Bestandes eingesetzt worden. Während 70 Tagen wurde eine Wachstumskurve aufgenommen, in der die täglichen und nächstlichen Einflußgrößen in ihrer Auswirkung differenziert wiedergegeben sind, so daß der jeweiligen Gesamtmasse auch die einflußnehmenden Parameter zugeordnet werden können. Die Gesamtmasse des Feldes wurde gravimetrisch bestimmt und mit der durch Absorption gemessenen verglichen. Innerhalb der erzielten Meßgenauigkeit von 5% gibt das zerstörungsfrei arbeitende Verfahren das Gesamtgewicht des Bestandes ausreichend gut wieder. Beim Transport von Wasserdampf in Böden infolge von Diffusion, Evaporation und Rekondensation sind die physikalischen Eigenschaften der Bodenmatrix von grundlegender Bedeutung. Sie bestimmen im wesentlichen die Dampfdurchlässigkeit des Bodens. Besonders wichtig ist die Wasserdampfbewegung in Böden in ariden Gebieten. Aus einem Löß und einem Feinsand wurden fünf anteilig verschiedene Böden gemischt und ihre Poren- und Korngrößenverteilung bestimmt. In einer senkrechten Säule wurden bei konstanter Temperatur Transportprozesse in diesen Böden durch Markierung der Mittelschicht bei pF 2,5 mit HTO untersucht. Die darüber und darunter befindlichen Schichten wurden jeweils auf pF 6 entwässert. Dadurch waren die Wasserspannungsgradienten für alle Bodenarten gleich.

Ergebnisse des Projekts Nr. 1

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Dr. W. Kühn, Dr. H.P. Schätzler

Titel des Projekts: "Mass determination on Plantations"

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The development of growth of a cereal plot have been studied with the scanning device already described in 1974. The whole measuring equipment has been installed on foundations of 50 cm height with a rye plot of 1,5 x 4 m<sup>2</sup> size in between. The plot consists of 56 pots of 10 l volume equally distributed of the whole area. The procedure of measurement extended over a period of a about 70 days, beginning from the blossom-time until the harvest. The growing curve of the first 55 days including the climatic course is shown in fig. 1. The nearly stagnant growth rate during the blossom-time, the great influence of irrigation and humidity on the biomass including the day and night cycle, the strong increase of mass during the grain development as well as the decrease of mass due to the loss of water during the maturity period is obvious. The accuracy of the mass determination always was better than 5%. So alterations of mass greater than 5% could be detected with 95% probability even when the culm density is as low as it has been. The accordance of the gravimetrically determined crop yield and the measured one was within the two fold statistical error of 5%, too.

In connection with  $\gamma$ -absorption measurements on plants experiments concerning the appearance of internal tipburn of white cabbage, the correlation between transpiration and Ca-uptake of a cabbage plant has been investigated. The transpiration of the cabbage head during the diurnal climatic variations has been measured gravimetrically and by the gamma absorption method using <sup>241</sup>Am (60 KeV). The Ca uptake of the plant was studied in vivo by measuring the  $\beta$ -intensity of <sup>45</sup>Ca which was added to the nutrition solution. The daily added activity was rather high, exceeding 1-2 mCi <sup>45</sup>Ca per day depending on the transpiration loss of the plant. The  $\beta$ -measurements were performed with two small Si surface barrier detectors attached within light tight capsules with thin Al windows. The  $\beta$ -intensity was determined continuously at an outer leave and at an inner head leave. The results show a high uptake of Ca by the outer leaves and a nearly stagnant concentration of Ca within the head during the day, when the transpiration was high. A strong increase of Ca within the head and a nearly constant concentration in the outer leaves could be observed during the night, when the transpiration was low. A Ca-uptake of the outer leaves could also be detected, when the transpiration during the night was kept artificially high, whereas the concentration within the head remained nearly constant.

Literature

- 1) Kühn, W., Schätzler, H. P.:  
 "Non-destructive Determination of the Biomass of a Cereal Plot by Gamma-Radiation"  
 Journal of Plant Breeding (in print)
2. Schätzler, H. P.:  
 "Bestimmung der Biomasse lebender Pflanzenbestände durch Absorption von Gammastrahlen"  
 Gesellschaft für Strahlen- und Umweltforschung,  
 GSF-Bericht BT 245 (1975)

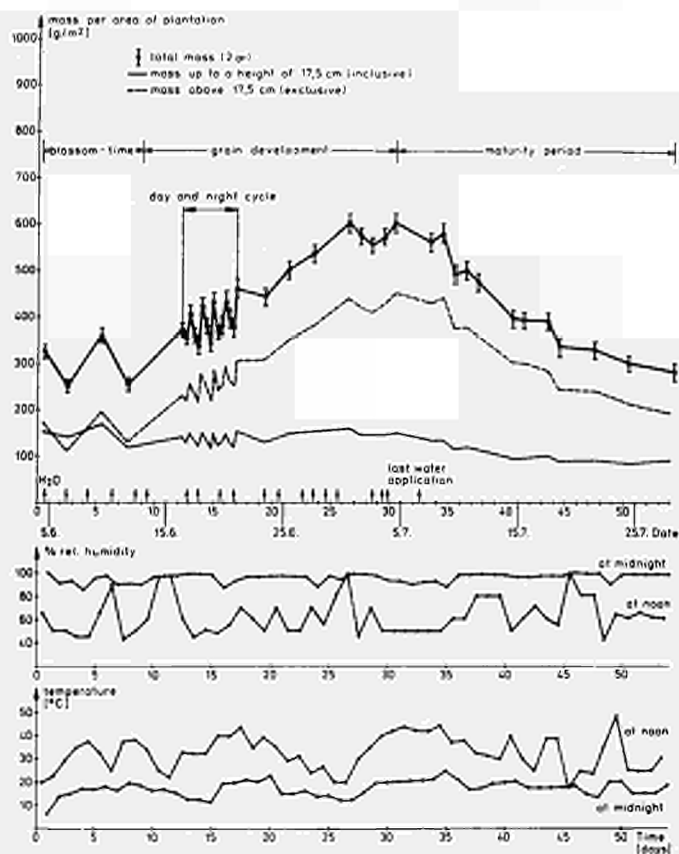


Fig. 1 Growing curve of a rye plot (1,5 x 4 m<sup>2</sup>) including the climatic course.

Ergebnisse des Projekts Nr. 3

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Dipl. Phys. C. Bunnenberg, Dr. W. Kühn, Dr. J. Handl

Titel des Projekts: "Messung von Evaporation und Transpiration im Boden"

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For the transport of water vapor by diffusion, evaporation and re-condensation in the pores of soils and at the surface of soil particles the physical parameters of the soil matrix, as pore volume, distributions of pore and particle sizes, free cross section of the porous material and its active surface etc., are of basic importance. They determine the penetrability of soils for vapor, whereas temperature, concentration and pressure gradients built up driving forces causing such movement.

In order to describe the movement of gases in porous media under specific boundary conditions, certain diffusion coefficients are defined as factors between the flux of substance through unit area and the concentration gradient:

$$\int \frac{\partial \theta}{\partial t} dx = D \frac{\partial \theta}{\partial x} \Big|_{x=x_0}$$

For arid climates the movement of water vapor in soils of very low moisture content is of special interest, and efforts are made to realize relations between vapor movement and physical properties of soils.

Five soils were mixed for investigations taking different proportions of a loess and a sandy soil. Vapor movement processes were studied in vertical soil columns at constant temperature. The middle layer of each column was homogeneously labelled with tritiated water to a water pressure of pF 2.5, while the soil above and below the middle layer was dried to a pressure of pF 6, achieving the same pressure gradient for all five soils at the start of the diffusion process, and moisture contents being limited to a range where liquid movement is negligibly small.

At a certain time the dry soil layers were brought in contact with the moist middle layer to start the process of vapor transport. The labelled vapor was collected in horizontal tubes positioned at different distances in the soil above and below the middle layer and measured with a special H-3 flow counter. The amount of moisture collected in a certain period of time yields the flux  $\int \frac{\partial \theta}{\partial t} dx$  at distance  $x_0$ . From those measurements the moisture distribution  $\theta(x)$  and the moisture gradient  $\frac{\partial \theta}{\partial x}$  at  $x_0$  can be calculated.

During the diffusion process the water pressure at the measuring points changes from pF 6 to about pF 3. Thus the amount of mobile moisture increases, while the free cross section for vapor transport decreases due to the filling up of small pores. The diffusion coefficients will be calculated for all five soil types by means of a computer

program considering the specific boundary conditions of the experiments. Furthermore, functional relations between diffusion coefficients, water pressure and physical properties of the soil matrix are to be investigated.

Further experiments are planned to study the temperature dependence of those functions. All the information is meant to finally provide a basis for a mathematical simulation of water vapor movement in soils, when the physical parameters of the soil and the climatic conditions are known.





Commission's associate: University of Modena  
Contract No.: SC/11-I/094-72-1 BIAN  
Head of the research group: Ernesto Carafoli  
General theme of the contract: Transport of calcium and strontium in chloroplasts and plant mitochondria

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The research has been carried out by Dr. E. Carafoli, and Dr. M. Montaguti. Dr. R. Tiozzo, and Dr. F. Crovetti, have also contributed to some of its phases.

The scheme presented at the beginning of 1975 has been pursued with particular attention to a) the reconstitution of  $\text{Ca}^{++}$  transport in artificial lipid bilayer membranes using the  $\text{Ca}^{++}$ -binding glycoprotein isolated from mitochondria and b) the chemical and biochemical characterization of the  $\text{Ca}^{++}$ -binding glycoprotein. A research has also been completed on the cellular site of biosynthesis of the glycoprotein.

The experiments on the "reconstitution" of the  $\text{Ca}^{++}$  transport in artificial lipid bilayer systems have represented an extension of the investigations carried out in 1974, in which it had been found that the glycoprotein increased the conductance of lipid bilayers (black films) in the presence of  $\text{Ca}^{++}$ . It has now been possible to conclude that the glycoprotein does not act as a  $\text{Ca}^{++}$  carrier, but rather as a  $\text{Ca}^{++}$  receptor, which becomes associated with the bilayer under the influence of the cation. The conclusion is based on 1) the failure to measure  $\text{Ca}^{++}$ -dependent Nernst potentials, 2) the requirement for  $\text{Ca}^{++}$  to demonstrate the increased conductance in lipid bilayers (black films) and 3) the inability of the glycoprotein to increase the efflux of  $\text{Ca}^{++}$  from  $\text{Ca}^{++}$ -loaded liposomes. The experiments on liposomes have occupied a substantial amount of time, and have been carried out in a large variety of conditions. In no case, however, has it been possible to observe increases in  $\text{Ca}^{++}$  permeability induced by the glycoprotein. The chemical characterization of the glycoprotein has centered on the phospholipid components, which have been extracted, quantified, and analyzed.

It now appears that lecithin (phosphatidyl-choline) represents by far the most abundant phospholipid. It has also been found that removal of the phospholipid results in the inactivation of the  $\text{Ca}^{++}$ -binding ability of the glycoprotein. The role of the phospholipid in the binding of  $\text{Ca}^{++}$  is at present being investigated.

Double labelling experiments with radioactive amino-acids ( $^{14}\text{C}$ -leucine,  $^3\text{H}$ -valine) on growing yeast, in the presence of inhibitors of either the cytoplasmic, or the mitochondrial protein synthesis system, have indicated quite clearly that the  $\text{Ca}^{++}$ -binding glycoprotein is translated on cytoplasmic, and not mitochondrial, ribosomes.

Contractor: Danish Atomic Energy Commission

Contract number: 138-74-1 BIO DK

Head of research team: Jens Sandfær

General subject of contract: IMPROVEMENT OF THE NUTRITIONAL  
VALUE OF BARLEY SEED PROTEIN

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Like the protein of most other cereals, barley seed protein has too low a content of some of the amino acids, e.g. lysine and threonine, that are essential for the growth and maintenance of non-ruminant animals. The nutritive quality of the barley protein is sought improved by means of induced mutations. Mutants with an increased lysine content of the protein have been selected, and their agronomic properties and nutritive value are studied. Furthermore, the mutants are investigated biochemically, genetically and ultrastructurally to obtain a better knowledge of the formation and composition of barley seed protein.

Results of Project No. 1:

Scientific staff: Hans Doll, Arna J. Andersen and Bertel Køie

Title of Project: Screening, genetics and agronomic properties  
of high lysine barley

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The search for high lysine mutants was continued by analysing 5000 plants derived from a mutagenic treatment with sodium azide. Nitrogen and DBC analyses of 2000 plants revealed 3 potential high lysine mutants with an increased DBC/N ratio. The remaining 3000 plants were analysed by a single-seed method employing electrophoresis on a protein extract from the endosperm. Mutants with an altered electrophoretic protein pattern were selected for further evaluation.

Eight of our high lysine mutants have each a single gene causing the increased lysine content of the protein. Preliminary results indicate that the eight genes belong to six different loci. The eight mutants all have more or less shrunken seeds. This character has so far been completely linked with the high lysine character, indicating that these high lysine genes restrain normal grain filling and thereby reduce the grain yield.

The effect of different high lysine genes on important agronomic properties is studied by means of lines derived from chromosome-doubled monoplastids made on crosses between mutants and normal barley. The testing of 100 of such lines in the field showed that the high lysine gene in mutant 29 reduces the grain yield by about 15%, while the gene in mutant 56 causes a nearly 30% yield reduction.

The relation between the amount of nitrogen fertilizer and the yield and composition of protein was studied. The highest yield of both grain and protein was obtained with a moderate amount of nitrogen given in two steps, one at sowing time and one 7 weeks later. Neither in the high lysine mutant 1508, nor in its normal parent variety Bomi, was the composition of the protein affected by the time of fertilizer supply.

Results of Project No.: 2

Scientific staff: Bo Büchmann, Bertel Køie and Hans Doll

Title: Nutritional value of barley seed protein

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A method for determination of the protein digestibility in vitro has been established. The sample is incubated with pepsin at pH 2.0 followed by pancreatin at pH 7.6 so that the method simulates in vivo conditions. The results obtained are highly reproducible. However, since the variation in protein digestibility among different barleys is very limited, it has not been possible to make a detailed test of the correlation between the in vitro and the in vivo results. A collection of 345 barley varieties is now being analysed in vitro in an attempt to select types with a better protein digestibility.

The in vivo nutritive quality of the protein in the high lysine mutant 1508 and in the parent variety Bomi was investigated by feeding at the National Institute of Animal Sciences, Copenhagen. A pig-feeding trial showed that the nutritive value of the protein in mutant 1508 was substantially increased compared with that of normal barley. About one third of the normal soya meal supplement could be saved by using mutant 1508 instead of Bomi in the feed. Trials with poultry revealed that the additional lysine in the mutant has the same nutritional value as supplementing the feed by synthetic lysine. However, both pig and poultry experiments indicated that the mutant has a slightly reduced content of digestible carbohydrates.

Results of Project No.: 3

Scientific staff: Anders Brandt, John Ingversen and Bertel Køie

Title: Synthesis, composition and storage of proteins in the endosperm

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The formation of barley endosperm proteins has been followed by amino acid analysis and SDS-polyacrylamide electrophoresis during kernel development of wild-type and high lysine barley, mutant 1508. In the mutant, only minor differences in the amino acid composition and electrophoretic patterns of the albumins and the globulins, respectively, were observed, but three times more free amino acids were present at all stages of endosperm development compared to the wild type. Hordein formation was severely impaired and the synthesis of two components of the fraction was inhibited in the mutant. The high lysine content of the mutant endosperm is probably due to this reduction in the lysine-poor hordeins, a reduction of lysine-poor components of the glutelin fraction, and a compensating increase in the amount of lysine-rich glutelins as well as free lysine.

The incorporation of  $^{14}\text{C}$  into the endosperm proteins following injection of  $^{14}\text{C}$ -lysine into the top internode of wild type and mutant 1508 was studied. Albumins are preferentially labelled early in endosperm development, whereas the hordeins and glutelins are labelled later in endosperm development. The label did not move between the protein fractions. Most of the  $^{14}\text{C}$ -label incorporated into the albumins was recovered as  $^{14}\text{C}$ -lysine, but a substantial part of the  $^{14}\text{C}$ -label incorporated into the hordeins and glutelins was recovered as glutamic acid and proline. At the advanced stage of endosperm development the mutant did not incorporate glutamic acid and proline derived from the conversion of labelled lysine, whereas wild-type hordein did reveal such an incorporation.

In order to make a detailed study of the mechanisms in protein synthesis in the barley endosperm, a cell-free protein synthesizing system was recently established. We plan to study the translation of mRNA from the wild type and the high lysine mutants at different developmental stages of the endosperm and of mRNA from the free and membrane-bound polysomes.

A reproducible method for extraction, separation and quantitative determination of the lysine-poor hordein was developed. The earlier described

classification of the hordein into three groups, A, B and C, has proved appropriate. The extremely lysine-poor B and C hordeins seem to be present in the endosperm as two well-defined proteins, each consisting of a fixed number of polypeptides. There is a great genetic variation in these polypeptides with respect to electrophoretic mobility.

All the high lysine mutants have a reduced amount of B and/or C hordein compared with the parent varieties. In normal barley the lysine concentration of the protein decreases with increasing supply of nitrogen fertilizer, since the B and especially the C hordeins are preferentially produced in response to nitrogen addition.

A substantial part of the endosperm protein is stored in protein bodies after their synthesis on the endoplasmic reticulum. A procedure was developed to isolate protein bodies from immature endosperms of Bomi barley and mutant 1508. Protein bodies from the endosperm of Bomi are mainly homogene in structure, while those from mutant 1508 have a predominant component of granular structure. However, some of the homogene components are also present in the mutant. The protein bodies of Bomi have a high content of the lysine-poor B and C hordeins, while the protein bodies from mutant 1508 contain both hordein and other proteins, probably glutelins. These results indicate that the homogene component of the protein bodies acts as the store for the hordein, and that at least part of the glutelin is stored in the granular structure.

Publications in 1975.

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Contractor: Danish Atomic Energy Commission

Contract No.: 139-BIO DK

Head of research team: Jens Sandfær

General subject of contract: INDUCED MUTATIONS FOR POWDERY  
MILDEW RESISTANCE IN BARLEY

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The general aim of the research is to elucidate the value for disease resistance breeding of barley (Hordeum vulgare) of induced mutant genes conferring resistance to the powdery mildew fungus (Erysiphe graminis f.sp. hordei). Ten independently induced resistant mutants and some spontaneously arisen resistant barleys having the allelic resistance genes designated ml-o 1 through ml-o 11 (or Reg 6a through Reg 6k) are being studied with respect to the genetics of the mutants, the agronomic properties of the mutants, and their usefulness in cross-breeding.

Results of Project No.: 139-BIO DK

Head of Project and scientific staff: J. Helms Jørgensen and  
H. P. Jensen

Title of Project: Induced mutations for powdery mildew  
resistance in barley

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Genetics of the mutants. Ten independently induced mutant genes and one gene of spontaneous origin are designated ml-o 1 through ml-o 11 (or Reg 6a through Reg 6k). The ml-o locus is mapped relative to four marker genes on chromosome 4 in two-, three- and four-point linkage tests. The order of the genes is f9, K, Ml-g, br2, ml-o. Three additional marker genes, yh, min and cer-j, failed to show reliable linkage. A study in progress on inter-allelic recombination has so far yielded 41 susceptible recombinants in F<sub>2</sub> populations from mutant x mutant crosses indicating that there are at least three mutational sites within the ml-o locus. The recombination percentages obtained vary from 0 to 0.15.

Resistance characteristics of the mutants. Studies on this aspect were completed in 1975 and the results compiled. Over the years the ml-o resistant barleys have been tested at the seedling stage for reaction to 51 cultures of the powdery mildew fungus from Europe, Israel, U.S.A., Canada, and Japan. They were resistant with infection type O/(4) in all tests. They were also resistant to field populations of the pathogen in disease nurseries at more than 74 locations in 28 countries in Europe, the Near East, North and South America, New Zealand, and Japan. This indicates that the 11 genes confer the same world-wide range of powdery mildew resistance. These results fall in line with those from a histological study. This showed that the 11 ml-o genes do not differently affect the primary infection of the barley seedlings by the pathogen and the frequency of the occasional mildew colonies that may be observed on heavily inoculated seedlings. The frequency of mildew colonies may, however, be altered when the genetic background for the ml-o genes is altered. It has also been shown that the ml-o genes have no effect on several other barley diseases, such as stripe rust and leaf rust.

Agronomic properties of the mutants. The exploitation of the ml-o resistance genes in barley breeding depends on the extent to which their

pleiotropic effects, necrotic/chlorotic leaf flecking and reduced grain yield, can be modified or suppressed. A preliminary experiment was completed in 1975 in which homozygous resistant populations from several crosses were exposed to successive cycles of selection against necrotic leaf spotting. It was found that the spotting can be reduced or eliminated by crossing and subsequent selection, that the flecking is affected by several or many modifier genes, and that the reduction in spotting may be accompanied by an increase in grain yield.

A new experimental material comprising about 500 chromosome-doubled monoplloid barley lines with or without three selected ml-o genes is being developed. We aim at an elucidation of possible specific pleiotropic effects of specific ml-o genes and of their modification.

Publications in 1975.

Jørgensen, J. Helms, 1975: Identification of powdery mildew resistant barley mutants and their allelic relationship. - In: Proceedings of the 3rd International Barley Genetics Symposium, München, July 7-12, 1975 (in press).

Jørgensen, J. Helms, 1975: Studies on recombination between alleles in the ml-o locus of barley and on pleiotropic effects of the alleles. - In: Proceedings of the 3rd FAO/IAEA/SIDA Research Co-ordination meeting, Ames, Iowa, September 15-19, 1975 (in press).

Jørgensen, J. Helms and K. Mortensen: Primary infection by Erysiphe graminis f.sp. hordei of barley mutants with resistance genes in the ml-o locus. - Phytopathology (submitted).



Contractor : An Foras Taluntais.

Contract No. : 141-74-1 BIOEIR

Head of research team : F. O'Connor.

General subject of Contract : To study the possibility of using radiometric detection of bacteria in milk as a rapid grading index.

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Initial studies were concerned with modifications to the procedure developed by Buddemeyer, 1974. Appl. Microbiol 28: 177-180 for the radiorespirometric determination of  $^{14}C$ CO<sub>2</sub>. Particular emphasis was placed on the amount of scintillation cocktail taken up by the paper strip. In addition it was felt that diffusion of CO<sub>2</sub> from a deep liquid through a narrow orifice could effect the rapidity of the determination.

Head of Project and scientific staff : F. O'Connor and T. Cogan.

Title of Project : To study the possibility of using radiometric detection of bacteria in milk as a rapid grading index.

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Initial results, with the temperature of the scintillation counter cabinet set at 30°C, showed a large background count. This was eventually traced to the contamination of the paper with radioactivity during preparation. It was also found necessary to increase the fluor concentration in the cocktail to 25 times normal amounts to ensure that the paper absorbed 45±2 mg PPO-POPOP as recommended.

30 samples of raw milk were analysed for initial bacterial counts and for the rate of development of  $C^{14}O_2$  with the addition of 0.5% glucose (V/V) containing 0.5 uCi uniformly labelled glucose. Three different volumes of milk were studied (viz 4, 1.0 and 0.4 ml) each of which contained the same relative concentration of glucose plus label. A more rapid evolution of  $C^{14}O_2$  was obtained in the lower volume of milk presumably because of more rapid diffusion. The correlation coefficient (r) between the  $\log_{10}$  of the initial count and the time to reach a count of 1000 cpm was -0.90 (using 0.4 ml milk). This appeared to be independent of the volume studied since the r values for 4 and 1.0 ml were -0.87 and -0.91 respectively.

The number of samples analysed to date is small and it is hoped that the correlation would be better when a greater number of samples are examined.

Production of  $CO_2$  could also occur from decarboxylation of amino acid or breakdown of formate. The use of  $^{14}C$  amino acids and formic acid in addition to the glucose will also be studied during the next year.

Due to non functioning of a liquid scintillation counter which was delivered last August (1975) we could not commence this research project until the end of October consequently our results to date are limited. Much of the time was spent in developing the method but there are still variations we must examine. During 1976 we hope to examine a wide range of milk samples and by the end of the year we hope to have a large bank of data available.





ZELLKULTUREN

CELL CULTURE

CULTURE DE CELLULES



Commission Associate: Istituto di Genetica della Università-Pisa

Contract Number: 106-72-1 BIOI

Head of research team: Prof. F. D'Amato

Co-workers: Drs. S. Baroncelli, A. Bennici, M. Buiatti, P.G. Cionini, R. Cremonini, M. Durante, L. Giorgi.

Subject of the Contract: Cytology and genetics of plant tissues and cells grown in vitro.

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During 1975 the following investigations have been carried out:

1) Genetics of growth and differentiation in plant tissue cultures.

Studies on the genetic control of callus growth and root differentiation in vitro were continued using node and root explants of ditelocentrics and substitution lines of hexaploid wheat. Callus growth analyses carried out with 26 ditelocentric stocks of the cv. "Chinese Spring" showed that group 1 chromosomes were of relevance in controlling cell proliferation both in the case of nodes and roots, whereas group 7 did not seem to play any important role. Moreover, groups 4,5 and 6 influenced callus formation from roots but not from nodes with the notable exception of the stock 6B<sup>S</sup>. The observed effect of deletions for nucleolar chromosomes prompted a more thorough analysis of growth in varieties and substitution lines known to differ in ribosomal cistron multiplicity. The existence of a rough but significant correlation between growth and amount of rRNA-DNA hybridization was thus shown. Finally, preliminary data on the control of root formation suggest that the majority of genes relevant for this character belong to genome D chromosomes (Baroncelli, Bennici, Buiatti).

2) Cell dedifferentiation in plant tissues grown "in vitro".

Studies on the amplification processes in the early phases of dedifferentiation of pith tissue in Nicotiana spp. were concentrated on the inhibitory effects of BrdU. Analytical ultracentrifugation of BrdU-substituted DNA showed a shift in buoyant density up to 1.738 (from 1.695) Thermal denaturation profiles of heavy peaks along with <sup>3</sup>H-BrdU labelling experiments however suggested incorporation of the analogue in A-T rich regions and not in the G+C rich satellite which was known to be formed during

the BrdU sensitive period. Cytological analyses confirmed on the other hand inhibition of the formation of micronucleoli and their extrusion in the cytoplasm thus suggesting that BrdU substituted sequences may play some regulatory role in the amplification process: an hypothesis supported also by the differential inhibition observed when tumorous and non tumorous N. glauca × N. lundsdorffii stocks were compared (Buiatti, Durante, Giorgi, Parenti).

### 3) Molecular biology and physiology of the embryo suspensor of Phaseolus coccineus.

In work on the cytological localization of genes in the suspensor polytene chromosomes of Phaseolus coccineus, homologous tritiated 5.8S RNA and iodinated (<sup>125</sup>I) 5S RNA with a high specific activity were hybridized in situ in competition with an excess of 18S and 25S rRNA to eliminate the risk of erroneous localizations. As expected, the DNA sequences complementary to 5.8S RNA were found to be localized in the same regions which were previously shown to contain ribosomal cistrons (Avanzi et al. in Chromosoma, pp. 191-203, Vol. 39, 1972). DNA sequences complementary to 5S RNA have shown a multi-site distribution (probably four major sites) (Avanzi, Cionini, Cremonini, D'Amato, Durante).

To study the physiological role of the suspensor during embryogenesis, gibberellins, auxins and growth inhibitors were extracted from embryos and suspensors of P. coccineus at the heart-shaped and the cotyledonary stage. It was shown that the suspensor of heart-shaped embryos contains a high level of gibberellin-like substances, which undergoes a dramatic reduction in transition to the cotyledonary stage, indicating a major role of gibberellins in embryogenesis in P. coccineus (Alpi et al. 1975). That some GA<sub>3</sub> concentrations can completely replace the suspensor has been demonstrated by experiments on the in vitro culture of intact embryos and embryos deprived of suspensor (Alpi, Bennici, Cionini, D'Amato).

It has been found that the most proximal portion of the suspensor, consisting of nuclei with a low degree of endopolyploidy, can be induced to form a callus when excised embryos are transplanted on a hormone-free solid medium containing 120g/l sucrose and kept in culture under continuous light (Bennici, Cionini).

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- 4) Buiatti M., Baroncelli S. and Bennici A. (1974). Genetics of growth and differentiation in vitro of Brassica oleracea var. botrytis. IV. Genotype-hormone interactions. Z. Pflanzenzüchtg. 73, 298-302.
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Vertragspartner der Kommission: Prof. Dr. J. Reinert  
Inst. f. Pflanzenphysiologie  
u. Zellbiologie, F.U.B.

Nr. des Vertrags: 117-72-1 BIO D

Leiter der Forschungsgruppe: Prof. Dr. J. Reinert

Allgemeines Thema des Vertrags: "Growth and differentiation  
of cells and plant tissues  
growing in vitro."

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During 1975 investigations were continued on the following aspects:

- 1)
  - A. Factors influencing the yield of haploid tobacco (*Nicotiana tabacum* cv. Badischer Burley) plants by anther culture.
  - B. Factors controlling the induction and yield of androgenesis in cultures of isolated microspores.
  - C. Study of the genetic stability of haploid tissues and cell culture of tobacco and *Atropa belladonna*.
  
- 2)
  - A. Isolation, culture and induction of embryogenesis in protoplasts of *Atropa belladonna* and *Daucus carota*.
  - B. Isolation and culture of haploid protoplasts from *Lycopersicon peruvianum* and *Atropa belladonna* pollen tetrads and mesophyll protoplasts from haploid plants of *Nicotiana tabacum* and *Atropa belladonna*.
  - C. Protoplast fusion and somatic hybridisation studies on various plant species.

Ergebnisse des Projekts Nr. 1

Leiter des Projekts und wissenschaftliche Mitarbeiter:  
Prof. Dr. J. Reinert, Dr. Y.P.S. Bajaj; E. Heberle;  
A. Gröbler; S. Sorvari.

Titel des Projekts: "Production of haploid embryos  
from anthers and cell cultures growing in vitro."

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1 A Factors influencing the yield of haploid tobacco (*Nicotiana tabacum* cv. Badischer Burley) plants by anther culture.

A high yield and a method for reproducible production of haploid plants is highly desirable for various purposes in plant breeding. Based on earlier results (1974) on the effect of charcoal and cold treatment, various other factors have been investigated in more details.

Addition of various aminoacids and chelating agents as citrate and EDDHA did not show any enhancing effect in the production of haploid plants by the anthers. Cold treatment is supposed to increase the number of two similar "vegetative - type nuclei". This effect could not be observed in the used cultivar, but after a cold treatment of 3 days at 5°C and 7 days of culture at 28°C the number of viable units was increased to 80% compared to 30% in the control.

B Factors controlling the induction and yield of androgenesis in cultures of isolated microspores.

Androgenesis in cultures of isolated microspores can be subject to considerable variations. Therefore various endogenous and exogenous factors have been studied in order to establish a high reproducibility. In these experiments it turned out that the optimal density of the microspores in suspension was  $3 \text{ m } 10^4/\text{ml}$  and that the yield of haploid plantlets could be increased from 0.03% to 0.2% by raising the sugar (sucrose) concentration in the media up to 8%. Experiments on the separation of "induced" microspores from "non-induced" and dead ones were up to now partly successful.



C Study of the genetic stability of haploid tissues and cell culture of tobacco and Atropa belladonna

Haploid cells are known to be genetically unstable. Experiments have been conducted to maintain the haploid level of the cells and to study the factors causing instability. The effect of a stabilizing substance, p-Fluorophenylalanin (PFP) was tested on haploid and diploid tissue of *Atropa belladonna* and *Nicotiana tabacum*. The addition of PFP in appropriate concentrations showed different effects to the different plants and origin of material. Haploid pith segments as well as callus cultures showed a stimulation of growth on agar medium supplemented with 15 and 20 mg/l PFP during 3 weeks of culture, whereas diploid pith segments and callus cultures remained unaffected and were inhibited respectively. *Atropa* callus cultures reacted similarly. PFP at 20mg/l showed a clear-cut reduction of endomitosis by haploid and diploid cell cultures. The experiments were conducted by measuring the DNA content using microspectrophotometric techniques after a culture period of 3 weeks.

Ergebnisse des Projekt Nr. 2

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Prof. Dr. J. Reinert; G. Gosch; Dr. Y.P.S. Bajaj;  
M. Ottma; A. Weber.

Titel des Projekts: "Production and fusion of  
protoplasts from cells growing in vivo and in vitro."

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2 A Isolation, culture and induction of embryogenesis in  
protoplasts of Atropa belladonna and Daucus carota.

The successful work with protoplasts of *Daucus carota* in 1974 could be extended to another species. i.e. *Atropa belladonna* cv. *lutea* Döll cell cultures. Isolated protoplasts obtained from actively growing cell suspensions of *Atropa belladonna* have been induced to divide into masses of callus and undergo embryogenesis.

About 80-90% of the cells yielded protoplasts when incubated in enzyme solution, consisting of 1% cellulase Onozuka R10, 0.5% macerozyme R10 and 11% mannitol for 5 hours at 30°C. The protoplasts were purified from debris by filtration through a 40 µm nylon mesh, followed by floating on 15% sucrose solution. The protoplasts, cultured in synthetic liquid medium, regenerated cell walls within 2 days followed by repeated divisions after 3 days of culture. During 2 weeks 56% of the protoplasts had formed small colonies, which grew into masses of callus. The regenerated calli when transferred to an auxin free medium underwent embryogenesis in 3-4 weeks, and eventually regenerated plantlets.

B Isolation and culture of haploid protoplasts from *Lycopersicum peruvianum* and *Atropa belladonna* pollen tetrades and mesophyll protoplasts from haploid plants of *Nicotiana tabacum* and *Atropa belladonna*.

Haploid protoplasts were isolated from pollen tetrades of *Lycopersicum peruvianum* and *Atropa belladonna*, as well as from haploid plants of *Nicotiana tabacum* and *Atropa belladonna*, obtained by anther culture.

The isolated mesophyll protoplasts were plated on agar medium as well as into thin layers of liquid medium, they regenerated cell walls within 2 days divided repeatedly and formed colonies. Tobacco protoplasts showed a plating efficiency of 60%. Clones obtained from single protoplasts were induced to undergo morphogenesis and the regenerated plants are being employed for cytological studies.

C Protoplast fusion and somatic hybridisation studies on various plant species.

It is a basic requirement for the culture of somatic heterokaryons, that the method of fusion yields in a high rate of heterokaryocytes and on the other hand does not adversely effect the viability of the protoplasts. For the evaluation of the efficiency of various methods of fusion, protoplasts from cell suspensions and mesophyll cells of *Atropa belladonna*, *Daucus carota*, *Nicotiana tabacum* and *Petunia hybrida* were used. The different colour (red, white, green) of the protoplasts was used as a visual marker; and in addition, differential staining of the nuclei was employed as a criterion for the identification of heterokaryons.

With sodium nitrate a low fusion value of 1.5% was obtained as compared to 5.5% with the  $\text{CaCl}_2$  - high pH method. PEG, however, was found to be superior, with increased frequency of adhesion, tight agglutination, 17% fusion and an optimal value of 8% of heterokaryon formation of all viable protoplasts.

The protoplasts from mesophyll cells and the callus cell suspensions exhibited differential sensitivity to the PEG treatment. Whereas the carrot and *Atropa* protoplasts remained unaffected when they were treated with 25% PEG (final concentration), the mesophyll protoplasts were distorted and bursted at this concentration. In order to obtain protoplasts with a high viability the PEG solution had to be diluted slowly.

The protoplasts were fused by the microdrop technique, and cultured in thin layers of liquid or on agar medium. Heterokaryocytes, which were formed, assumed a round shape and could be identified by their colour. They regenerated walls after 3 days and occasionally they divided. Experiments concerning growth and the ability for plant regeneration of the heterokaryons are in progress.

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*Naturwiss.* 62, 327-339.
3. Gosch, G.; Y.P.S. Bajaj and J. Reinert, 1975: Isolation, culture and fusion studies on protoplasts from different species. *Protoplasma* 85, 327-336.
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Associato della Commissione: Comitato Nazionale  
per l'Energia Nucleare, Laboratorio Applicazioni  
Agricoltura.

N° del contratto : Euratom-Cnen 107-72-1 B101

Capo del Gruppo di ricerca : Prof. T.Cervigni

Tema generale del contratto : Applications of the  
in vitro cultures to radiobiological researches  
and mutagenesis of higher plants.

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The researches carried out in 1975 were  
principally oriented in three directions:

1) The use of in vitro culture techniques in  
relation to self-incompatibility problems.

2) The research at the macromolecular level  
of the modifications induced by in vitro culture  
in different plant tissues.

3) Genetical studies on isogenic lines of  
tobacco derived from anther cultures and muta-  
genic treatments of the microspores from a diploid  
isogenic line.

Risultati del progetto n. 1

Capo del progetto e collaboratori scientifici :

M.Devreux, U.Laneri, K.Sree Ramulu, P.De Martinis,<sup>⊗</sup>  
M.R.Celestre<sup>⊗</sup>.

Titolo del progetto: The use of in vitro culture techniques in relation to self-incompatibility problems.

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1.1. Eight self-incompatible diploid and two self-compatible tetraploid genotypes of Lycopersicum peruvianum were used for in vitro culture of anthers and stem internodes. The results showed that the genotypes responded differently in respect of callus induction and regeneration. Cytological analyses revealed that among the 82 regenerated plants, there were not only diploid and tetraploid plants, but also cytochimeric plants with respect to the ploidy of  $L_1$ ,  $L_2$  and  $L_3$  layers showing both periclinal and mericlinal types of chimeras. Genetic analyses of diploid and tetraploid plants coming from anther cultures, using self-incompatibility locus as a marker gene, indicated that at least 51 plants out of 52 plants derive from somatic tissue of the anthers. Cytophotometric analyses of DNA content in the nuclei, isolated from the calli derived by anther cultures, showed the occurrence of different ploidy levels; with the increase in the age of cultures, there was a clear increase in the ploidy level of cells. Analyses also showed that there was an influence of the composition of the medium, particularly the ratio between kinetin and NAA, on the relative proportion of diploid and polyploid cells. The results further indicated that the material after culturing for certain time on one medium might get stabilized. From these results, it appears that the presence of cells with different ploidy

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⊗ At no cost for the contract.

level in the calli support the possibility that two or three heteroploid cells lead to the formation of chimerism in the regenerated plants.

1.2. Attempts were made utilizing the periclinal chimeras of constitution  $4n-2n-2n$  and  $2n-4n-4n$  to ascertain the ontogenetic origin of the in vitro regenerated plants from stem internode cultures. The analyses were carried out on the size measurements of stomata and pollen grains and on chromosome counts in PMC's and root meristems representing respectively the three ontogenetic layers  $L_1$ ,  $L_2$  and  $L_3$ . The data show that all the plants regenerated from periclinal chimera of constitution  $2n-4n-4n$  had tetraploid  $L_1$ ,  $L_2$  and  $L_3$  layers in all the shoots. On the other hand, the plants regenerated from periclinal chimera with  $4n-2n-2n$  show diploid  $L_1$ ,  $L_2$  and  $L_3$  layers in all the shoots. Thus, the results reveal that the in vitro regenerated plants originate from the inner layers  $L_2$  or  $L_3$ . Genetic analyses were carried out by selfing as well as by crossing with the original mother clone in two ways in order to ascertain if any changes have occurred at the S-locus in the regenerated plants. All the regenerated plants, diploid and tetraploid, were as expected in the case of normal diploids and tetraploids, i.e., self-incompatible and self-compatible respectively; the results of two-way crosses were also as expected. Only one plant coming from periclinal chimera  $4n-2n-2n$  showed some fruit and seed-set upon self-pollination and also gave rather a high number of seeds when crossed as pistillate parent with the mother clone. Further studies are being made with the progeny to know the type of change at the S-locus in this plant.

1.3. The in vitro culture of tissues coming from different self-incompatible genotypes of L.peruvianum was pur-

sued in order to constitute a "bank of tissues" characterized by known S-alleles. We are trying to regenerate plantlets from these tissues with the aim to find out if some changes could occur at the S-locus during a prolonged in vitro culture.

1.4. With regard to the biological screening method for the mutated pollen grains at the S-locus, the in vitro culture of excised pistil which was pollinated with irradiated pollen carrying the same S-alleles as in the diploid tissue of the style was pursued with Oenothera organensis. The irradiation applied on whole plants were this year of 400, 800, 1200 and 2400 R of gamma rays, with an exposure rate of 80 R/min. The results showed that, with these irradiation conditions, only the flower having overcome the first haploid mitosis remain attached on the plant and among them, during the successive developmental stages of the binucleate male gametophyte, it appears clear differences in the radiosensitivity of the S-locus in the vegetative nucleus. At a stage which seems corresponding to the end of the  $G_1$  stage of the nuclei, many pollen tubes are able to pass through the style without any stop, (till 2.68 pollen tubes per style for flower buds irradiated at 32 mm length while the unirradiated pollen gave 0.13 pollen tubes per style). All the attempts to obtain seeds in vivo from flowers pollinated with irradiated pollen and maintained on the plants failed.



Risultati del progetto n. 2

Capo del progetto e collaboratori scientifici:

A. Brunori, G. Ancora, K. Sree Ramulu, P. De Martinis,<sup>\*</sup>  
M. Devreux.

Titolo del progetto: The research at the macromolecular level of the modification induced by *in vitro* culture in different plant tissues.

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2.1. Some attempts were made in order to produce genetic transformation in higher plants. DNA was extracted from a line of Pea characterized by several dominant alleles and experimentally given to the seedlings of the corresponding recessive line. No positive results were obtained till now and this research will be pursued.

2.2. The analysis of the DNA synthesis during the process of cell dedifferentiation in an explant of tobacco tissue cultured *in vitro* was continued (see report of 1974). The data so far obtained indicated that the dedifferentiated process in this species does not involve the synthesis of particular components of the genome (DNA satellite). Contrary to what reported in other species, all the genome is regularly replicate when the DNA synthesis is reinitiated.

2.3. With the aim to studying the influence of the culture medium, duration of culturing and genotypic constitution of the materials on *in vitro* callus induction and plant regeneration, investigations were carried out by performing anther cultures in two different genotypes, namely  $S_{12}S_{13}$  and  $S_1S_3$ , and stem internode cultures in other two genotypes, namely  $S_4S_5$  and  $S_1S_5$ , of Lycopersicum peruvianum.

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\* At no cost for the contract.

In stem internode cultures, it was observed that with the same culture conditions, the two genotypes responded very differently: whereas in  $S_1S_5$  there was good and rapid callus induction and abundant plant regeneration, there was very poor and late callus formation and very slow plant regeneration in  $S_4S_5$ . In  $S_1S_5$  the cytophotometric analyses of DNA content showed that there were no differences in composition of the different classes of ploidy between the calli cultured on the same medium with or without kinetin.

For anther culture, differences were observed between  $S_1S_3$  and  $S_{12}S_{13}$  with respect to their responses to callus induction medium and plant regeneration medium. In both the genotypes, two types of calli were found of which one grows rapidly and appeared earlier whereas the other grows very slowly and formed later. Cytophotometric analysis of DNA content in the nuclei of both these calli showed that the first appearing calli are more polyploid. In addition, it was confirmed from the present analysis that with the increasing duration of culture on the medium containing high concentration of kinetin, there was an increase in the ploidy level of cells. Further analyses are being carried out.

2.4. To study the variation of DNA content in the nuclei isolated during one cycle of culture in the callus of L. peruvianum, maintained since several transfers in L.S. medium, cytophotometric analyses were made at different intervals of time up to 54 days since the last transfer. The results showed that during the period of culture, there was no significant difference in the ratio of different classes of ploidy. In this respect, it seems that this callus can be analysed at any time during its development.

It is also true with the calli in which even very few mitoses are present. In addition, the analyses of DNA contents of mitoses showed that there is a constant relationship between the distribution of the classes of the ploidy of nuclei and the ploidy level of mitoses.

Risultati del progetto n. 3

Capo del progetto e collaboratori scientifici:

M.Devreux, U.Laneri, K.Sree Ramulu, S.Marchetti<sup>‡</sup>,  
R.Chirila<sup>‡</sup>, E.Gacek<sup>‡</sup>.

Titolo del progetto : Haploid induction, genetical studies  
on homozygous lines derived from anther cultures and  
mutagenic treatments of the microspores.

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3.1. With the aim to induce the regeneration of plantlets from anthers with any doubt about their origin, free microspore cultures were performed with different species: Datura innoxia, Nicotiana tabacum, Lycopersicum peruvianum and Capsicum annuum following the technique recommended by C.Nitsch. Positive results were obtained till now with Datura free microspores from which a very large number of haploid embryoids were obtained. In our anther cultures, the positive effect of the charcoal added in the culture medium was confirmed as increasing the number of haploid plantlets in liquid and solid media.

3.2. Irradiation treatments were carried out on anthers of Datura just before culturing the free microspores in order to evaluate the radiosensitivity of this material. Also after irradiation, numerous haploid embryoids were obtained but the plantlet development was very abnormal due to some hormonal unbalance.

3.3. Attempts to induce haploids in Triticale and in Capsicum annuum are performed and callus induction media

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<sup>‡</sup> At no cost for the contract.

have been defined. With Capsicum, several genotypes and various culture conditions were tried, large variations were observed in their responses.

3.4. In pearl millet (Pennisetum typhoides), anther cultures were performed using so far eleven different media which have previously adopted in various crop species of Gramineae and with other modifications concerning composition of the media and cultural conditions; but up to now, there was no callus induction with any of these media and attempts are being continued.

Embryo culture of mature seeds was undertaken in pearl millet using the medium of Cheng and Smith (1975) modified in sucrose and kinetin composition. Good and rapid callus development was obtained from very young seedlings and, transferred after 20 days to freshly prepared medium, these calli developed so fast that their size was almost doubled after one week. Attempts are being made, utilizing these calli, to prepare cell suspension cultures on one hand, and to obtain regeneration on the other.

In rye (Secale cereale) embryo culture of mature seeds was undertaken with the same media adopted for pearl millet; the callus formation is however lesser and slower.

PUBLICATIONS

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Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung mbH., München

Nummer des Vertrages: 118-72-1 BIO D

Leiter der Forschungsgruppe:

Prof. Dr. H. Gaul, Leiter der Abteilung für Pflanzengenetik

Allgemeines Thema des Vertrages:

Cell culture genetics in barley, wheat, potato and maize

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For practical plant breeding the production of haploid plants from anthers, as compared to cross breeding, has the following advantages: Mutants can be detected in the  $M_1$  generation; the  $F_1$  hybrids are homozygote immediately after diploidization; new varieties can be obtained in considerably shorter time.

Experiments with barley anthers were carried out with the primary intention of inducing callus growth. The resulting calluses should then be regenerated into whole plants. The experimental series conducted so far varied in their culture conditions, i.e. temperature, light, and composition of the culture medium.

## Ergebnisse des Projekts

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Prof. Dr. H. Gaul  
Dr. B. Foroughi-Wehr

Titel des Projekts:

Development of techniques for the production of haploid plants through the use of anther culture.

Darstellung der Ergebnisse:

Pollen development has been investigated from the formation of tetrad up to the two nuclei stage in vitro and in vivo. CLAPHAM (1971) reported that cultured anthers with pollen in these developmental stages are most productive with regard to callus formation. The pollen develop in vivo is shown in Figure 1. We were able to determine six different stages of pollen development. At the end of pollen meiosis four young microspores form a tetrad (stage 1). Each nucleus is half the size of the cell. The tetrad falls apart and each cell forms a round microspore (stage 2). Each microspore grows almost to the size of a tetrad and begins to develop a thick wall (stage 3). The nucleus moves to the periphery of the cell and at the opposite side a vacuole starts to develop (stage 4). The big vacuole pushes the nucleus against the wall (stage 5). The microspore undergoes the first mitotic division (stage 6).

We included nineteen varieties of Hordeum vulgare in our investigation and all of them showed a similar microspore development, but some abnormal arrangements were observed in several varieties.

These consisted of (a) different arrangement of the four microspores of the tetrad, (b) more than one nucleus per microspore, (c) abnormally enlarged microspore in stage 2 to 4.

Our experiments indicate that these abnormalities are caused by environmental factors, and are emphasized by the differences between varieties. In vitro the microspores developed to mature starch-filled pollen grains or ruptured and lost their cytoplasmic contents. Sometimes it was possible to see intact microspores after several months in culture.

In our studies on anther culture microspores of all the stages 1 to 6 were able to produce callus. Mostly callus formation occurred when the microspores were in stages 3 or 4.

The investigation with Hordeum vulgare were continued. Up to now ca. 70 000 anthers of different barley varieties have been cultured using many different media.

The investigations show that anthers of all the varieties are able to produce callus, but there appear to be differences between the varieties.

Thus the variety Dissa gave the best results with an anther response of 14 %, while e.g. less than 1 % of the anthers of the variety Milu were responsive. Callus tissues of five varieties differentiated green and white plants, while those of ten more varieties produced only albinas. Typically when the plants were white adventitious root development was very poor but tillering was extensive, while the green plants usually showed relatively vigorous root growth and few tillers.

The callus tissues derived from 42 different barley anthers were able to differentiate green plants. Some of the green plants are already potted and are tillering. The cytological studies of the root tips of both green and white plantlets made at an early stage in their development revealed, in all cases, cells with the haploid chromosome complement as well as a number of aneuploid cells.

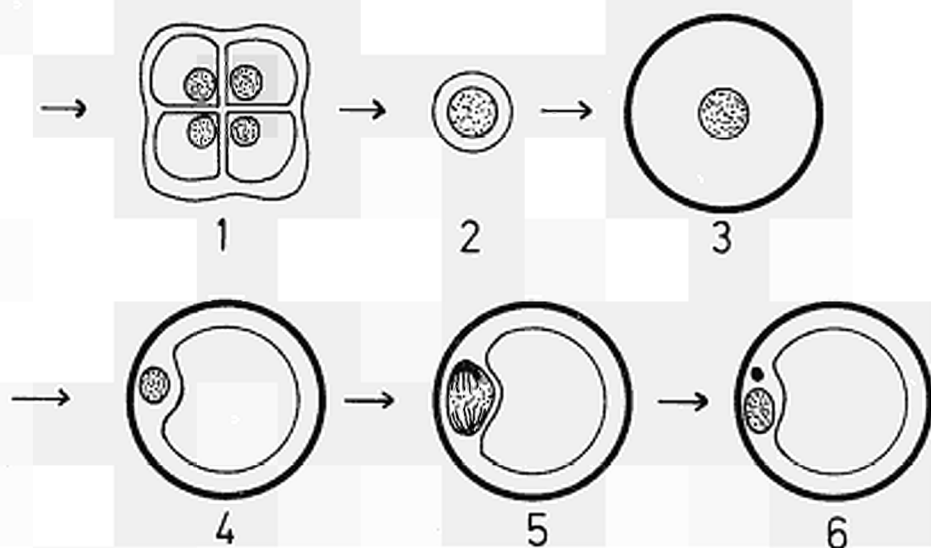


Fig.1

Schematic representation of the six pollen grain stages

Publications 1975

BUIATTI, M.; B. FOROUGHI-WEHR; S. BARONCELLI; B. GIORGI;  
G. MIX; H. GAUL:

Genetic Control of Callus Growth in Hexaploid Wheat  
Experientia, in press

FOROUGHI-WEHR, B. u. G. MIX:

Der Einfluß von Außenfaktoren auf die Kallusbildung  
von Hordeum vulgare-Antheren

Arbeitstagung für Genetik der GSF, April 1975  
GSF-Bericht B 626; P. 21-22

FOROUGHI-WEHR, B. u. G. MIX:

Zell- und Gewebekulturen

GSF Jahresbericht 1974, P. 76-80

GAUL, H.; G. MIX; B. FOROUGHI-WEHR; M. OKAMOTO:

Pollen Grain Development of Hordeum vulgare

Z.f.Pflanzenzüchtung; in press

MIX, G.; B. FOROUGHI-WEHR; H. GAUL:

Pollen development to the binucleate stage of  
different barley varieties

Third Int. Barley Genetics Symposium, 7.-12. July  
1975, Munich-Garching

Proceedings of the Third International Barley  
Genetics Symposium; in press

OKAMOTO, M.; H. GAUL:

Quartet abnormalities of a cultivar of six-rowed  
barley, Asse

Pollen Science No. 8 1975, P. 1-5

OKAMOTO, M.; H. GAUL:

Abnormalities of Pollen Tetrads in Asse, a four-  
rowed cultivar of Hordeum vulgare

Third Int. Barley Genetics Symposium; 7.-12. July  
1975, Munich-Garching

Proceedings of the Third International Barley Genetics  
Symposium; in press



RADIOENTOMOLOGIE

RADIOENTOMOLOGY

RADIOENTOMOLOGIE





Vertragspartner der Kommission: Freistaat Bayern, vertreten durch die  
Bayerische Landesanstalt für Boden-  
kultur und Pflanzenbau, München  
Bundesrepublik Deutschland

Nr. des Vertrags: 114 - 72 - 1 BIO D

Leiter der Forschungsgruppe: Dr. Albert Haisch

Allgemeines Thema des Vertrags: Ökologie, Aufzucht und Sterilisation  
der Kirschenfliege (Rhagoletis cerasi L.)

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Rearing, sterilization and ecology are the three main topics of the research project the goal of which is to test the applicability of the insect sterilization technique to control the European cherry fruit fly (*Rhagoletis cerasi* L.) under the special circumstances of the cherry cultivation conditions of South Germany.

The main problem of the artificial mass production of the European cherry fruit fly is the difficulty to raise the larvae. A pupal diapause lasting half a year is added to the difficulty of rearing the larvae due to the delayed development. Finally it has to be mentioned that the way of egg collection is still insufficient. The technique used up to now is too time consuming and needs further improvement.

The point of the radiobiological work is to diminish the somatic damages caused by the sterilizing radiation dose.

The ecological work deals with the flying behaviour of the European cherry fruit fly, because the size of the living area of one population and the frequency of migrations between two different population areas influence the practical feasibility of the insect sterilization technique. With other words it has to be checked if there exist isolated population areas despite of absent geographical borders of a population.

Ergebnisse des Projekts Nr. 1

Leiter des Projekts:

Dr. Albert Haisch

Titel des Projekts:

Laboratoriums- und Massenzucht der  
Kirschenfliege (Rhagoletis cerasi L)

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68 different mixtures of larval substrates have been tested. 140.588 larvae have been used for that. They brought 14 % pupae on the average. This result includes also the raising results unfavourable only due to special experimental designs. This rearing results from standard substrates showed the actual stage of development. 22 % of 23.378 larvae put on standard substrates pupated, with confidential limits of 9 up to 35 %. With these tests a series of experiments lasting several years is going to be finished, the sense of which was to improve the faults of the feeding substrate concerning its nutritive and physical quality. The latter one apparently proved to be favourable if paper pulp together with weed brans were used. Also the conservation of the feeding substrate by propionic acid and by formaldehyd yielded good results. However, the mean rearing result remained unsatisfying. No change of the mixture of the feeding substrate concerning the components carbohydrates proteins stuffs with vitamine like character and minerals improved permanently the yield of pupae. Therefore, it may be concluded that the larvae have been damaged already by the way of egg collection or by the treatment of the neonate larvae. This suggestion is confirmed by the observation that the main larval mortality occurs during the first instar. First experiments to eliminate such detrimental treatment of young larvae gave promising results.

Our knowledge on the induction mechanism of the diapause in strictly univoltine species as for instance in the European cherry fruit fly is unlike to the multivoltine species with a facultative diapause very poor. Up to now no photoperiods applied to adults, eggs and larvae effective in preventing the diapause could be found. This lasts about six months. Its intensity can slightly be influenced by changing the temperature during the dormant stage (HAISCH 1975 a). It could also be showed that the diapause character of population from climatically different regions is variable (HAISCH und FORSTER 1975). Further low temperatures (15°C) at the time of puparium formation weaken significantly the diapause intensity. These observations indicate apparently that the dormancy of the cherry fruit fly is not unchangeably fixed.

## Ergebnisse des Projekts Nr. 2

Leiter des Projekts: Dr. Albert Haisch  
Titel des Projekts: Ökologie und Sterilisierung der  
Kirschenfliege (*Rhagoletis cerasi* L.)

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### 1. Irradiation

The cherry fruit fly becomes unable to propagate after irradiation with a dose of 9 kr. The vitality of these flies is diminished. In a field experiment sterile flies of both sexes copulated less than the untreated control flies did. A cage experiment in the laboratory confirmed this result.

A further feature of radiation symptom is the lowered flight disposition of these flies, how it could be shown by special equipment (HAISCH et al. 1975 a). On the average of 5 independent replications the flying propensity of irradiated pairs fell from one to  $0.77 \pm 7.5\%$  in a statistically significant way. Also the stage of radiation is of importance. If pupae and adults are irradiated the flying propensity of the first ones is  $0.9 \pm 8\%$  compared with that flies irradiated as adults. The radiation has a stronger impact on the flight disposition of the females than on the males. The female flew only three thirds of that of the males did.

### 2. Ecology and distribution

The releasing of labelled flies in the field and there recapture showed that under the special experimental circumstances the released flies did not leave the 500 m range. A small portion of the flies could be caught in a farer distance from the release point and that soon after releasing. The direction of distribution was influenced by trees and by the prevailing wind direction.

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### Literature:

- HAISCH, A. 1975 a: Zur Puppendiapause der Kirschenfliege, *Rhagoletis cerasi* L. I. Beeinflussung der diapausierenden Puppen durch unterschiedliche Temperaturen und verschieden lange Kälteexpositionen. Z. ang. Ent. 79, 1 - 11
- 1975: Raising and multiplying of the European Cherry fruit fly, *Rhagoletis cerasi* L. CONTROLLING FRUIT FLIES BY THE STERILE-INSECT TECHNIQUE pp. 120 - 121, IAEA, Vienna

- 1975: Observations on the flying behaviour of the European Cherry fruit fly (*Rhagoletis cerasi* L.) STERILITY PRINCIPLE FOR INSECT CONTROL 1974 pp. 191 - 198, IAEA, Vienna
- u. S. Forster 1975: Zur herkunftsspezifischen Diapause-Ausprägung der Europäischen Kirschenfruchtfliege, *Rhagoletis cerasi* L. (Diptera Trypetidae)  
Entomologica Germanica Dezember
- , H. Stärk u. S. Forster 1975: Markierung von Fruchtfliegen und ihre Erkennung durch Indikatoraktivierung  
Ent. exp. appl. 18, 31 - 43
- , S. Forster u, I. Kamm 1975 a: Experimentelle Bestimmung der Flugdisposition von Kirschenfliegen, *Rhagoletis cerasi* L.  
Anzeiger für Schädlingskunde, Dezember

Associato alla Commissione: Istituto di Entomologia agraria della Università di Padova.

N° del contratto 105-72-1 BIOI

Capo del gruppo di ricerca: Prof. Sergio Zangheri

Tema generale del contratto: Ricerche sulle metodologie e le tecniche per l'allevamento in laboratorio del Dacus oleae Gmel. e di altri insetti di interesse agrario in vista della produzione in massa e ricerche radioentomologiche e biologiche, con particolare riguardo ai ditteri Tripetidi.

General Theme of the contract: Researches on methods and techniques for laboratory rearing of Dacus oleae Gmel. and other insects of agricultural interest for mass-production and radioentomologic and biologic researches with particular regard to fruitflies.

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A brief outline of the work carried out in 1975.

In the picture of the researches relative to Project No. 1, through an investigation into the symbiotic aspects of the family of the Tripetides, it has been possible to observe that the role carried out by the symbiotic bacteria on the larval growth may be secondary in respect to the action exercised on the vitality of adults.

In these last, endocellular bacterial colonies have frequently been observed, as a further support of the hypothesis that the cycle of transmission of the hereditary symbiosis of Tripetides may be more complex than hitherto known.

In relation to Project No. 2, to the end of arriving at the isolation of the chemical principles which stimulate the oviposition of the D.oleae, the researches have been directed towards the purification of the enzymes interested in the biochemistry of oleoeuropine. Interesting technical innovations have been directed to the artificial media of the phytophagous Lepidoptera.

The investigations have reached conclusion, relative to Project No. 3, on the dynamics and on the factors limiting development of the population of D. oleae in the area of the lake of Garda.

As the investigations were carried out at the northern limit of distribution of the species the new acquisitions can constitute the basis for further investigations in more temperate areas.

In the ambit of Project No. 4 tests of radio-sensitivity have been carried out on Coleoptera harmful to stored products by means of rigorous parameters of valuation.

For the first time the radiosensitivity of the Cryptolestes turcicus has been investigated and the researches have been extended also to insects of agrarian interest.

Interesting observations have finally been carried out on the consumptions of wheat treated with differing doses of gamma radiation by means of the Sitophilus granarius.

As regards Project No. 5, researches have been carried out on the use of sub-sterilizing doses on the Ceratitis capitata, with the end also of inducing chromosomic translocations.

Fast neutrons have been shown to be more efficacious than gamma radiations, increasing, among other results, the competitiveness of sterilized males.

Investigations have also been carried out on D. oleae in nature, on the survival, predation and periods of appearance of that species. Interesting differences in behaviour have been encountered between the more temperate areas near the sea and the hill-side olive groves.

Finally a continuous mathematical model has been set up for the population dynamics of polyvoltine insects.

Risultati del progetto n. 1

Capo del progetto e collaboratori scientifici: Prof. S. Zangheri, Prof. L. Masutti, Dott. V. Girolami.

Titolo del progetto: Influenza dei batteri simbiotici sul ciclo vitale del Dacus oleae Gmel.

The influence of symbiont bacteria on life cycle of Dacus oleae Gmel.

(Researches made in 1975)

The poor vitality of D. oleae adults, which grow on artificial media, is likely due to the loss of symbiotic bacteria.

No strict evidence has so far been reached on this matter as it has been impossible to multiply microorganisms in vitro and restore symbiosis.

Much evidence however has been found which supports that the difficulties met rearing D. oleae are connected with symbiosis interruption. One is the observation that C. capitata, which is easily reared, does not lose its symbiotic microorganisms. Other fruit flies on the contrary - as R. cerasi and pomonella - which are not fit for rearing, also lose their symbiotic bacteria when they are reared on artificial media.

The complex relations binding fruit-flies to their symbionts therefore involve problems in the applied field, so it seems interesting to show some aspects of the symbiosis of the whole fruit-fly family, partly pointed out in the course of recent investigations.

One first observation may ensue from the consideration that bacterial colonies are not often found in larval stages (for instance in Tephritinae), in the adult stage, on the contrary, examined species seem without symbionts only in the little tribe of the Euribiini (genus Euribia or Urophora) together with a few species of Tephritinae as E. sonchi and X. miliaria.

Therefore the presence of bacterial colonies in the adults cannot be only considered as a permanence in order to warrant the transmission of symbionts to the larvae, for the growth of which they seem necessary.

On the contrary we may reasonably suppose that the presence of bacteria has an essential role for the vitality of the same adults. A brief picture of the situation may be found in tab. 1.

The fact that no bacterial colonies are seen in the microscope in larvae shows that there are still unknown stages in the transmission of symbionts. In fact we can observe that symbionts regularly appear in adults - for instance in C. capitata - in spite of using intestinal disinfectants in previous larval stages. It is therefore reasonable to admit that there is - for instance in the Trypetini - an endo-cellular or introcoelomatic stage of the symbiotic bacteria. This assumption seems moreover to be supported by the fact that endocellular bacteria are regularly observed in the mesenteric cells of the Tephritinae.



Subfamilia Tribus Genus Species	Vesicle type	Symbionts inside:		
		Vesicle	Midgut	
			inside peritro- phic membr.	outside peritro- phic membr.
<u>Dacinae</u>				
<u>Dacini</u>				
<i>Dacus oleae</i> Gmel.	Dacus	x	x	
<u>Trypetinae</u>				
<u>Euribiini</u>				
<i>Euribia solstitialis</i> L.	Ensina	-	-	-
" <i>quadrifasciata</i> Meig.	"	-	-	-
" sp.	"	-	-	-
<u>Trypetini</u>				
<i>Ceratitis capitata</i> Wied.	Ceratitis	x	x	
<i>Rhagoletis cerasi</i> L.	"	x	x	
<i>Trypeta zoë</i> Meig.	"	x	x	
<i>Acidia cognata</i> Wied.	"	x	x	
<i>Philophylla heraclei</i> L.	"	x	x	
<i>Hemilaea dimidiata</i> O. Costa	"	x	x	
<i>Myolia caesio</i> Harr.	"	x	x	
<i>Cryptaciura rutundiventris</i> Fall.	"	x	x	
<u>Tephritinae</u>				
<u>Terelliini</u>				
<i>Chaetorellia jaceae</i> Rob. -Desv.	Orellia	x	x	
" <i>hexachaeta</i> Loew.	"	x	x	
<i>Terellia fuscicornis</i> Loew.	"	x	x	
<i>Orellia falcata</i> Scop.	"	x	x	
" <i>colon</i> Meig.	"	x	x	
" <i>ruficauda</i> Fabr.	"	x	x	
<u>Xyphosiini</u>				
<i>Xyphosia miliaria</i> Schr.	Ensina	-	-	-
<u>Tephritini</u>				
<i>Paroxyna punctella</i> Fall.	"			
<i>Oxyna flavipennis</i> Loew.	"			
<i>Sphenella marginata</i> Fall.	"			
<i>Ensina sonchi</i> L.	"	-	-	-
<i>Tephritis arnicae</i> L.	"			
" <i>conjuncta</i> Loew.	"			
" <i>formosa</i> Loew.	"			
<i>Trypanea amoena</i> Frfld.	"			
" <i>stellata</i> Fuessl.	"			
<i>Acanthiophilus helianthi</i> Rossi	"			
<u>Ditrichini</u>				
<i>Noëta pupillata</i> Fall.	"			

Fig. 1 - Principal types of pharyngeal vesicle and bacterial symbiosis in the adults of the family of Tripetides.

Risultati del progetto n. 2

Capo del progetto e collaboratori scientifici: Prof. S. Zangheri, Prof. L. Masutti, Dott. V. Girolami, Dott. ssa L. Panizza, Dott. ssa G. Pellizzari.

Titolo del progetto: Ricerche su substrati artificiali per gli stadi larvali del Dacus oleae Gmel. e di Lepidotteri defoliatori e sulle tecniche di allevamento.

Researches on artificial media for the larval stages of Dacus oleae Gmel. and of phytophagous Lepidoptera and on rearing techniques.

(Researches made in 1975)

The investigations into the chemical principles of the olives which stimulate the egg-laying of the D. oleae Gmelin have been directed mainly towards the purification of the enzymes relative to the biochemistry of oleoeuropine.

It has emerged from previous researches that the principles stimulating ovi-position are products of spontaneous degradation of the above-mentioned glucoside and in a greater measure of the aglucone obtained enzymatically. The enzymes of animal origin, however, do not give constant yields and therefore the purification of the above-mentioned enzyme present in the acetonic dursts of olives has been attempted.

The difficulties encountered, also because of the presence of fats and pigments, have suggested directing the investigations towards the acetonic dusts of the leaves. The researches are in course of execution.

Risultati del progetto n. 3

Capo del progetto e collaboratori scientifici: Prof. S. Zangheri, Prof. L. Masutti, Dott. V. Girolami, Dott. ssa G. Pellizzari.

Titolo del progetto: Ricerche sulle cause di mortalità del Dacus oleae Gmelin e sulla dinamica delle popolazioni in natura ed in laboratorio.

Researches on mortality causes of Dacus oleae Gmelin and on population dynamic in field and in laboratory.

(Researches made in 1975)

The researches on the population dynamics of the D.oleae Gmelin in the Garda lake area have reached a first conclusive phase and the relative publications are in course of being drafted.

As a basis for the study of the population dynamics the biological cycle has been brought to light, which furthermore, being relative to the northern limit of distribution of the species, can constitute the basis for further investigations in more temperate areas.

The first (summer) generation on the new olives of the year begins around the middle of the month of August and can be protracted for the whole of September. The second generation follows (autumnal/winter) with ovi-positions protracted as long as November. In the winter period, mature ♀♀ also being present, ovi-position ceases in that temperatures sufficiently high are not reached (17°C.).

The adults which have survived (of the second generation) start laying again at the end of February on the olives still hanging giving origin to the first 'spring' generation. Development is completed in about two months, and with the new depositions in mid-May the second spring generation begins, the adults of which lay on the new olives, closing the cycle.

The larvae that have emerged in late autumn grow during the winter as far as to transform into pupae. The latest, however, die by the beginning of February without having been able to complete their development.

From the pupae formed in autumn adults are obtained up to January. The latest and the winter ones are destined to die. Therefore, the survival of the species during the winter period is in the end left to the adult stage.

In the biotope considered the D. oleae does not generally complete all the generations in the same olive grove in that the first summer ones are observed only in the warmer areas, while the second spring generation takes place as a general rule in cooler areas where the olives remain on the trees for a long time and where the first summer generation does not appear. Finally, a third area exists, at the limit of the vegetation of the olive trees, in which is developed only the second 'autumnal' generation. The study of the population dynamics has therefore been carried out on one entire valley isolated from the contiguous olive-growing area in which the above-cited biotopes are present.

From the quantitative investigations relative to the immature stages, the following has emerged:

1. Between the first and second summer/autumn generations there is a ten-fold numerical increase of the population and the highest annual density of population (millions of individuals in an area of 4 km<sup>2</sup>) is reached.
2. During the winter the population is drastically reduced, in the order of hundreds of thousands (as a ratio between the immature autumn stages and the first 'spring' generation) to the point where the first 'spring' generation can be observed with difficulty even when it is possible to collect all the drupes present in entire olive groves.
3. In the successive spring generation the populations undergo a notable increase, (remaining however inferior to the first summer generation) for which in May a considerable presence of larvae is noted in the few olives still hanging.

In figure 1 are reported the data relative to an olive grove in which, given the particular climatic course, all the generations of D. oleae were found in 74-75.

Winter survival in such a case is relatively high given that, dif-

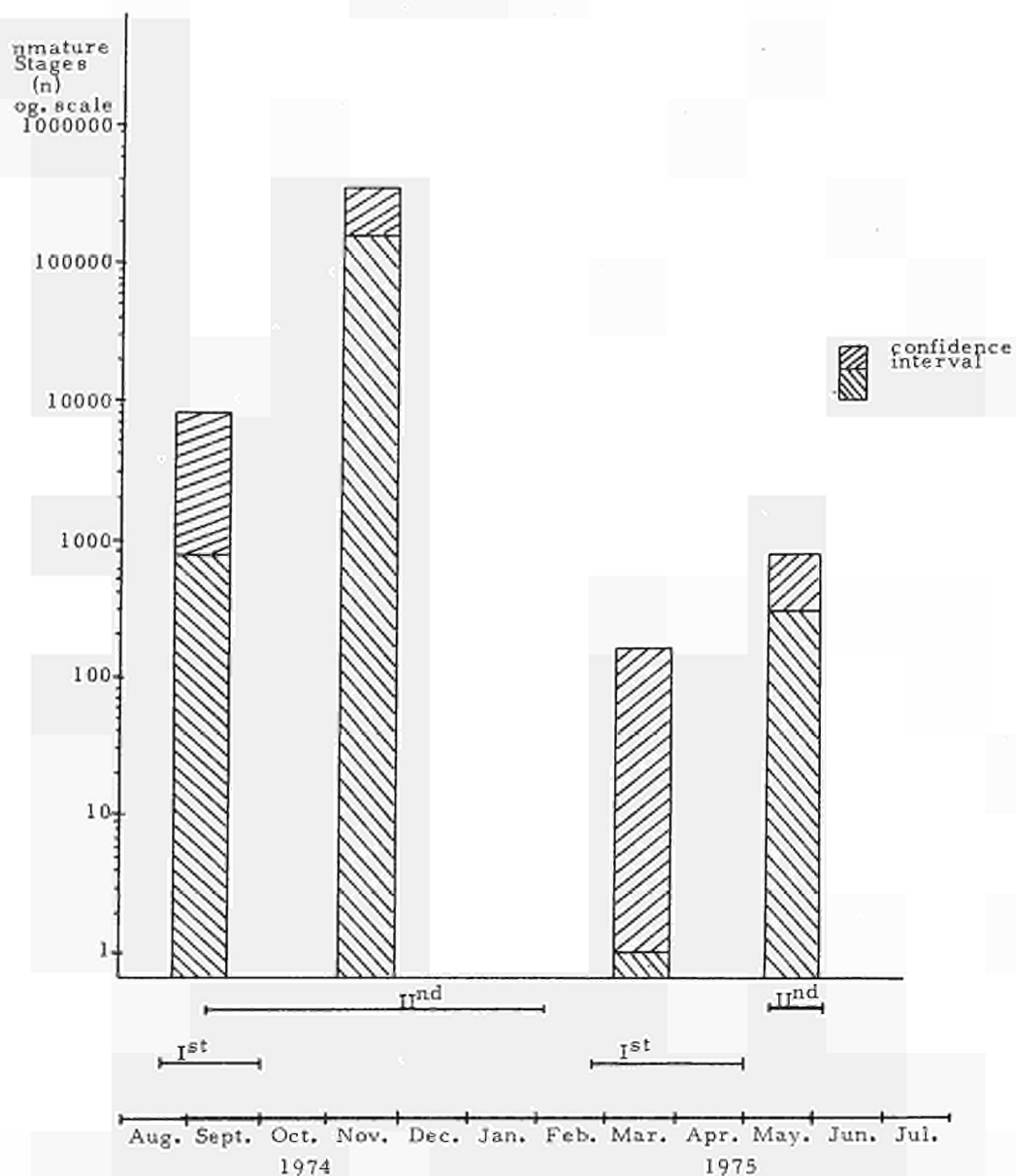


Fig. 1 - Number of immature stages present in an olive grove in correspondence to the various annual generations. The lines represent the periods in which the larvae of the various generations are present in the field.

ferently from the 2nd 'autumnal' generation, the first spring generation is absent in the majority of olive groves examined. (The data relative to the entire area, and thus comprehensive of the global valuation are in course of elaboration).

In the biotope considered, the biotic factors of limitation of the population have been relatively of little importance. The drastic reduction of the populations during the winter is linked principally with the protraction of the period of low temperature during which the adults have difficulty in finding food: in fact at least 7°C are necessary because they pick up food at 14°C, as they can look for it flying. Survival, without food, is of about twenty days at 7°C, and death overtakes them within shorter periods at temperatures both higher and lower.

An important factor of self-regulation of population of Dacus oleae has been brought to light, linked to a drastic reduction in fecundity when the olives at the disposition of the ♀♀ are already, at least in part, attached. There is not, therefore, a direct proportionality between the numbers of the adults and the eggs laid. This suggests, among other things, that, at least from a theoretical point of view, even a high mortality of the insects does not affect to any considerable degree the numerical entity of the successive generation when an appreciable level of infestation is reached.

Risultati del progetto n. 4

Capo del progetto e collaboratori scientifici; Prof. R. Cavalloro, Dott. E. Ratti.

Titolo del progetto: Effetti biologici comparati delle radiazioni gamma, neutroni e chemosterilizzanti sui diversi stadi di vita degli insetti.

Comparative biological effects of gamma radiations, neutrons and chemosterilants on different life stages of insects.

(Researches made in 1975)

A. Tests of radio-sensitivity to gamma radiations have been carried out on six species of Coeloptera, with the aim of verifying, in homogeneous conditions of environment, the real difference in radio-sensitivity manifested by the various species. As a parameter of comparison the dose which halves the time of survival by 50% of the normal population was selected ( $ST_{50}$ ) to the end of obtaining data closely linked to the average longevity of the populations not treated. Thus, it has been possible to recognize a scale of radio-sensitivity for the species examined.

Tab. 1 - Average values of four repetitions, each of 100 insects; sexes not separated.

Species	$ST_{50}$ of the population not treated	Dose which halves $ST_{50}$ of the population
<u>Sitophilus oryzae</u> (L.)	22 weeks	about 7 krad
<u>Sitophilus granarius</u> (L.)	23 "	" 7 krad
<u>Cryptolestes turcicus</u> (Grouw.)	15 "	" 8.5 krad
<u>Oryzaephilus surinamensis</u> (L.)	25 "	" 13.5 krad
<u>Rhizopertha dominica</u> (F.)	27 "	" 14 krad
<u>Stegobium paniceum</u> (L.)	12 days	" 180 krad

The parameter of comparison utilized was shown to be applicable to other orders of insects, and thus lends itself to being used to compare the diverse radio resistance manifested by diverse systematic groups of insects; this will possibly permit the clarification of the still obscure cause of the phenomenon.

Furthermore, a study has been initiated on the radiosensitivity in the various stages of life of the Cryptolestes turcicus a Cucujidae (Coleoptera) harmful to flour, not yet studied from this point of view. The work now in course has already permitted the precisation that the species is completely controlled at a dose of 12.5 krad. This dose provokes a 100% rate of mortality in two weeks and complete sterility, but also at 10 krad there is a mortality rate of a good 90% of the adults and almost complete sterility.

A preliminary research on the influence of a diet of irradiated wheat on the untreated population of Sitophilus granarius has led to interesting results (Fig. 1): the course of the experiment seems to demonstrate a progressively minor consumption of irradiated wheat in respect to that not irradiated.

B. An experiment has been conducted on the radio-sensitivity to gamma radiations of a Coleoptera Chrysomelidae harmful to the hazel tree, Altica brevicollis Foudras. Among the results obtained should be emphasized: for the eggs, complete devitalization at 1.000 rad; for larvae and pupae, the dose of 4.000 rad prevents respectively the pupation and the development into adults; for imagines a major radio-sensitivity emerged for females in respect to males. The sterilizing dose (Table 2) for females is less than 2.000 rad, for males it seems to be greater (about 4.000 rad); a residual fertility (10%) in females mated with males treated at 4.000 rad and over, is probably due to the intervention of sperm of previous matings with normal males.



Tab. 2 - Egg hatching % of A. brevicollis laid 12 days after the irradiation of irradiated adults mating with one another or with normal individuals.

DOSE (rad)	0 (test)	500	1000	2000	4000	6000	8000
$\sigma^- \times \text{♀}^N$	74%	63%	37%	24%	9%	8%	4%
$\sigma^+ \times \text{♀}^-$	74%	31%	3%	1%	0%	-	-
$\sigma^N \times \text{♀}^-$	74%	45%	13%	0%	0%	-	-

C. Another preliminary investigation regards chestnuts attacked by larvae of the Coleoptera Balaninus elephas Gyllh. and of four species of Lepidoptera (Cydia splendana Hb., C. amplana Hb., as well as two species not yet identified). As regards radio sensitivity, Balaninus was shown to be the most sensitive species, (at 10 krad there is total mortality); the Lepidoptera, on the other hand, showed a much higher resistance and only with the largest doses utilized (50 and 100 krad) was a high mortality obtained.

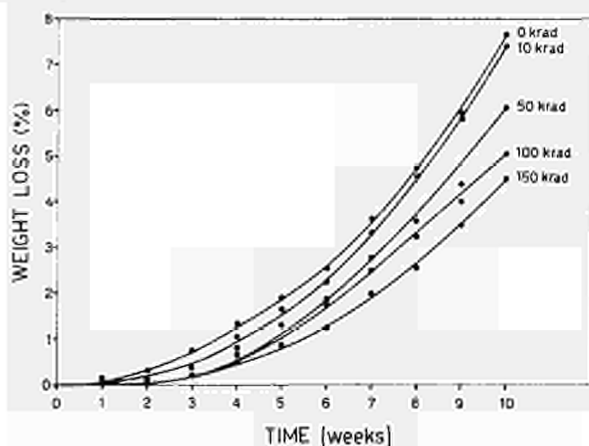


Fig. 1 - Weight loss (%) of wheat irradiated at different dose levels caused by untreated Sitophilus granarius.

Risultati del progetto n. 5

Capo del progetto e collaboratori scientifici: Prof. R. Cavalloro, Dott. E. Ratti, Dott. C. Ricci.

Titolo del progetto: Studi sul comportamento di femmine e maschi normali e sterili.

Studies on the behaviour of normal and sterile females and males.

(Researches made in 1975)

A. The investigations into the population of Dacus oleae Gmel. have been continued in certain experimental stations in an olive-growing area of Liguria. The capture of adults by means of yellow chromotropic traps has shown that, in the olive groves half way up the hills, the first adults appear in May, giving rise to a first peak of capture in the month of August, and to a second, considerably greater in the first half of October.

Checks have been effected, by means of the direct examination of samples of olives collected periodically, of the percentage of infestation of the olives and the rate of parasitisation of the larvae; the data are in course of elaboration. Furthermore, studies have been initiated on site, on the dynamics of population of Dacus, by means of the release and re-capture of adult insects marked with fluorescent pigments.

In consideration of the importance of taking pupae that have wintered in the ground for the forecast of new attacks of Dacus oleae, an investigation has been conducted in the winter period, on the horizontal and vertical distribution of the pupae in the ground, as well as on the abiotic and biotic factors influencing their survival. It has been possible to observe that in sandy silt ground over 90% of the pupae are found at a depth of from 1 - 4 cm., in stony ground, on the other hand, a part of the larvae pupate at the surface, generally in the vicinity of stones or low walls. Furthermore, it is to be emphasised that a more superficial pupation corresponds to an increase in the humidity of the ground.

As regards the spatial distribution of the pupae, this is very

heterogeneous, with an increasing pupal density little by little as one proceeds to the outer foliage of the tree, with the maximum in a southerly direction.

The following work has been published on this subject:

Cavalloro R. e Delrio G. - Osservazioni sulla distribuzione e sopravvivenza delle pupe di Dacus oleae Gmelin nel terreno. Redia, Firenze, LVI, 1975, 167-175.

Researches similar to those conducted on Dacus oleae on site have been carried out on the factors influencing the pupation of the Ceratitis capitata Wied., correlated with the emergence into butterflies and thus with the diffusion of the species and the fluctuation of population. The studies have regarded types of ground of diverse chemical composition and physical structure and with various degrees of relative humidity.

Pupation does not appear to be influenced by the composition of the ground, but by the structure, and above all, by the degree of humidity existing in the ground in which the mature larvae bury themselves for the metamorphosis. The following publication on this subject is in course of being printed:

Cavalloro R. e Delrio G. - Fattori edafici influenzanti l'impupamento di Ceratitis capitata Wiedemann. Boll. Lab. Ist. Ent. Portici.

B. A continuous mathematical model has been elaborated for the dynamics of a population of polyvoltine insects, with the aim of forming an estimate of the particular characteristics of the species once known the experimental observations relative to the density of the various stages of life and the times of development. A population of Dacus oleae Gmelin was submitted to examination, studied with a direct collection of the olives for the immature stages, and by the capture of adults by means of chromotropic traps. The model permitted the interpretation of certain processes regulating the dynamics of population, and the estimate of certain parameters not observable using experimental means; in particular, the model permits one to

know in a sufficiently realistic and complete way some of the factors regulating the dynamics of the population (mortality rate, fertility of the species, etc.) and to render coherent measures obtained by diverse methods of sampling and to interpret the larval and pupal parasitism. The following publication on this subject is in course of being printed:

Cavalloro R., Delrio G., Di Cola G. - Un modello matematico continuo per la stima di parametri biologici di una popolazione di insetti polivoltini. *Re- dia, Firenze.*

C. In the context of the genetic control of Ceratitis capitata Wied. researches have been conducted for the first time on the possibility of applying the method of semi-sterility caused by chromosomic translocation or pericentric inversions.

The data obtained confirm the utility, not yet brought into evidence, of having recourse to sub-sterile males in the control of Ceratitis, thanks to the greater competitiveness with respect to completely sterile males, obviously in comparison with the natural population; furthermore, the influence of sub-sterile males can have a certain value, also in successive generations, under the aspect of deferred sterility.

The following work on this subject is in course of being printed:

Delrio G., Cavalloro R. - Semisterility in the Mediterranean Fruit Fly Ce- ratitis capitata Wiedemann. *Genetica Agraria, Roma.*

Finally, radio-sensitivity investigations have been conducted on all the stages of life of the Ceratitis capitata Wied. using fast monoenergetic neutrons of 4.96 MeV.

It has been discovered that resistance increases with the age of the insect. The dose which prevents the transition into a butterfly of the adult is 761 rad per egg and newly-born larva; while doses of over 1.521 and 2.979 rad are necessary for mature larvae and 3-day pupae respectively. A dose of 2.979 rad causes a high percentage of sterility in the adult irradiated whether pupa or adult, without on the other hand influencing longevity. Males

are much more sensitive than females.

Neutrons have been shown to be much more efficacious than gamma radiations, both in causing mortality in the immature stages and in inducing lethal dominants in adults, especially in the male germinal cells. Males sterilized by fast neutrons display a greater sexual competitiveness than those irradiated by gamma rays, compared with normal insects. The following publication on this subject was presented at the 8th International Congress of Plant Protection:

Cavalloro R., Delrio G. - The effect of fast neutrons on the Mediterranean Fruit Fly (Ceratitis capitata Wiedemann). Reports of VIII Int. Plant Protection Congress, Moscow 1975, Sect. V, pp. 53-62.



Contractual Partner of the Commission:

Institut für Genetik, Johannes Gutenberg-Universität, Mainz, Germany

Contract No.: 115-72-1 BIO D

Head of the Research Group:

Prof. Dr. H. Laven, Director of the Institut für Genetik

General Topic of the Contract:

Development of genetical control systems in pest insects.

Project No.1 : Mosquitoes

The closing of the "WHO-ICMR Research Unit for Genetical Control of Indian Mosquitoes" in Delhi (India) in July 1974 is a severe drawback for the sake of genetical control. Our own work with Culex fatigans, especially the development of efficient translocation lines for application in India, has now become somewhat obsolete since there is no possibility left for testing such strains under natural conditions in field experiments. Under these circumstances we have shifted our aims to the development of genetical control systems in Anopheles stephensi, the main vector of malaria in vast areas in the Near East and Southeast Asia, and Aedes vexans, the predominant nuisance mosquito of the northern hemisphere.

Project No.1

Title: Genetical systems for control of mosquitoes.

Research workers: Prof. Dr. H. Laven, J. Kutsche-Ohmann, M. Ashraf-Choudhary, R. Kuhn, K. Zimmer, G. Schuler, R. Steffens, K. Gollnisch.

1.1. Anopheles stephensi

After the development of a pure colony of this species without inherited lethality due to recessive lethal factors, a dose response curve for dominant lethality after X-ray irradiation has been worked out. It is not much different from the curve for Culex pipiens or Aedes aegypti. A first attempt to isolate lines with partial sterility due to chromosomal aberration gave promising results. Out of 138 irradiated (4.000r) sperms tested 28 (= 20.2%) led to lines with inherited sterility between 19 and 86%. These lines were followed through several generations.

The most stable ones were selected and will be tested for the mode of inheritance of the semisterility, i.e. male-linked, female-linked or autosomal.

A second experiment to produce more translocation lines has been initiated and shows also good results.

Cytological investigations with the aim to elucidate the nature of the chromosomal aberrations in the different stable lines have been initiated.

### 1.2. Aedes vexans

After initial difficulties in our attempt to colonize this species we are now well on the way. We have now a colony in the fifth generation maintained in the laboratory under an appropriate light and humidity regime. For the time being this colony is kept in a large cage. Subcultures are tested in ever smaller cages for copulation. It can be expected that in the near future the development of genetical control systems can be started.

Parallel with these laboratory investigations detailed studies on the ecology of this species have been carried out. We have studied the distribution of eggs in the field and their ability to hatch under various circumstances. These investigations are fundamental for future attempts to control this species with genetical systems.

### Project No.2

Title: Production of inherited semisterility in the Mediterranean fruit-fly (Ceratitis capitata).

Research workers: Prof.Dr.H.Laven, H.Bernd.

A laboratory colony of the medfly has been established. Before irradiation experiments could be started it was necessary to investigate this colony for the content of recessive lethal factors in order to get a lethal-free subculture. This aim has been accomplished. Other activities were directed to the development of a method for single pair matings, since it is necessary for the future work on the genetics of this species,



Project No.3

Title: Production of inherited semisterility in the rice weevil  
(Sitophilus oryzae).

Research workers: Prof.Dr.H.Laven, R.C.Sharma.

After the successful development of a lethal-free colony irradiation experiments were carried out, first to develop the dose-response curve for dominant lethals and second for the isolation of lines with inherited semisterility. Up till now 13 lines with suspected chromosomal aberrations have been isolated. Out of these only 4 lines were maintained as they show a stable and high degree of inherited semisterility. Further experiments to produce more lines with semisterility are in progress. It is worth mentioning that these experiments have shown for the first time, that genetical control systems can also be developed in Coleoptera.

Project No.4

Title: Production of inherited semisterility in the cockroach Blatella  
germanica.

Research workers: Prof.Dr.H.Laven, G.Ackermann.

This project is still in the initial stage of colonization of the species and development of suitable methods for single pair matings.



Contractant van de Commissie : Instituut voor Plantenziektenkundig  
Onderzoek (IPO), WAGENINGEN, Nederland

Nummer van het contract : 098 - 72 - 1 BIO N

Hoofd van het researchteam : dr. ir. J. Ticheler

Algemeen onderwerp van het contract : Control of the onion fly,

Hylemya antiqua (Meig.), by means of the sterile-male technique.

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Publications :

Theunissen, J., M. Loosjes, J.Ph.W. Noordink, J. Noorlander and J.  
Ticheler, 1975 Small scale field experiments on sterile-insect control  
of the onion fly, *Hylemya antiqua* (Meigen). in "Controlling fruit flies  
by the sterile-insect technique", 83-91, IAEA, Vienna.

Ticheler, J., 1975 Possibility of insect control by sterile males in  
tropical agriculture. Meded. Fac. Landbouww. Rijks Univ. Gent 40 421-425.

Project no. 1

Control of the onion fly, *Hylemya antiqua* (Meig.), by means of the sterile-male technique

General (J. Ticheler)

1974 marked the end of the research phase in its strict sense. The aim with which the study was started, namely to investigate the possibility of using the sterile-male technique as a control measure for the onion fly, was achieved. The mass production of a competitive sterilized insect is possible and the ecological characteristics of the species are such that efficient control can be obtained by releases of sterile insects.

1975 was used to further analyse the results and make them ready for publication, to prepare for the development phase and to initiate research which has a direct bearing on the practical application of the method. Calculations have shown that economically genetic control of the onion fly is competitive with chemical control and, moreover, avoids the risks of the latter : development of insecticide resistance and contamination of the environment.

During the year many reports and plans have been drawn up and a workable base has been found for the development phase, for which the final decision, we trust, will be taken shortly.

Mass rearing (J. Noorlander)

As this year no release experiments were planned, and therefore no mass rearing was required, the attention could be focused on the determination of optimal rearing conditions. This means that biological factors were weighed against their financial consequences.

+ Improvement of the diet of the adult flies was one of the subjects. The quality of this food is very important as flies cannot lay eggs without eating fairly large amounts of protein rich nutrients. The influence on reproduction of other components of the diet has been studied simultaneously.

+ An experiment has been carried out to determine the maximal feasible fly density which is still economically desirable. The results of this experiment, which links up with the results of Houwing (Ann. Rept. 1973, p. 751) are being analyzed.

+ Experiments about the nature of the illumination and the light intensity above fly cages indicated that increased light intensity enhanced egg production. Some additional work is required.

+ Development of a new type of fly cage met with difficulties as far as egg deposition is concerned. The flies seem to be well at ease in this cage which permits the achievement of a higher fly density per unit of space than before.

+ It was not possible to replace the anti-microbials in the larval diet, K-sorbate and Nipagin, by Allyl cystein sulfoxide, a substance with anti-biotic action which occurs naturally in onions.

+ A few preliminary experiments were carried out in view of reducing evaporation of the larval medium, but no positive results were obtained sofar. The maintenance of the optimal humidity of the larval diet is very time consuming, but has on the other hand an important bearing on the quality of the pupae in the end.

#### Radiobiology and radioisotopes (J.Ph.W. Noordink)

In order to verify whether semi sterility in the F<sub>1</sub> generation upon irradiation with substerilizing doses as it occurs in mosquitoes, does also occur in the onion fly, the following experiment was set up. Two groups of pupae were irradiated on a Röntgen machine with doses of 750 and 1500 R respectively (complete sterility is achieved with 3 000 R). A thousand males from each of the groups were caged in large size cages with the same number of females. A similar number of not irradiated flies served as control. In the three cages, Control, 750 R and 1 500 R eggs were produced with a fertility of 90,0 , 18,5 and 4,2% respectively. From these eggs the next generation was reared. For each of the groups of flies that became available three cages were set up with population of :

- a normal females and male progeny of irradiated parents
- b normal males and female progeny of irradiated parents
- c male and female progeny of irradiated parents,

with one control seven cages in all. The fertility of the eggs produced was checked regularly, and the following figures were obtained :

control	89,6 %		
750 R <u>a</u>	85,5 %	1 500 R <u>a</u>	83,7 %
750 R <u>b</u>	80,5 %	1 500 R <u>b</u>	83,9 %
750 R <u>c</u>	78,7 %	1 500 R <u>c</u>	76,7 %

From these figures it is clear that the doses applied did not induce a meaningfully increased sterility in the F<sub>1</sub> generation.

Towards the end of the year an experiment was set up to evaluate the effects of <sup>60</sup>Co radiation on adult flies. The results will be available for the next report. When competitiveness of these insects should be better, then a method is required for labelling flies when they are

still in the pupal stage. To this end four colours of fluorescent dyes were mixed in different concentrations with pupae and the emerged flies were checked on the presence of the dye mark by squeezing the ptilinum and by squashing the head under UV light. It appeared that 10 mg of dye per 100 pupae was sufficient to have the label on the ptilinum of all the flies.

#### Ecology of the onion fly (M. Loosjes)

As this year no field experiments have been carried out, we were able to analyse in depth the results of the foregoing years. Unhappily illness caused considerable delay. The following points merit attention:

- + On the field on Flakkee where sterile insects were released in 1974 not only a selective elimination of the onion fly was obtained, but also a drastic reduction of the seed-corn maggot, *Hylemya cilicrura*. Quantitatively this maggot is, therefore, not a primary parasite of onions.
- + Refuse heaps of onions are unimportant for the population dynamics of the onion fly, but they are of importance for various fly species, whose larvae live in rotting onions, such as *Ortalis urticae*.
- + The occurrence of *Entomophthora* infection in recaptured flies, dependant upon their age, can be explained by the assumption of a rather constant chance of infection for the healthy flies. For the second flight in 1974 this chance amounted to about 5%. This will have reduced the reproduction factor by about half.
- + From experiments with release methods, carried out in order to limit in the field concentrations of young flies liable to predation, it appeared that onion flies can be kept for 1 - 2 days after eclosion in densities up to 4 flies per cm<sup>3</sup> without increased mortality.
- + For the sampling of damage a rule of thumb could be devised that the number of days between two checks should not exceed the number of cm the onions are tall at the first of these two checks.
- + Prediction of the moment at which 5% of the onion flies have emerged in spring can be carried out by calculation of temperature sums (above 6°C) or from the correlation of the emergence with the eclosion of the summer oak. In view of the time required to react by releases of sterile flies, this forecast needs to be available at least 10 days beforehand. It appeared that, due to the variations in the sequence of soil temperatures, the 5% emergence point can be predicted  $13 \pm 3$  days in advance, if the required temperature sum is exactly known. The accuracy which seems to be obtainable with the eclosion of the summer oak as a crite-

tion is similar to that of a temperature sum within a range of 10 day degrees.

+ Damage by the onion fly in the field does not show a border effect. The damage on the head ends of the field is about a factor of 4 higher than in the rest of the field. The distribution of the damage over randomly chosen sample rows can be adequately described by a negative binomial distribution.

#### Histological studies (J. Theunissen)

The project on the histopathology has been concluded with a description of the normal gametogenesis and of the histopathological reactions of the reproductive organs on various doses of hard X-rays.

Normal spermatogenesis has been studied in light- and electron microscopic preparations as well as in living germinal cells. The germinal and somatic cell types and structures have been described and identified. Moreover, the ontogenetic development of the testis, the dynamics of the spermatogenic processes and an aspect of comparative spermatogenic study have been dealt with. As oogenesis in insects has been studied more carefully and extensively than spermatogenesis, for *H. antiqua* we could limit ourselves to the study, under the light microscope, of the germinal and somatic cell types and the ontogenetic development of the ovary.

Based on the study of normal gametogenesis the histopathological reactions of cell types, cell populations and reproductive organs as a whole on radiation have been investigated. The pathogenesis of the observed symptoms has been analyzed, and, further, the pathological reactions on low, sterilizing and higher doses of radiation. The development of the symptoms was, for the various doses, followed in insects of different age. It appeared possible to combine these qualitative data in such a way that radiation effects on the germinal cell population of the testis could be listed in the form of a table. This table gives for each dose and/or age group an estimate of the damage caused in the male germinal cell populations. In this way radiation effects can be compared, in principle also with other insect species.

Descriptions of the normal gametogenesis and the radiation pathology are being published.





Associato della Commissione: Comitato Nazionale per  
l'Energia Nucleare, Laboratorio Applicazioni Agricoltura

N° del contratto: Euratom-CNEN 107-72-I-BIOI

Capo del gruppo di ricerca: prof. T. Cervigni

Tema generale del contratto: Biological control of Ceratitis  
Capitata Wied. by nuclear technique.

Capo del progetto e collaboratori: U. Cirio, I.D. De Murtas,  
F. Barbera

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Conclusion of the Procida Medfly pilot experiment: results and  
evaluations.

#### General

The necessity to have definitive, practical and economic data on the application of the sterile insect technique against the Medfly convinced CNEN, under the auspices of FAO/IAEA, and with the collaboration of EURATOM, the Italian Ministry of Agriculture and Forestry, and the Institute of Entomology of Naples University, to begin in 1971 a five year pilot experiment. The pilot area chosen was again Procida island for the following reasons:

- a long term of ecological information available;
- an abundance of Medfly plant-hosts;
- an advantage for a ground release program;
- a good collaboration with the local farmers.

The objectives of this project, characterized by ecological studies, during and after the planned two years of sterile fly releases, were mainly:

- to transform the technological application of the "SIT" (Sterile Insect Technique) into practical ecology;

- to study the impact of this technique on the Procida ecosystem;
- to evaluate the real cost of such method of control in order to integrate it with other control techniques;
- to act as a model for the "SIT" programs under way in other areas of Italy.

A general information on the Island of Procida and the Medfly ecology is given in figure I.

#### Materials and methods

The flies were reared at the Casaccia insectary where the development and the improvement of the mass rearing have permitted a production of about 10 million flies per week (DE MURTAS et al., 1972; CIRIO et al., 1976). The sterilization of insects was studied and accomplished at the Co<sup>60</sup> irradiation plant of the Agriculture Application Laboratory of CNEN - Casaccia Center- with a dose of 10,000 rads (DE MURTAS et al., 1972). A practical system for sterilization and handling a great number of flies was also developed (DE MURTAS et al., 1975). The experimental pilot project began with a pre-releasing phase devoted to massive trapping of medfly adults and to the collection of about 90% of the sour oranges. Releases started in March and were continued until October 1972. A total of 85,4 million irradiated insects were released on the island (CIRIO et al., 1974 a). In 1973, last year of planned releases, the strategy of medfly control was changed on the basis of the new ecological information collected (CIRIO, 1974). The attempt to achieve the same 1972 results with a cheaper control program using only 40 million of sterile flies was done with success (CIRIO, 1975). However in 1974 the CNEN, under the strong request of the farmers to continue the releases of




ISLAND of PROCIDA			
GENERAL INFORMATION	CHANGES IN MEDFLY POPULATION		
	WINTER	SPRING - EARLY SUMMER	SUMMER - AUTUMN
Area 3,7 Km <sup>2</sup> Maximum height above sea level 91m Distance from the mainland by 3 Km Annual rainfall max 800 mm Mean temperature of the coldest month 17°C Mean annual temperature 15 - 17°C Type of agriculture intensive family farming of fruit trees Number of host species 12 Total number of host trees 25.000 Main other pests 1) <i>Anastreba brevitarsis</i> 2) <i>Gnathobothris melastri</i> 3) <i>Luspeyrisa camaronella</i>			
Density (Adults / Ha)	max 20	max 200	max 30.000
Population (Adults / No.)	max 7000	max 60.000	max 10.000.000
Population growth factor		max 7,5	max 42,5
Number of generations per year	max 7		

Fig 1 - Distribution and abundance of medfly in the Procida island

SUBJECTS	1971	1972	1973	1974	1975
1-MASS REARING	ONEN - Casaccia	ONEN - Casaccia	ONEN - Casaccia	ONEN - Casaccia	ONEN - Casaccia
2-MARKING	—	ONEN - Casaccia Ultra violet Fluorescent powders	ONEN - Casaccia Ultra violet Fluorescent powders	ONEN - Casaccia ultra violet Fluorescent powders	—
3-PACKAGING	—	Pupae in paper bags	Pupae in release insect cages	Pupae in paper bags	—
4-IRRADIATION					—
Insect stage	Adults	Adults in paper bags	Adults in release insect cages	Adults in paper bags	
Type	Gamma rays and neutrons	Gamma rays	Gamma rays	Gamma rays	
Total dose (kRad)	75 - 120	82-130	90-110 (in 40 min)	90-110 (in 40 min)	
Exposure rate (kRad/min)		32	From 0-32 to 32-0	From 0-32 to 32-0	
5-SHIPMENT	—	ONEN - Casaccia Adults in paper Bags by car and ferry boat	ONEN - Casaccia Adults in release insect cages by car and ferry-boat	ONEN - Casaccia Adults in paper Bags by car and ferry-boat	—
6 QUALITY CONTROL	ONEN - Casaccia	ONEN - Casaccia	ONEN - Casaccia	ONEN - Casaccia	ONEN - Casaccia
Laboratory gage tests	Longevity Fecundity No matings	Longevity Fecundity No matings	Longevity Fecundity No matings	Longevity Fecundity No matings	Longevity Fecundity
Field gage tests (with laboratory & wild)	—	Longevity Fly movement Selective mating Activity time	Longevity Fly movement Selective mating Activity time	Longevity Fly movement Selective mating Activity time	—

Fig 2-Laboratory data on the application of "SIT" in the Procida island

irradiated insects on Procida, was induced to supply sterile flies for an other year. The new field programme was very limited by the poor financial support. About 19 million of sterile flies were sent to Procida from the end of June to October. The releases were carried out only in the main citrus crops of the island .

During the five years of the experiment the evaluation of effectiveness of medfly control was based on the following controls:

- examination of fruit infestation;
- trapping of adult flies;
- determination of egg hatch taken from oviposition on peaches.

From 1973 onwards larvae and adults of two inter-specific competitors of the medfly (larval stage) were also checked in Procida by fruit examination and captures of the adults with pheromone traps (CIRIO et al., 1974 b).

#### Results and evaluations

Figure 2 and 3 present the data on the laboratory and field work involved in the application of sterile insect technique on Procida island. The most critical points in these major research areas were:

- to improve the technique of the adult sterilization using cloth cages in order to have less mortality and more competitive insects;
- to develop techniques for evaluation of the quality of the produced flies and of the behavioural performance;
- estimation of the low population densities of Ceratitis by different methods (the overwintering period should be included);
- accurate studies of the influence of the climate on the medfly population especially in relation to its seasonal appearance.

SUBJECTS	1971	1972	1973	1974	1975
<b>1- PRE-RELEASE PHASE</b>					
Intensive trapping	Yes	Yes	Yes	—	—
Sour oranges collection	—	Yes (90%)	Yes (90%)	—	—
<b>2- RELEASE PHASE</b>					
	—	24 Mar — 30 Oct	3 May — 30 July 28 Aug — 30 Oct	25 June — 27 Oct	—
No medfly (collins)	—	0.87	60.3	27.6	—
% emergency	—	66.3	65.0	70.0	—
No adults released (millions)	—	85.4	40.8	19.3	—
Stage insect released	—	Adults from paper bags	Adults from rearing cages	Adults from paper bags	—
Type of release	—	Ground	Ground	Ground	—
Mode	—	Randomly flow 650 pairs	From all Procida roads	Only the citrus crops	—
Carrier out by	—	FARMERS & CNEN	CNEN personnel	FARMERS & CNEN	—
<b>3- EVALUATION TECHNIQUES</b>					
Adults trapping	With black traps	With black traps	With black traps	With black traps	With black traps
Fruit infestation	In selected areas	In selected areas	In selected areas	In selected areas	In selected areas
Punctures examination	—	Yes	—	Yes	—
<b>4- QUALITY CONTROL</b>					
Longevity	—	Yes	Yes	Yes	—
Dispersion	—	Yes	Yes	Yes	—
Host finding	—	Yes	Yes	Yes	—
Fruit localisation	—	Yes	Yes	Yes	—
Activity time	—	Yes	Yes	Yes	—

Fig 3 - Field data on the application of "SIT" in the Procida island

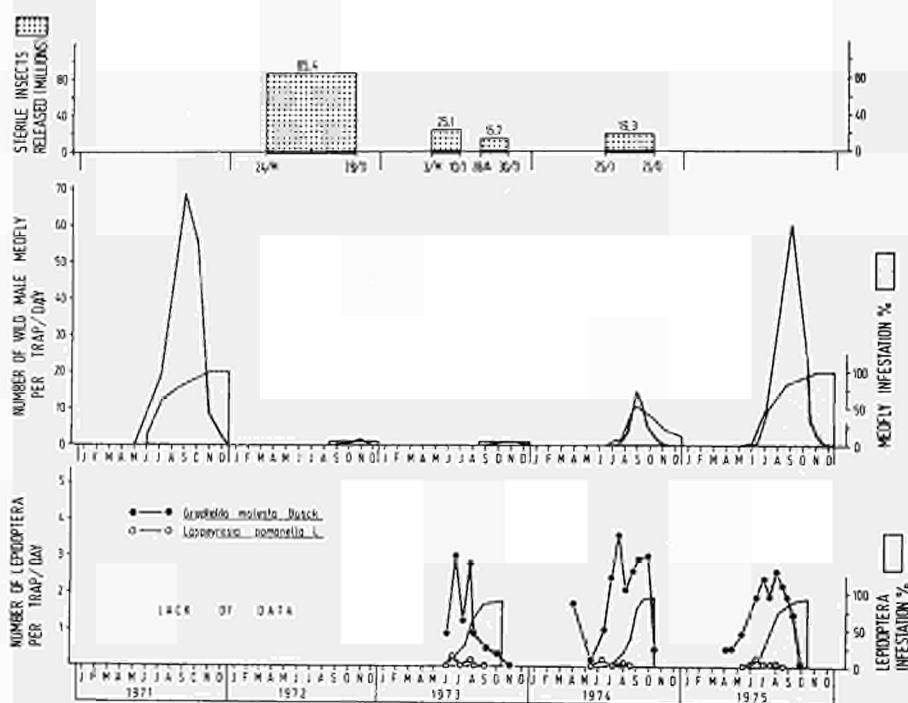


Fig 4 - Abundance of medfly and two lepidoptera populations in relation to the number of sterile flies released in Procida island during the pilot experimental project

Figure 4 shows the results of the application of "SIT" on Procida and its impact on some Lepidoptera competitors of medfly from 1971 to 1975. During the first two years of releases the effectiveness of the technique was clearly demonstrated by repeatedly short period of eradication of flies from the island. The medfly nevertheless re-invaded Procida through immigration of gravid females from the nearby mainland and from Ischia island. The capture of wild flies however never exceeded more than one fly per day and trap and the fruit infestation always remained below 10%. By contrast, before and after the application of SIT in Procida the number of wild flies captured was more than 60 per day and trap and the infestation practically was 100% by the end of August.

In 1974 the control of the native medfly population was not completely positive because the number of sterile insects used was less than planned, the releases limited to the citrus crops, and the pre-release work (fig. 3) omitted.

Concerning the study of the impact of the SIT on Procida ecosystem it seems that suppression of medfly does not cause and increase of the abundance of two Lepidoptera species (fig. 4). It is reasonable to hypothesize that the density of these populations is scarcely influenced by competition for food.

As a first approximation, before accurate data are available, the total cost of medfly Procida project could be estimated about \$ 140 per hectare and year (exclude the purchase of  $^{60}\text{Co}$  source). Nevertheless this cost should not be taken in consideration for plant protection programmes in which SIT is used or is suggested.

On the basis of this pilot experiment some coordinated programs were initiated between CNEN and regional organizations. In the Latium region a demonstration of medfly control by SIT was carried out in the Fiorano area and considerable ecological data were collected along the coast (FIDEGHELLI *et al.*, 1976; SCIROCCHI *et al.*, 1976). In the Sardinia region CRAI (pest control regional Centre) is collecting background information on the species in the framework of the international programme designed for the eradication of the medfly from the island (MELLADO, 1975). Recently CNEN had preliminary contact with the Sicily region for regional medfly control programs. A summary on the projects concerning the application of SIT in Italy is presented in figure 5.

REGION	LOCATION	AREA ha / km <sup>2</sup>	PROGRAMS		MAP	
			OBJECTIVES	PRESENT STATUS		
CAMPANIA	1 CAPO	ca 1000	967	- Attempt of eradication - Control area for Praxeda experiments		
	2 OCHA MONTE di PRACIDA	ca 2000	1988	- Control areas for Praxeda experiments		- Possibility to continue ecological studies
	3 PRACIDA	370	1658/88	971		- Application of SIT based on ecological grounds - A four year pilot project to initiate SIT programs under way in other areas of Italy
LAZIO	1 LATTI COAST	ca 15000	1970	- Ecological information to assist future application of SIT in the mainland	- Ecological studies finished	
	2 FREGENE	ca 15000	1972	- Attempt of eradication	- Ecological studies still in progress	
	3 FIORANO	73	1973	- Medfly control and improvement of SIT	- Medfly control achieved	
SARDEGNA	1 BOIA	ca 1000	1973	Eradication of medfly in Sardegna (1, 2, 3, 4, 5 candidate areas to start the applications of SIT in the island & 7, 8, 9, 10, 11, 12, 13 control areas)	Ecological studies still in progress	
	2 S SPERATE	ca 1000				
	3 MURAVIARA	ca 1000				
	4 VILLA IGRO	ca 100				
	5 S. VERO MILIS	ca 300				
	6 PULA	ca 300				
	7 S. ANTONIO	ca 100				
	8 S. PIETRO	ca 30				
	9 ARBONIA	ca 35				
	10 CHIA	ca 50				
	11 TEGUADA	ca 50				
	12 QUARTU	ca 1000				
	13 VILLA SIMUS	ca 500				

Fig.5 - Objectives and present status of programs aiming to control the medfly by the Sterile Insect Technique in Italy

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V.

FORSCHUNGSTÄTIGKEIT ANWENDUNGEN MEDIZIN

RESEARCH ON APPLICATIONS IN MEDICINE

RECHERCHES RELATIVES AUX APPLICATIONS MEDICALES



NUKLEARMEDIZIN

NUCLEAR MEDICINE

MEDECINE NUCLEAIRE



Vertragspartner der Kommission:

Universität Ulm, Abteilung Nuklearmedizin (RadiologieIII)

Nr. des Vertrages 116 - 72 - 1 bio D

Leiter der Forschungsgruppe: Prof. Dr. W. E. Adam

Wissenschaftliche Mitarbeiter: Dr. F. Bitter, Dr. H. Geffers,

Doz. Dr. H. Kampmann, Dr. G. Meyer und Ing. grad. R. Weller

Allgemeines Thema des Vertrages:

Entwicklung eines nicht traumatisierenden Verfahrens zur Analyse der Herzkinetik mit Hilfe der quantitativen Funktionsszintigraphie und Anwendung simulierender Verfahren.

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Im Jahre 1975 wurden die schon vorhandenen Methoden zur Analyse der Herzkinetik im Steady state (Kamera-Kinematographie) erweitert durch ein interaktives Verfahren zur automatischen Festlegung der Herzbereiche und zur Bestimmung des enddiastolischen und des systolischen Volumens des linken Ventrikels (a). Weiterhin wurden Methoden zur Analyse der regionalen Motilität des linken Ventrikels und zur Erkennung von Bewegungsstörungen entwickelt (b, c). Die Software-Arbeiten zur routinemäßigen Anwendung in der Klinik wurden weitgehend abgeschlossen.

- a) Es wurde ein Programm entwickelt, welches es gestattet, die Herzbereiche halbautomatisch in Form eines interaktiven Dialogs festzulegen. Das Verfahren beruht auf einer Analyse des 1. Fourierkoeffizienten der Zeit-Aktivitätskurve an jedem Punkt der Bildmatrix. Durch die Vorgabe von zwei Parametern, nämlich der unteren Grenze für die Amplitude und eines Phasenintervalls kann einerseits der Untergrund, welcher sich lediglich stochastisch ändert, getrennt werden von dem Herzbereich mit einer synchronen Bewegung, andererseits kann aufgrund der Phase der Bewegung eine automatische Festlegung der Bereiche "Ventrikel" und "Vorhöfe" erfolgen. Die enddiastolische Fläche des linken

Ventrikels wird berechnet. Dies gestattet zusammen mit der Bestimmung der großen Achse und des Abbildungsmaßstabes die Berechnung des enddiastolischen Volumens. Die endsystolische Begrenzung wird entweder als Knicklinie aus den entsprechenden Phasenbildern oder als Gratlinie in der Matrix der enddiastolisch-endsystolischen Differenzen berechnet. Die Volumenbestimmung erfolgt wie oben beschrieben.

- b) Es hat sich weiterhin herausgestellt, daß die Phase des 1. Fourierkoeffizienten ein wichtiges Kriterium zur Erkennung von regionalen Bewegungsstörungen des linken Ventrikels ist. Dieser Parameter erlaubt eine automatische Festlegung von Bereichen mit asynchroner Bewegung. Zusammen mit den Zeit-Aktivitätskurven für die Vorhöfe und beide Ventrikel, werden für den linken Ventrikel regionale Zeit-Aktivitätskurven für normale und dyskinetische Bereiche berechnet.
  
- c) Zur weiteren Analyse der regionalen Kontraktion des linken Ventrikels wurde ein Darstellungsverfahren entwickelt, welches es gestattet, Zählratendifferenzen zwischen beliebigen Phasenbildern des Herzens als Graustufen in Form von Höhenlinien darzustellen zusammen mit der berechneten Kontur der Ventrikel. Die Zählratendifferenzen, welche ein Maß sind für Änderungen des Wandbestandes zwischen der dem Detektor zugewandten und der dem Detektor abgewandten Wand, geben einen unmittelbaren räumlichen Überblick über die Kinetik des linken Ventrikels. Bewegungsstörungen können als Abweichungen vom normalen Muster sofort erkannt werden.

#### Klinische Ergebnisse:

Die klinische Arbeitsgruppe setzte ihre Untersuchungen an herzkranken Patienten fort. Bisher wurden 30 Patienten nach Herzinfarkt, 20 Patienten mit Herzvitien und weitere 20 Patienten mit unterschiedlichen klinischen Diagnosen einer kamera-kinemato-

graphischen Untersuchung unterzogen. Dabei ließen sich alle Patienten mit einem Zustand nach Vorderwandinfarkt, Vorder-Seitenwandinfarkt und Vorderwand-Septuminfarkt anhand eines abnormen Motilitätsverteilungsmusters identifizieren, und zwar anhand dyskinetischer, akinetischer oder hypokinetischer Wandbereiche des Myocards. Bei 15 der Herzinfarkt-Patienten wurde die Kamera-Kinematographisch, also nicht-invasiv bestimmte Größe und Lage der dyskinetischen Bereiche durch eine konventionelle Ventrikulographie überprüft. In allen Fällen stimmten ventrikulographischer Befund und kamera-kinematographischer Befund überein. In 2 Fällen nach Hinterwandinfarkt mit kleineren Narben konnten kamera-kinematographisch keine Veränderungen gefunden werden, die ventrikulographisch darstellbar waren. Anhand einer Bewegungsanalyse, die die regionale Wandbewegung in Form einer Zeit-Volumenkurve erbringt, lassen sich Narbenbereiche der Vorder-Seitenwand des linken Ventrikels von aneurysmatisch veränderten Bereichen abgrenzen, die eine deutliche Phasenverschiebung zeigen, während Narben "stumm" sind. Nach tierexperimentellen und echocardiographischen Untersuchungen ist als erstes Zeichen einer Coronarinsuffizienz eine Änderung des Bewegungsablaufes in den minderperfundierten Myocarbereichen ohne Minderung der Amplitude zu erwarten. Die gegenwärtige und zukünftigen Arbeiten der Klinischen-Gruppe wenden diesem Problem spezielle Aufmerksamkeit zu. Dabei soll die Frage geklärt werden, ob mit dem vorgestellten nicht-invasiven Verfahren regionale Motilitätsstörungen in einem recht frühen Stadium erkannt und somit ein wesentlicher Beitrag zur frühen Diagnostik einer Myocardperfusionsstörung geleistet werden kann.

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Scuola di Medicina Nucleare Università di Pisa

Contract No. 110-72-1 B101

Director: Prof. Luigi DONATO

subject of contract: Research and Development of diagnostic techniques and pathophysiological methods using in vivo and in vitro radioactive tracers.

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In 1975 investigation continued with the aim of developing new radioactive methods and improving existing ones for early detection of disease states and quantitation of function.

In the radiochemical field (Projects 1 & 2) it is of particular importance the development of methods directed to the assay of steroids in serum, the synthesis of  $^{125}\text{I}$  labelled steroids, the development of RIA for LDH isoenzymes, and the better understanding of the protein-ligand interaction in radioimmunoassay.

Methods for investigation of insulin metabolism (Project 3) have attained significant goals, with the preparation of  $^{125}\text{I}$  monoiodinated monocomponent zinc free insulin, retaining full biological and 95% immunological activity. Definition of the role of hepatic extraction and testing of methods for simultaneous evaluation in humans of the secretion and the catabolism of the hormone, represent important and original results.

In the lung field (Project 4) an original method using the cyclotron produced isotopes  $^{123}\text{I}$  has been investigated: it shows promise for simultaneous measurement in man of lung blood volume, extravascular and interstitial lung water. An entirely new method for the study of the distribution of ventilation using  $^{99\text{m}}\text{Tc}$  minimicrospheres has also been developed and tested.

In the field of diagnosis of coronary diseases (Project 5) further progress has been attained in the investigation of myocardial perfusion by  $^{133}\text{Xe}$ , the evaluation of spontaneous angina using the cyclotron produced isotope  $^{201}\text{Tl}$  thallium, and the study of myocardial necrosis using  $^{99\text{m}}\text{Tc}$  pyrophosphate scintigraphy.

Finally, the field of renal diagnosis (Project 6) has seen the demonstration of the reliability of  $^{99\text{m}}\text{Tc}$  DTPA for the measurement of glomerular filtration rates, and the confirmation of the value of the divided renal function studies in renal hypertension by the previously developed technique.

The above activity has resulted in 31 papers published or currently in publication.

## RESULTS OF PROJECT NUMBER 1

Head of the project and scientific co-workers:

Umberto Rosa, Giancarlo Zucchelli, Renzo Malvano e PierPaolo Cozzani

Title of the project: Development of RIA methods for protein and non-protein plasma components

The experimental program developed during 1975 was centered on the following lines:

- a) relationship between chemical structure and specificity in the case of synthetic immunoagents (e.g. non antigenic small molecules coupled to proteins).

The high specificity conferred to antisera against estrogens, aldosterone and progesterone by coupling at C<sub>6</sub>-C<sub>3</sub>-C<sub>11</sub>, respectively, has been exploited in setting up simplified RIA methods.

Direct measurements of serum extracts were optimized and standardized for estriol in pregnancy, estradiol, progesterone and aldosterone: for the last steroid a procedure not including the extraction step was developed for hydrolyzed urine samples.

- b) Synthesis of <sup>125</sup>I-labelled steroids.

Using different approaches based either on direct iodination of phenolic or imidazolic steroid derivatives or on coupling of <sup>125</sup>I-thyramine to active steroid esters, the <sup>125</sup>I tracers were prepared for estradiol, estriol, testosterone, aldosterone, progesterone and digoxine. The analytical performances of such tracers in the corresponding RIA systems were compared with those of tritiated tracers used as a reference.

- c) Measurements of circulating concentration of isoenzymes by RIA.

The H<sub>4</sub> and M<sub>4</sub> isoforms of lactate dehydrogenase were chosen as models to identify the problems connected with the RIA of circulating isoenzymes.

The antisera elicited in rabbits, showed a good specificity, being the tetrameric forms M<sub>4</sub> and H<sub>4</sub> completely discriminated in the H<sub>4</sub> and M<sub>4</sub> systems, respectively, while the hybrid forms<sup>4</sup> were found to cross-react proportionally to the relative abundance of the H and M monomers in either cases. <sup>125</sup>I-labelled isoenzymes were prepared by electrolytic iodination: a double-antibody solid-phase procedure was developed for the B/F separation.

RESULTS OF THE PROJECT NUMBER 2

Head of the project and scientific co-workers:

Umberto Rosa, Renzo Malvano, Ermano Rolleri and Anilla Massaglia

Title of the project: Studies of the properties of antibody sites  
in homegenous and eterogeneous phases.

The work carried out during 1975 was centered on the following lines:

a) Properties of antibody sites.

The steroid-antisteroid systems were assumed as models to investigate a nature of binding forces. In particular, using cortisol antibodies covalently coupled, to ~~CN-Br~~-activated cellulose the contributions of enthalpy and entropy in stabilizing the bonds were evaluated. The pravailing role of hydrophobic components, observed in this case as well as in general for steroid molecules, is seemingly to be regarded as a peculiar feature of the steroid-antisteroid interactions.

In comparison with the interactions with plasma carried proteins, a much less-strict interdependence of conformational structure and binding-function is suggested for immunoglobulin by the small effects associated to pH and temperature changes. The situation was exploited in practical terms for a selective inhibition of carried proteins in setting up a direct RIA of plasma cortisol.

b) Study of interaction models for the ligant-protein systems.

The interactions with proteins were taken into consideration under the twofold aspects of the definition of the binding parameters and the interpolation of dose-response curve in the immunological methods. The different approaches so far proposed were tested for validity in different systems, and the model based on the transcription in terms of protein interactions of the isotherm of Brunauer, Emmet and Teller was eventually applied. This model, not implying any simplificative assumption on the site nature and distribution, proved perfectly adequate in describing the interactions and in fitting the response curves of the analytical systems using protein reagents.

Results of project number 3

Head of the project and scientific coworkers:

Prof. Renzo NAVALESI, Eleuterio FERRANNINI,  
Alessandro PILO, Lorenzo CITTI, Anna PACI,  
Franco MANESCHI.

Title of the project: Development of tracer  
techniques for the study of the kinetics of  
insulin.

The project has developed towards the following lines:

- A: Preparation and characterization of  $^{125}\text{I}$ -moniodoinsulin;
- B: Quantitative evaluation of the role of the liver in insulin metabolism;
- C: Description of the kinetics of insulin in overt diabetes;
- D: Investigation of insulin metabolism in chronic uremia.

A: Mono-component, zinc-free pork insulin was labelled with carrier-free  $^{125}\text{I}$  by the lactoperoxidase method, at pH 5.6, using a ratio insulin: iodine: hydrogen peroxide of 1:0.01:0.01, and with a labelling yield between 70% and 90%. The labelling mixture was purified by ion-exchange chromatography on DEAE Sephadex A-25, which allows for an efficient separation of  $^{125}\text{I}$ -insulin from cold hormone. The tracer obtained by this procedure has the following properties

- a) specific activity ranging from 200 to 300 Ci/g;
- b) precipitability superior to 95% by the double antibody method;
- c) di-iodo-tyrosine content less than 1%;
- d) full biological activity as tested on rat epididimal fat pad;
- e) metabolic behaviour indistinguishable from that of native insulin.

This  $^{125}\text{I}$ -insulin has proved a suitable tracer of endogenous insulin for in vivo kinetic studies.

B: Insulin metabolism in man is usually investigated by peripheral injection of the hormone, whereas native insulin undergoes hepatic extraction prior to mixing in the general circulation.

To quantitate this difference, in 10 dogs  $^{125}\text{I}$ -insulin was injected into a peripheral vein, and the initial distribution volume (IDV) the metabolic clearance rate (MCR) and the mean transit time ( $\bar{t}$ ) were computed from the plasma disappearance curve of the immunoprecipitable activity. The splenic vein was then cannulated under pentobarbital anesthesia and the parameters were again computed from the peripheral activity after portal introduction of the tracer.

The MCR after portal injection ( $15.1 \pm 1.1$  ml/min/kg) was greater ( $p < 0.01$ ) than the MCR after peripheral administration ( $13.4 \pm 0.9$  ml/min/kg). Also IDV was larger ( $p \sim 0.01$ ) after portal injection ( $167 \pm 12$  vs.  $138 \pm 10$  ml/kg). Mean

transit times did not change significantly. Insulin secretion rate ( $0.29 \pm 0.04$  mU/min/kg) and body insulin mass ( $7.03 \pm 1.5$  mU/kg) were also measured.

An estimate of hepatic extraction was obtained from the difference between the clearance rate values calculated following portal and peripheral injection.

Under our experimental conditions, hepatic retention of insulin was found to be 19.6% (range 9.6-36.2%).

The method is suggested for investigations in man.

C: Insulin secretion is usually evaluated on the basis of plasma hormone levels, which, in contrast, depend not only on production, but also on distribution and catabolism.

A tracer technique was therefore developed to measure metabolic clearance rate (MCR), basal post-hepatic delivery rate (IDR), initial distribution volume (IDV), exchangeable body insulin mass (BIM), and fractional turnover rate (FCR) from the plasma disappearance curve of immunoprecipitable  $^{125}\text{I}$ -insulin.

30 overt diabetics who had never received insulin and were normal body weight, and normal subjects were studied.

MCR in diabetics ( $336 \pm 18$  ml/min.m<sup>2</sup>, mean + SEM) was slightly ( $0.05 < p < 0.1$ ) reduced in respect to normals ( $410 \pm 18$  ml/min.m<sup>2</sup>). IDR was similar in normals and diabetics ( $193 \pm 17$  vs.  $187 \pm 20$  mU/min.m<sup>2</sup>); also IDV was similar in both groups ( $1.9 \pm 0.06$  and  $1.9 \pm 0.05$  l/m<sup>2</sup>) and approximately equal to plasma volume, BIM was significantly ( $p < 0.05$ ) larger in diabetic ( $84 \pm 10$  mU/m<sup>2</sup>) than in normals ( $57 \pm 5$  mU/m<sup>2</sup>), whereas FCR was highly significant ( $p < 0.001$ ) reduced in diabetics ( $4.0 \pm 0.3$  vs.  $5.8 \pm 0.3$  % min<sup>-1</sup> of normals). MCR showed a significant negative correlation with the duration of diabetes, whereas it was not age-related either in normals or in diabetics.

These results demonstrate that:

- a) insulin clearance rate is slower in diabetics, mainly as a result of the progression of the disease.
- b) The basal post-hepatic delivery rate in diabetics, although similar to that of normals, is associated with hyperglycemia, thus suggesting peripheral insulin-resistance.
- c) The exchangeable insulin mass is expanded in diabetics.

D: Previous work has shown that chronic uremia is associated with marked depression of both insulin removal and secretion, consequent to lack of renal tissue and accumulation of toxic metabolites. Maintenance hemodialytic treatment removes, at least in part, the toxic inhibition of insulin metabolism.

The present study was aimed at establishing whether the changes brought about by dialysis are acute, i.e. occurring after each dialysis, or very gradual ones, consequent to a regular dialytic program.

Eight patients (6 males and 2 females, mean age = 44 years) on maintenance hemodialysis from 1 to 36 months (except case No. 4) were studied an average of 15 hours after dialysis, and then again an average of 78 hours after dialysis. Each study consisted of measurement of basal plasma values for non-proteic nitrogen, creatinine, methylguanidine, glucose, insulin (IRI), growth hormone, total proteins, cholesterol, triglycerides and electrolytes.  $^{125}\text{I}$ -insulin was then injected i.v. as a single bolus, and the plasma disappearance curve recorded for 150 minutes; a standard IvGTT was then immediately performed, the glucose and insulin levels being measured for 60 minutes.

The plasma disappearance curves of labeled insulin were analyzed by methods described elsewhere to obtain the metabolic clearance rate (MCR), the fractional catabolic rate (FCR), the basal post-hepatic insulin delivery rate (IDR), and the total insulin mass (TIM). The IRI curves after glucose load and the labeled insulin disappearance curves were analyzed by a deconvolution technique to obtain the post-hepatic secretion function. Its integral between zero time (i.e. the glucose injection) and 60 minutes (IvGTT-ID) is therefore the cumulative output of insulin during that time interval in response to a standard secretory stimulus.

Table I summarizes the most relevant results available at the present times. In discussing our findings, it should be kept in mind that only three metabolite (namely, nitrogen, creatinine and methylguanidine) known to be more or less toxic when retained in excessive amounts, were measured; the effect(s) of other known toxic factors (e.g. phenols, uric acid, etc.) and of possible unknown ones cannot be evaluated. In addition, it is well established that comparable degrees of intoxication affect the metabolic functions of uremic patients to a very variable extent. Nevertheless, some common features were evident in our series.

In all patients, azotemia was decreased at the first reading after dialysis; in 6 of them creatininemia was also found to be lower at this reading, but in cases No. 2 and 4 slightly higher values were found. Plasma methylguanidine levels always paralleled the creatinine concentrations.

In all cases, the fractional catabolic rate increased when creatinine and methylguanidine decreased; the same was true of the metabolic clearance rate, with the exception of case No.7.

Thus, the accumulation of creatinine and methylguanidine seems to depress the mechanisms of insulin removal.

In the 5 cases where the IRI values were available, insulin secretion, both basally (IDR) and in response to the glucose load (IvGTT-ID), improved as azotemia went down.

Massive retention of nitrogen seems therefore to be a main factor in the depression of insulin secretion.

In these same 5 cases, when both secretion rate and clearance rate increased, body insulin mass was reduced; where (cases No. 2 and 4) the secretion rate was higher but the clearance



rate was lower, a remarkable increase of the insulin mass was found. It can be noted that in these two cases, although azotemia was reduced, creatinine and methylguanidine levels were higher.

Thus, in all the patients for whom this information was available, body insulin mass directly followed the level of intoxication by creatinine and methylguanidine.

Glucose utilization, as estimated from the disappearance rate (K value) of an intravenously administered load, improved (in 7 out of 8 cases) with the lowering of creatinine and methylguanidine concentrations.

TABLE I - Results in 8 uremic patients (partial)

Case No.	1		2		3		4		5		6		7		8	
Hours after dialysis	108	12	22	14	65	15		16	72	17	76	22	109	13	109	13
AZ	174	60	167	89	221	94	172	131	142	95	169	110	220	125	189	59
CR	11	5.5	9.1	12	12.8	5.9	10.1	12	8.7	6.2	13.2	8.3	20.3	10	14.5	6.6
MG	50	22	120	160	64	60	30	120	70	63	130	67	168	92	67	45
PPG	74	105	109	116	99	99	62	93	99	105	112	104	74	83	73	75
IRI	3	3	6.8	13.3	7.3	11.2	3.4	13.2	6.0	5.9	-	-	-	-	-	-
MCR	96	194	766	539	460	559	473	321	323	511	315	407	422	323	440	473
FCR	0.70	1.21	3.41	2.43	1.36	2.75	2.49	1.74	1.42	2.67	1.26	1.75	1.93	1.99	2.56	3.66
IDR	47	47	313	430	201	376	96	254	116	181	-	-	-	-	-	-
TIM	111	64	153	295	247	227	64	243	137	113	-	-	-	-	-	-
IvGTT-ID	215	347	1174	2113	912	1793	674	1011	462	651	-	-	-	-	-	-
KG	1.8	3.5	3.9	2.4	1.6	1.9	2.0	1.9	4.1	4.5	1.5	2.2	3.7	5.0	2.9	2.7

This patient had never been dialyzed when the first study was conducted.

AZ = plasma non-protein nitrogen (mg/100 ml); CR= plasma creatinine (mg/100 ml); MG= plasma methylguanidine (ug/100 ml); PPG= fasting plasma glucose (mg/100 ml); IRI = plasma immunoreactive insulin (uU/ml); MCR= metabolic clearance rate (ml/min m<sup>2</sup>); FCR= fractional catabolic rate (% min<sup>-1</sup>); IDR= insulin delivery rate (mU/hour m<sup>2</sup>); TIM= total insulin mass (mU/m<sup>2</sup>); IvGTT-ID= insulin output after IvGTT (integrated over 60 minutes) (mU/m<sup>2</sup>); KG = disappearance rate of plasma glucose increments above fasting level (% min<sup>-1</sup>).

Results of project number 4

Head of the project and scientific coworkers:

Prof. Carlo GIUNTINI, Dr. Anna Maria SANTOLICANDRO, Dr. Massimo PISTOLESI, Dr. Carlo MARINI, Dr. Antonio PALLA, Dr. Giuseppe PISTELLI.

Title of the project: Development of the use of short-lived radioisotopes for the quantitative evaluation of lung function by imaging techniques.

The project has been directed toward the development: A. of a method to measure accurately the volume of distribution at equilibrium within the lung of a suitable short-lived isotope such as  $^{123}\text{I}$ ; and B. of a method to visualize the lung deposition of inhaled albumin micromicrospheres labelled with  $^{99\text{m}}\text{Tc}$ .

A. Study of regional lung blood volume, extravascular and interstitial water.

Depending on the chemical combination with albumin, antipyrine, and sodium the space of distribution of  $^{123}\text{I}$  will represent at equilibrium that of blood volume, vascular and extravascular water, and interstitial water, respectively. Hence, the possibility of measuring the space of distribution of  $^{123}\text{I}$ , in its various combinations, within an organ, amounts to measure the regional blood volume, total water, and interstitial water in that organ.

In the specific case of the lung and the surrounding chest wall,  $^{123}\text{I}$  exhibits some useful features. In 83% of its disintegrations emits a gamma-ray with a peak energy of 159 KeV. Furthermore, gives off for each disintegration x-ray with an energy peak around 28 KeV. The possibility of distinguishing by height pulses analysis the radiations of the two peaks makes it possible to separate the amount of  $^{123}\text{I}$  in the chest wall from that in the lung. This stems from the different absorption and penetration of the two kinds of radiations.

This possibility has been tested using a realistic reconstruction of the lung (sawdust with different water contents to simulate the variable density of the lung tissue according to the degree of pulmonary congestion and edema) surrounded by an appropriate phantom of the chest wall. Various combinations of  $^{123}\text{I}$  distributions in the lung and in the chest wall have been tried. The imaging devices employed have been a planiscanner with focusing collimator and a gamma-camera with a high resolution high energy collimator. The gamma-camera has been modified to make it sensitive to the low energy radiations. The two kinds of radiations are so widely apart that the higher peak does not interfere at all with the lower one. Thus distinct images can be obtained for the two types of radiations. This makes it possible to write for each kind of radiation a simultaneous linear equation as if the two kinds of radiations represented two different positions of measurement. We can then

arrange the simultaneous linear equations into a matrix that allows to compute the distribution of  $^{123}\text{I}$  in the lung and in the chest wall.

The model experiments have shown that the computed distribution is a close approximation of the actual one.

B. Lung deposition of nebulized minimicrospheres and sequential gamma-camera visualization of bronchial clearance and alveolar residual activity.

Inhalation of nebulized albumin minimicrospheres labelled with  $^{99\text{m}}\text{Tc}$  has been employed to investigate bronchial mucociliary transport and ventilatory dynamics. Inhaled particles have a mean diameter of 0.85 micron and an upper diameter less than 2 micra. Particles of this size show a high rate of deposition in peripheral airways and alveoli. The lung deposition of the minimicrospheres has been followed by a large field gamma-camera. Radioactivity accumulation over any lung field has been linear with time since the very start of the nebulization. Life size images of the lung deposition have been obtained on x-ray films during inhalation, at the end of it, and several hours later. Using a light transmission densitometer the films have been quantitatively analyzed. Thus the mucociliary clearance of large bronchi and trachea may be followed. Once this is over, the residual radioactivity reflects the deposition of the minimicrospheres in the alveoli.

In assessing ventilatory dynamics this method presents several advantages over the  $^{133}\text{Xenon}$  gas technique. The energy of  $^{99\text{m}}\text{Tc}$  is more suitable for the external detection. The particulated tracer stays put in the air spaces for the time needed to take pictures with no problems of diffusing, dissolving in blood, accumulating in the thoracic wall, etc. The subjects need only breath quietly.

Ten normal subjects and fifteen patients with chronic obstructive pulmonary disease have been studied. In chronic bronchitis the minimicrospheres deposition is markedly uneven all over the lung fields. In asthma deposition prevails in the central lung regions (parahilar) and bronchial clearance is fast. In emphysema the pattern of deposition is still different. The method is more sensitive than that based on  $^{133}\text{Xenon}$  to disclose abnormal ventilatory dynamics. It correlates well with indexes of airways obstruction but shows regional irregularities of deposition when these indexes are within normal limits.

Results of project number 5

Head of the project and scientific coworkers:  
Prof. Attilio MASERI, Dr. Carlo CONTINI, Dr.  
Antonio L'ABBATE, Dr. Antonio PESOLA, Dr. Paolo  
MANCINI, Dr. Mario MARZILLI, Dr. Oberdan PARODI,  
Dr. Silva SEVERI.

Title of the project: Development and applica-  
tions of short-life isotopes to the functional  
evaluation of the myocardium.

The project was developed along three main lines based on the use of a gamma camera and, for study 1, also of a computer system:

1. Study of regional myocardial perfusion in patients with coronary artery disease by  $^{133}\text{Xe}$  (publications 20-22)
2. Study of regional myocardial perfusion in patients with spontaneous angina pectoris during the ischemic episodes by  $^{201}\text{Thallium}$  (publication 23).
3. Study of the evolution of myocardial necrosis in patients with acute myocardial infarction by  $^{99\text{m}}\text{Tc}$  pyrophosphate scintigraphy (publication 24).

1. We have developed new methods of analysis of  $^{133}\text{Xe}$  washout curves and applied them to the data collected in a group of patients (4 with normal coronaries and 14 with coronary disease in whom myocardial areas distal to a severely obstructed vessel were clearly separable from those normally perfused). We analyzed initial distribution, initial and final washout rates, and residual activity of  $^{133}\text{Xe}$  injected into the left coronary at rest and during angina pectoris induced by pacing. Constancy of the counting geometry was continually checked from the reference regional time activity curves of  $^{99\text{m}}\text{Tc}$  human albumin microspheres injected into the left coronary at the beginning of the study and recorded on the second channel of the gamma-camera.

At rest, while no consistent difference in initial washout rates was observed, residual activity was consistently greater in post-stenotic areas suggesting localized ischemia. Injections performed during pacing induced angina showed in post-stenotic myocardium: cold areas in the initial  $^{133}\text{Xe}$  distribution, smaller increase of initial washout rates and greater residual activity. Initial washout rates differed from those of normal myocardium less than predictable from the intensity of cold areas, suggesting that ischemia was no intense in some regions that too little indicator was deposited to contribute appreciably to

initial washout slope. Indeed angina induced during the course of the washout caused marked reductions of post-stenotic washout rates relative to control supporting the existence of functional factors which dramatically impair post-stenotic myocardial perfusion during angina.

2. In previous studies we demonstrated that "variant" angina could no be attributed to increase myocardial demands. In order to investigate whether a reduction of regional myocardial blood supply could be responsible for these ischemic episodes, we studied regional myocardial perfusion in 6 patients with "variant" angina admitted to our CCU. Myocardial scintigrams in LAO projection were obtained by a gamma camera 5-7 minutes following i.v. injection of 1 mC of  $^{201}\text{Tl}$  performed during an episode of S-T elevation.

In all a transmural deficit of tracer uptake was noted in the heart wall corresponding to the leads showing S-T elevation. These regional deficits of tracer uptake were no longer detectable 2 hours later because of subsequent  $^{201}\text{Tl}$  uptake in previously ischemic myocardium, nor were they present in scintigrams obtained a week later following injections performed in absence of acute ischemia. Tracer uptake in ischemic area of the scintigram was 60% to 85% of that observed a week later in the absence of symptoms. Because of  $^{201}\text{Tl}$  kinetics and of counting geometry problems, this represents a large underestimate of the actual flow reduction. Thus "variant" angina in these patients appear caused by a massive transmural reduction of myocardial blood supply.

3. Following the observation that  $^{99\text{m}}\text{Tc}$  pyrophosphate is deposited in the acutely infarcted myocardium (MI) and has been proposed and used for the visualization of the localization and extension of infarcted zone, we studied 15 patients with documented acute MI and 7 controls. Myocardial uptake of  $^{99\text{m}}\text{Tc}$  pyrophosphate was determined at three stages from onset of symptoms: 2-5 days, 10-20 days, 30-50 days, from 1 to hours after the i.v. injection of 9 mC of the traces by a jumboshiba gamma camera in three projections.

Maximal myocardial tracer uptake occurred 2-3 hours following injection. In all cases of MI tracer uptake was clearly detectable in areas corresponding to the electrocardiographic localization. Myocardial uptake was maximal in the very acute phase but in 7 patients was clearly detectable also at 30 days. Since these patients with persistence of myocardial tracer uptake could not be distinguished from the others on the basis of clinical, electrocardiographic or blood chemistry data,  $^{99\text{m}}\text{Tc}$  pyrophosphate scintigraphy should be considered as a possible objective means of evaluating the process of healing of acute MI.

Results of project number 6

Head of the project and scientific coworkers:  
Prof. Claudio BIANCHI, Dr. Mario BONADIO, Dr. Carlo DONADIO, Dr. Gianfranco TRAMONTI, Dr. Silvio FIGUS, Dr. Alessandro DALLE LUCHE.

Title of the project: Radioisotopes techniques in the functional and morphological study of the renal diseases and in the etiological evaluation of hypertension.

1. The reliability of DTPA-Tc<sup>99m</sup> for the determination of glomerular filtration rate in man.

This study was performed to evaluate the mechanism of the renal excretion of DTPA-Tc<sup>99m</sup> in man. For this purpose the renal clearance of DTPA-Tc<sup>99m</sup> and diatrizoate-I<sup>131</sup> have been simultaneously determined by means of vesical catheterization and of continuous venous infusion of the tracers. 31 clearance in 8 patients were performed. The mean ratio between the clearance of diatrizoate-I<sup>131</sup> and DTPA-Tc<sup>99m</sup> was  $1.04 \pm 0.05$ .

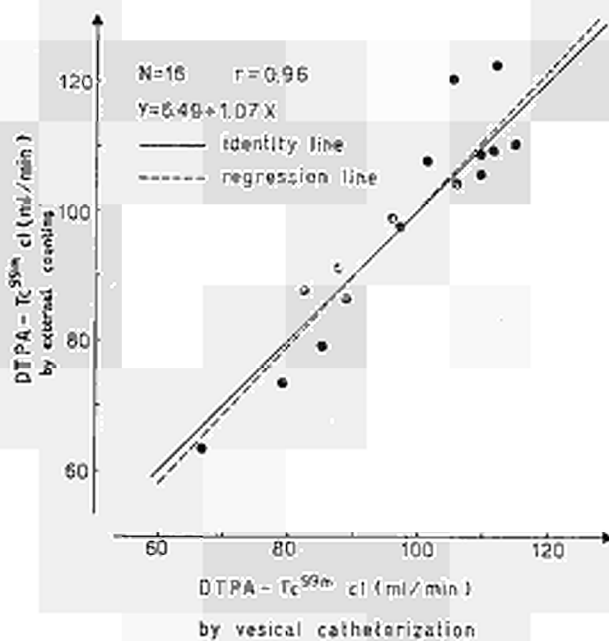
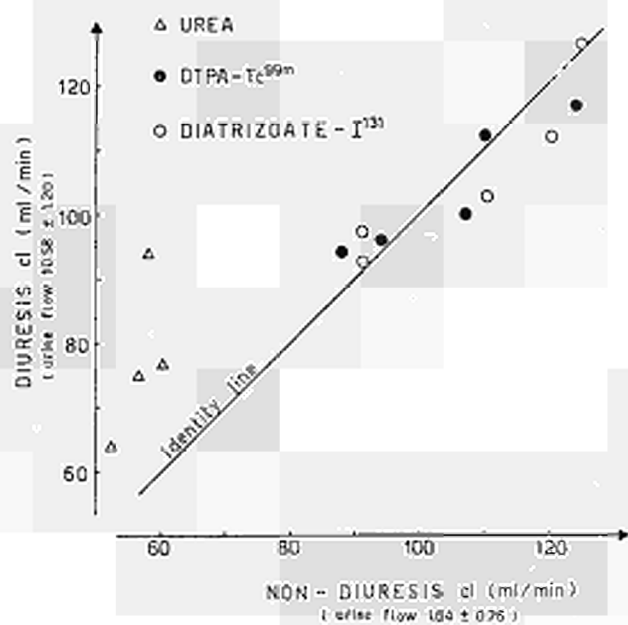
In order to demonstrate an eventual tubular reabsorption of the studied tracer, its clearance was determined simultaneously with those of diatrizoate-I<sup>131</sup> and urea in 5 patients at different urine flows, that is at basal condition (non-diuresis, mean urine flow  $1.64 \pm 0.76$  S.D.ml/min) and during hyperdiuresis induced by oral water administration (mean urine flow  $10.58 \pm 1.20$  S.D. ml/min).

On the contrary of the urea clearance, during both studied conditions, non diuresis and diuresis, the clearance of the radioactive tracers did not change significantly (figure 1). The clearance of DTPA-Tc<sup>99m</sup> has been also determined by means of external counting over the bladder in 4 patients. The mean ratio between the clearance performed by external counting and by vesical catheterization was  $1.00 \pm 0.03$  (figure 2).

In conclusion, the renal clearance of DTPA-Tc<sup>99m</sup> is very similar to the GFR measured by diatrizoate-I<sup>131</sup>. Like this, DTPA-Tc<sup>99m</sup> does not seem to be reabsorbed by the tubule. Its clearance can be easily determined by means of external counting over the bladder.

2. Renal haemodynamic changes in unilateral hypertensive kidney diseases

In 21 hypertensive patients, 16 with renovascular hypertension (RVH) and 5 with unilateral pyelonephritis and hypertension (PN), the divided filtration fraction (FF) was measured by means of the standard technique of ureteral catheterization.



Glomerular filtration rate and effective renal plasma flow of each kidney were determined using diatrizoate- $I^{131}$  and hippuran- $I^{125}$  respectively.

In patients with RVH, FF of the ischemic kidney appeared to be lower than that of the controlateral kidney (average difference 18.21%,  $P < 0.01$ ), while no difference of FF between the two sides was observed in PN.

This behaviour is likely to be referred to the glomerular-tubular imbalance of the ischemic kidney. It seems to be useful for the simultaneous attainment of the functional and etiological diagnosis of unilateral hypertensive kidney diseases.

	FF affected kidney	FF controlateral kidney	$\frac{\text{FF affected kidney}}{\text{FF controlateral kidney}}$
RVH	22.95 + 4.12	27.13 + 2.92	0.84 + 0.12
PN	27.72 + 4.03	27.61 + 3.81	1.00 + 0.01

3. The relation of serum haemoglobin to glomerular filtration rate in renal disease.

The relationship between GFR (determined by diatrizoate- $I^{131}$  and external counting over the bladder) and Hb (by auto-analyzer) has been analyzed statistically in 1738 renal patients. 370 patients had chronic glomerulonephritis (GN), 371 had pyelonephritis or urinary tract infections (PN) and 19 had polycystic kidneys (PK). Considered as a whole, the 1738 patients showed a progressive linear decrease of Hb when GFR was less than 70 ml/min. The table below shows this relationship, where  $HB = GFR \times b + a$

r = correlation coefficient

P = its significance

DF = degree of freedom

	all patients	GN	PN	PK
b	0.0255	0.0324	0.0200	-0.0131
a	11.9	10.59	11.28	12.66
r	0.246	0.284	0.163	-0.221
P	< 0.001	< 0.001	< 0.05	n.s.
DF	810	162	216	14

No correlation between GFR and mean corpuscular Hb was found.



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- 3) P.G. CROSIGNANI, L. TROISI, A. ATTANASIO, G.C. LOMBROSO-FINZI, R. MALVANO: Estradiol and estriol direct RIA in pregnancy procedures: validation and normal values.  
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- 4) R. MALVANO, S. ORLANDINI, P. COZZANI, P. DURANTI, N. SIMONINI, A. SALVETTI: Methodological simplification in RIA of urinary aldosterone.  
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- 5) R. MALVANO, G. GANDOLFI, P. GIANNOTTI, D. GIANNESI, P. GROSSO: RIA of aldosterone in crude plasma extracts.  
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- 6) G.C. ZUCHELLI, M.A. PIRO, N. SIMONINI, R. MALVANO: RIA of estradiol and progesterone in crude serum extracts.  
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- 7) R. MALVANO, M. ZANNINO, A. MASSAGLIA, V. CALI', U. ROSA: RIA of lactate dehydrogenase isoenzymes.  
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- 23) MASERI A., PAROSI O., SEVERI S., PESOLA A.; Transmural acute reduction of myocardial blood flow, demonstrated by  $^{201}\text{Thallium}$  scintigraphy, as a cause of "variant" angina (submitted to Circulation).
- 24) PARODI O., MASERI A., MARINI C., GIUNTINI C.: Valutazione scintigrafica della sede e dell'estensione dell'infarto miocardico acuto e della sua evoluzione. Boll. Soc. Ital. Cardiol. in press.
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- 26) C. BIANCHI, A. COLI, R. PALLA, M. BONADIO: Quantitative sequential renal scans to screen renovascular hypertension. Abstracts of free communications of the 6<sup>th</sup> International Congress of Nephrology (Florence, June 8-12, 1975).
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Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung  
mbH München  
Institut für Hämatologie

Nr. des Vertrages: O89-72-1 BIAD

Leiter der Forschungsgruppe:

Priv.-Doz. Dr. S. Thierfelder

Allgemeines Thema des Vertrages:

Nuklearmedizinische Hämatologie (Proj. 4-7)

(Proj. 1-3 über Strahlenbiologische Hämatologie und Immunologie sind unter Kapitel III "Forschungstätigkeit Strahlenschutz" aufgeführt)

Allgemeine Darstellung der durchgeführten Arbeiten:

With the installation of opposing Cobalt sources in the GSF the research group in Munich intensified their studies on the analysis and treatment of the consequences of radiation exposure. Combined conditioning treatment including cyclophosphamide and antisera against thymocytes found effective in mice are being studied in dogs. A first patient with leukemia was treated by antileukemic drugs and total body irradiation of around 850 r followed by bone marrow transplantation from an HL-A- and MLC-identical sibling. Induction of complete remission of the patients' leukemia which had no longer responded to conventional treatment could thus be achieved.

Bone marrow biopsies in locally irradiated patients with genital cancer revealed a defect of the irradiated tissues with a diminution of the marrow's sinusoides together with inconstant inflammatory changes up to 3 years later.

The enhancing conditioning effect of a treatment with ATG which reduces the radiation dose necessary for engraftment by about 150 r could clearly be demonstrated in mice. Because of the promising results in manipulating

secondary disease with anti-T-cell globulin in mice, considerable efforts were concentrated on the production of an anti-human T-cell globulin which no longer cross-reacted with hemopoietic precursor cells in vitro.

Collaborative research in histocompatibility typing was successfully continued on the european level. Our group contributed to the definition of 6 MLC alleles (HLA-DW1-W6) of the genetic locus HLA-D as defined on the 6th International Histocompatibility Workshop Conference 1975 in Aarhus/Denmark. Recently 3 further types were defined by our group: HLA-D type Bo, EI and RE.

Bone marrow transplantation in canine donor-recipient combinations with unilateral incompatibility permitted to dissociate the consequences of host-versus-graft- and graft-versus-host reactions.

The application of quantitative radioautography to the kinetics of bone marrow cell proliferation in preleukemia and overt leukemia permitted new conclusions concerning the mechanism of the amplification of the leukemic blast cell pool. Thus bone marrow transplantation will profit from a better understanding of the changes in the proliferation kinetics of leukemic and pancytopenic patients.

Ergebnisse des Projekts Nr. 4

Leiter des Projekts und wissenschaftliche Mitarbeiter:

S. Thierfelder, H. Rodt und M. Eulitz

Titel des Projekts:

Suppression of secondary disease with anti-T-cell antisera.

---

In earlier experiments we demonstrated complete suppression of acute secondary disease by treating the donor's cells with anti-T-cell globulin purified from antibodies crossreacting with stem cells. Anti-human-T-cell globulin is not commercially available so far. According to various authors it often suffers from a lack of specificity. We therefore tried to find techniques which permit the production of high titer anti-human-T-cell globulin which does not crossreact with hemopoietic precursor cells. These experiments finally led to a product of reliable anti-T-cell activity which did not inhibit human colony-forming cells in agar.

Ergebnisse des Projekts Nr. 5

Leiter des Projekts und wissenschaftliche Mitarbeiter:

S. Thierfelder, H. Rodt, E. Thiel und H. Huhn

Titel des Projekts:

T-B-cell concentration in normal athymic and chimaeric rodents.

---

In earlier experiments we had measured the number of T- and B-cells in normal and athymic mice as well as in radiation chimaeras. We also could quantify the amount of T-cell-antigen on single cells by automatic quantitative immuno-autoradiography, a method developed in this laboratory. Preliminary experiments in rats demonstrated a much higher number of T-cells in the bone-marrow of rats than found in mice. While less than 5% T-cells were found in murine bone marrow, more than 20% were found in rats. This confirms indirectly the observation of several authors that rat bone marrow causes a more acute type of secondary disease.



Ergebnisse des Projekts Nr. 6

Leiter des Projekts und wissenschaftliche Mitarbeiter:  
P. Dörmer, W. Brinkmann und E. Korge

Titel des Projekts:

Erythropoiesis and kinetics of erythroblasts in  
anemias and aplasias in man.

---

The kinetics of bone marrow cell proliferation have been reinvestigated in a patient in the phase of acute myelogenous leukemia who had been studied two years earlier in the preleukemic state presenting as pancytopenia. By comparison of the kinetics in preleukemia and in overt leukemia some conclusions could be drawn concerning the mechanism of amplification of the leukemic blast cell pool.

Erythropoiesis in this patient was found ineffective to the same extent in both stages of disease with most erythroid cell precursors dying at the polychromatic erythroblast stage. There was, however, some additional slowing down of the rate of erythroblast proliferation in manifest leukemia which had been found normal in preleukemia.

The rate of myeloblast proliferation was slightly reduced in preleukemia and a little more reduced in overt leukemia. Already in preleukemia the fraction of non-proliferative myeloblasts was found as high as in leukemia. Concerning the proliferative pattern in preleukemia there are two plausible explanations for the drastic increase in the blast cell number towards development of overt leukemia. The one is based on the assumption of a considerably increased rate of stem cell differentiation. Experimental data, however, derived from the CFU<sub>c</sub> technique speak against such a mechanism. The other is based on transition of steady state growth of leukemic myeloblasts to some degree of exponential growth. Results obtained in the patient lend support to the second explanation: out of 14 dividing myeloblasts only one cell passed over to the compartment of promyelocytes after division, the rest of 13 blast cells remained in the original compartment.

A model was constructed from these data suggesting that every increase in the leukemic blast cell pool is effected by an increasing fraction of exponentially growing myeloblasts. In other words: with expansion of the blast cell pool its degree of "stemness" increases.

Ergebnisse des Projekts Nr. 7

Leiter des Projekts und wissenschaftliche Mitarbeiter:  
H. Grosse-Wilde, W. Mempel, B. Netzel, E.D. Albert und  
S. Thierfelder

Titel des Projekts:  
Tissue typing for bone marrow transplantation:  
Definition of MLC-alleles in men and dogs.

---

Typing for MLC alleles was continued. Based on the results of the '6th International Histocompatibility Workshop Conference 1975 Aarhus/Denmark' the genetic locus HLA-D was accepted as the official WHO nomenclature. 6 alleles (HLA-DW1-W6) were defined. Our laboratory serves as reference laboratory for HLA-DW2. Recently 3 new types were found by our group: HLA-D Type B0, EI and RE. A computer program for defining and storing MLC-tests was developed. The MLC-typing was performed in 3 patients with aplasia or leucemia grafted with bone marrow in Munich.

NUCLEAR MEDICINE DEPARTMENT  
UNIVERSITY COLLEGE DUBLIN  
and  
ST. VINCENT'S HOSPITAL, DUBLIN

Contract No. : 140/74/1 BOIEIR  
Director : Dr. George Duffy  
Subject of Contract : The Evaluation of Radiochemicals  
in Localising Atheromatous Disease in  
Arteries

---

During 1975 investigations were begun into the possible detection and localisation of arterial disease using radiopharmaceuticals and external gamma ray detection.

Since Fibrin thrombi are frequently adherent to or incorporated into atheromatous plaques, the fibrinolytic enzyme streptokinase labelled with the radionuclide technetium 99m (TC-99m) is being considered as a possible useful localising agent. Although the use of this radiopharmaceutical has been reported in a number of studies in the detection of venous thrombosis, we have encountered great difficulty in satisfactorily labelling this agent with TC-99m. We report (Project 1) on some of the reasons for the unsatisfactory labelling of this agent and describe the methods we have used in evaluating the efficiency of labelling and on the optimum conditions we have found for labelling this enzyme protein.

Results of Project No. 1

Head of Project and Scientific Co-workers : M.J. Duffy, Ph.D.  
and  
G.J. Duffy, M.D.

Title of Project : Preparation of Tc-99m Streptokinase

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The main problems with the radionuclide labelling of streptokinase with Tc-99m were (a) reduction of Tc  $O_4^-$  to an active state at physiological pH. (b) development of a mild and rapid procedure for the separation of the free from the protein-bound Tc-99m. (c) labelling of non-streptokinase proteins in commercial preparations of the fibrinolytic enzyme (Streptase Hoechst).

Labelling of streptokinase was accomplished at pH 7.4 using stannous pyrophosphate. Optimum conditions for labelling at room temperature were incubation for three hours in the presence of 3mM stannous pyrophosphates. Using thin-layer chromatography in two solvent systems (acetone and 0.5% NaCl) it was shown that about 50% of the Tc-99m was protein-bound. Paper chromatography using methanol as solvent and trichloroacetic acid precipitation of protein were found to be unsuitable for separation bound Tc from the free species. The former technique overestimates the amount of bound Tc while the latter underestimates the bound radionuclide. Polyacrylamide gel electrophoresis showed that commercial streptokinase (Streptase Hoechst) contains a considerable amount of filler substance, proteins which are also labelled with Tc-99m. However, using purified streptokinase only one single protein-band was labelled. The results to date show that streptokinase can be labelled at physiological pH using stannous pyrophosphate, that thin-layer chromatography is a quick and easy method, to determine the amount of Tc-99m bound, and to separate free from bound Tc-99m.

Finally, for the detection of thrombi, whether venous or arterial, purified rather than commercial streptokinase is needed.

NEUTRONENDOSIMETRIE

NEUTRON DOSIMETRY

DOSIMETRIE DES NEUTRONS

Weitere Forschungsarbeiten zu diesem Thema werden auch in folgenden Jahresberichten beschrieben:

Further research work on these subjects will also be described in the following annual reports:

D'autres travaux sur ce thème de recherche sont également décrits dans les rapports annuels suivants:

094-BIAN      ITAL, Wageningen (De Zeeuw)

101-BIOC      Dosimetry Group

Biology Group Ispra

GESELLSCHAFT FÜR STRAHLEN- UND UMWELTFORSCHUNG MBH, MONCHEN  
Institut für Strahlenschutz, Neuherberg

Vertrag: 113 BIOC

Leiter der Forschungsgruppe: Dr. Georg Burger

Allgemeines Thema des Vertrages: Calculation and Measurement of Absorbed Dose and Neutron Spectra inside a Biological Object with Reference to Fast Neutron Radiotherapy

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The program is concerned with the determination of the biological effective neutron dose inside human phantoms. It includes neutron and gamma transport calculations and measurements with TE-proportional counters and various ionization chambers.

In addition to the scientific studies, the ENDIP intercomparison was performed. The main activities in this respect refer to the development and testing of the universal monitoring and data processing system MOSES (Monitoring System for the ENDIP-Service).

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References:

- /1/ Grünauer, F.:  
Dissertation TH München, 1975  
GSF-Report S 329
- /2/ Burger, G., F.Grünauer and E.Maier  
Mixed field phantom dosimetry  
Biochemical dosimetry, proc. of a IAEA symposium, Wien, 10.-14.3.75  
IAEA, Wien, IAEA-SM 193/18, 71-85 (1975); GSF-Bericht S 361
- /3/ Burger, G., F.Grünauer, A.Morhart und H.Schraube  
Entwicklung eines Neutronentherapie-Bestrahlungskopfes mit Neutronentarget für das DKFZ-Heidelberg, GSF-Berich S 346 (1975)
- /4/ Burger, G. and E.Maier  
Mixed field dosimetry with the twin chamber technique  
GSF-Bericht S 299 (1974)
- /5/ Maier, E., P.Knesewitsch and G.Burger  
Mixed field dosimetry with ionization chambers and proportional counters  
Proc. 2.symp. on neutron dosimetry in biology and medicine. Neuherberg, 30.9.-4.10.1974, EUR 5273, Luxemburg, 263-285 (1975); GSF-Bericht S 389

## Ergebnisse des Projekts

### Leiter des Projekts und wissenschaftliche Mitarbeiter:

G.Burger, E.Maier, F.Grünauer, H.Schraube

Titel des Projekts: Calculation and Measurement of Absorbed Dose and Neutron Spectra inside a Biological Object with Reference to Fast Neutron Radiotherapy

---

### 1. ENDIP-measurements

At the GSF neutron calibration hall, which was constructed in the past years under the sponsorship of EURATOM, the installations had to be extended for ENDIP in the beginning of 1975 by a monitoring and data collecting system (MOSES). The system includes primarily two parallel plate ionisation chambers and a gamma counter. In addition, a moderator counter for monitoring of fast neutron flux and a moderator detector with two counter tubes for spectral monitoring are parts of the system. A DePangher PLC serves as the total neutron fluence standard.

In addition to MOSES two devices for monitoring and positioning the participants' dosimeters were constructed, one at the calibration source and another at the accelerator target. An optical telescope allows the exact alignment of the dosimeters relative to the targetcenter.

Besides monoenergetic neutrons from target reactions at the 3 MV VanDeGraaff accelerator, also a 2 mg Cf-252 source was used. For storing, handling and positioning of this source a tube-system was developed. Between April and July 11 groups participated in the intercomparison program.

### 2. Phantom dosimetry

For mixed field dosimetry in phantoms irradiated with fast neutrons, the twin chamber technique is widely used and, for special purposes, TE proportional counters may be employed. The most recent data on W-values, stopping power ratios and kerma ratios, as well as their absolute uncertainty, energy dependence and variation inside the phantom were investigated for TE and C/CO<sub>2</sub> ionization chambers. From the uncertainties the total errors for the neutron and gamma dose components, the total dose, and an effective dose were determined by an extended error analysis (fig.1). Criteria were established for the allowed uncertainty in the determination of the gamma dose component. For a TE proportional counter, pulse-height distributions were measured and calculated. The separate determination of the neutron and gamma dose components was analyzed. By assuming reasonable RBE values as a function of neutron energy, the change of radiation quality for the neutron component was calculated for different radiation geometries inside a phantom. The calculations were made for several phantom depth and lateral distances from the beam axis. Proportional counter measurements were used together with a reasonable RBE versus LET relationship to verify the results of the transport calculations (fig.2).



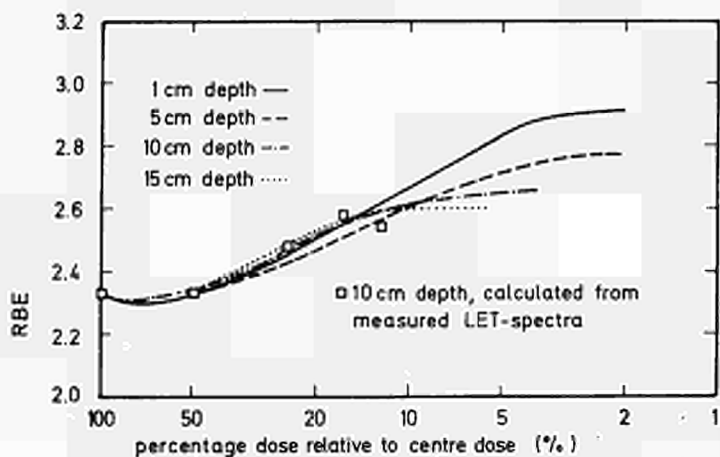


Fig.1 Relative uncertainties for the total ( $D_t$ ), effective ( $D_e$ ), neutron ( $D_n$ ) and gammadose ( $D_\gamma$ ) determined on the basis of twin chamber measurements.

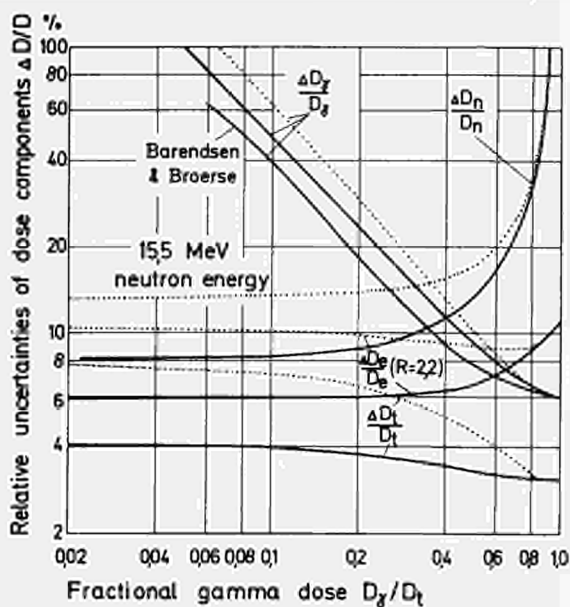


Fig.2 Calculated local variation of RBE in a phantom irradiated with a collimated beam of 15 MeV neutrons.



Vertragspartner der Kommission:  
Gesellschaft für Strahlen- und Umweltforschung  
München  
Nr. des Vertrages: 113-72-1 BIOC  
Leiter der Forschungsgruppe:  
Prof.Dr.W.Pohlit  
Allgemeines Thema des Vertrages:  
Calculation and Measurement of Absorbed Dose  
and Neutron Spectra inside a Biological Object  
with Reference to Fast Neutron Radiotherapy

---

The average energy expenditure  $W$  to produce an ion pair in different gases was determined for protons within an energy range of 0.1 - 0.55 MeV. The experiments are concluded. Preliminary values of  $W$  for tissue equivalent gas are given in the following Table. The evaluation of the final results with the systematic and statistical uncertainties are continued and will be given in a dissertation by T. Werba.

Table

Energy /keV	W/eV
114,8	31,95
252,2	32,24
401,7	32,11
550,7	31,53

Ergebnisse des Projektes

Nr. 113-72-1 BIOC

Leiter des Projektes und wissenschaftliche

Mitarbeiter: Dr.H.Kühn, Dipl.-Phys.T.Werba

Titel des Projektes:

Determination of the average energy expenditure to produce an ion pair in different gases for heavy ions

The determination of the energy expenditure  $W$  to produce an ion pair in a gas by charged particles was performed with an ionization chamber big enough to allow the primary ions and secondary particles to be stopped totally at the chosen gas pressure. The chamber was connected to the beam guide of a BBC-accelerator by a double pumping stage arrangement. The determination of the  $W$  value requires measurements of the primary ion particles entering the ion chamber and of the charge produced in the ion chamber. The entrance beam was monitored by a rotating Faraday cup. Further the energy of the primary ions must be known as accurate as possible. The energy was determined by measuring the gamma yield of nuclear resonance reactions for different accelerator voltages as a function of the magnetic field of the deflecting magnet within the beam guide. The magnetic field strength was determined by measuring the resonance frequency of a proton resonance magnetic field measuring device. The equation for the energy of the particles  $E$  and the resonance frequency  $f$  is given by  $E = a f^2$ . The constant  $a$  was determined by the calibration method using 7 nuclear resonance reactions with known resonance energies in the range of 224.4 keV - 505.5 keV. The measurements of the charges were performed with Keithley electrometers. Difficulties arose

due to the broadning of the primary beam by scattering of the particles within the double pumping stage arrangement. The measurements have been performed with tissue equivalent gas, methane, carbondioxide, and nitrogen irradiated with protons.

Some preliminary results are summarized in the following Tables:

Table I

W - value of methane

Energy /keV	W/eV
127.5	30.54
253.5	30.69
397.2	29.94
546.7	30.06

Table II

W - value of carbondioxide

Energy /keV	W/eV
125.1	35.62
250.9	35.92
403.0	35.04
550.0	34.80

Table III

W - value of nitrogen

Energy /keV	W/eV
105.2	36.86
233.6	36.78
405.4	36.60
555.4	36.45



Contractor : Medical Research Council

Contract : 113-72-IBIOC

Head of research team : Mr. D.D. Vonberg

General subject of Contract : Correlation of physical  
and biologically effective dose of fast neutrons.

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Description of the work carried out.

During 1975 the biological part of the work has been extended to an investigation of biological effect as a function of position across the beam in a phantom, both inside and outside the main irradiated area.

The measurements of the change in the neutron spectrum in the phantom compared with the spectrum with no phantom present using activation and fission detectors have been continued. They have been extended to include an investigation of the effect of field size on the spectrum in the phantom. A liquid scintillator spectrometer incorporating neutron-gamma pulse-shape discrimination is being developed in order to verify the results obtained so far.

Two papers on the work done under this contract have been accepted for publication and others are in preparation, but none have yet been published.

Results of Project No. : 1

Head of Project and scientific staff : D.K. Bewley  
B. Cullen

Title of Project : Biological effectiveness of neutron beam  
in a phantom as a function of position with respect to  
the beam edge.

---

Description of the work carried out.

Mouse Erlich acites cells are being used as biological dosimeters to study the beam profile in a phantom. The cells are uniformly suspended in a gel contained in a syringe. The syringe is placed across the edge of the field so that some cells are in the main beam, some outside and some in the penumbra. After irradiation the cylinder of gel is cut into sections, the cells cultured and clones counted. In this way the relative effective dose can be determined across the beam, i.e. a biologically effective beam profile. This is compared with a profile of physical absorbed dose.

This project is in a very early stage and so far only preliminary measurements have been made, not in a phantom.



Results of Project No. 2.

Head of Project and	D.K. Bewley
Scientific staff:	C.J. Parnell
	D.E. Bonnett

Title of Project: Study of modification of  
neutron spectrum by a phantom

Description of results

The set of activation detectors and pulse fission chambers have been used to investigate the neutron spectra at various depths in the phantom. These measurements have been made with three field sizes, 5 x 5, 9.5 x 9.5 and 21 x 21 cm<sup>2</sup>. The spectra obtained with the two smaller fields are similar in that there is an increase in low energy neutrons below 2 MeV and a gradual hardening of the spectrum with depth in the phantom. The main difference is that the peak of the low energy build up occurs at a smaller depth and has a smaller magnitude with the smallest field. The neutron spectrum has also been measured outside the main beam in air and on the phantom surface for the 9.5 x 9.5 cm<sup>2</sup> field. Results show that low energy neutrons form the dominant part of the spectrum, with only a small contribution from higher energy neutrons. On the phantom surface the low energy component shows an increase due to backscattered neutrons from the phantom. The measurements with the larger field size are nearing completion. No results have yet been obtained using the liquid scintillator spectrometer.



Contractor: Radiobiological Institute TNO  
Rijswijk, the Netherlands

Contract No.: 113-72-1 BIOC

Head of Research Team: J.J. Broerse

General Subject of Contract: Calculation and measurement of absorbed dose and neutron spectra inside a biological object with reference to fast neutron radiotherapy.

---

For the determination of the dose distribution in the human phantom, various experimental techniques have been employed including ionization chambers, Geiger-Müller counters and activation detectors. For the interpretation of the results obtained with the latter type of detector, it was necessary to determine the neutron energy spectra. In addition the spectrum information is essential in view of the dependence of the relative biological effectiveness (RBE) on neutron energy.

Neutron energy spectra have been determined with an organic liquid scintillator at different positions in and outside the beam behind a collimator free-in-air. The spectra measured behind the shielding show a considerable contribution of low energy scattered neutrons. Additional neutron energy measurements at various depths in a phantom are in progress.

Radiobiological Institute TNO, Rijswijk, The Netherlands

Contract No. 113-72-1 BIOC

J.J. Broerse, B. Hogeweg and J. Zoetelief.

Calculation and measurement of absorbed dose and neutron spectra inside a biological object with reference to fast neutron radiotherapy.

For radiotherapy with external sources of neutrons, the neutron beam has to be collimated to obtain a defined dose distribution at the site of the tumour. Interaction of the primary radiation with the collimator materials may change the original free-in-air energy distribution as emitted by the source. Moreover, changes of the spectrum with penetration depth in the patient can be expected. These changes in neutron spectrum can result in differences in the relative biological effectiveness (RBE), both longitudinal and transverse, over the beam. Knowledge of the neutron energy distributions is also required for calculations of the neutron dose from fluence measurements.

Neutron energy spectra have been measured for collimated and uncollimated neutron beams of 3 and 15 MeV energy with an organic scintillator proton-recoil spectrometer. The detector is a cylindrical cell NE 213 liquid scintillator (Nuclear Enterprises, type VH1) mounted directly on a photomultiplier (Philips, type 56 DVP). Discrimination between pulses produced by neutron-induced protons and gamma-induced electrons is provided by a pulse-shape discriminator circuit of usual design (Colvett et al., 1971). The resulting recoil proton pulse-height spectra are unfolded with a computer code, in cooperation with Chemtob and Nguyen from the Laboratoire de Dosimétrie Sanitaire, Centre d'Etudes Nucleaires du Fontenay-aux-Roses. The neutrons are produced with a Van De Graaff electrostatic generator by bombarding deuterium or tritium targets with a 400 keV deuteron beam. For collimation of the beams, the steel collimator with extra lucite shielding in front of the opening as earlier described is used (Hogeweg, 1974).

Measured differential flux density distributions free-in-air and in collimated beams for 3 and 15 MeV energy neutrons are presented in figures 1 and 2, respectively. The distributions are normalised to equal flux densities over the measured energy regions. The results demonstrate a slight broadening towards the lower energy part of the peaks in these distributions measured for the centre of the collimated beam compared to the free-in-air situation. This is due to small angle scattering of primary neutrons by the

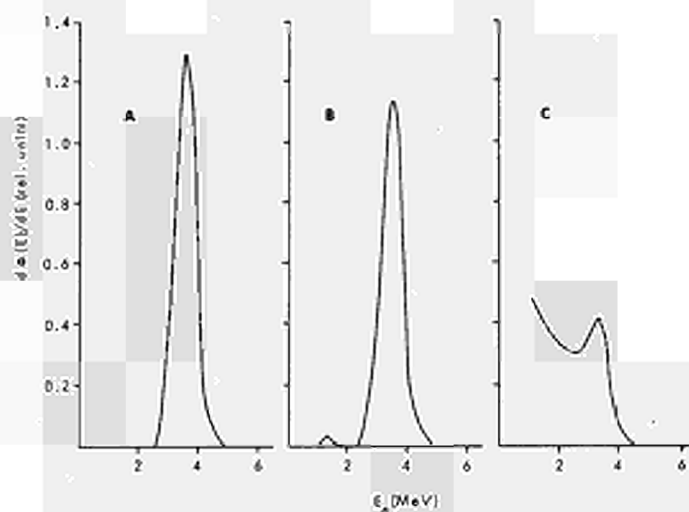


Figure 1. Differential distribution of the fluence for neutrons with primary energy of 3.3 MeV.

- a) free-in-air
- b) collimated beam, in centre of the beam
- c) collimated beam, behind the shielding

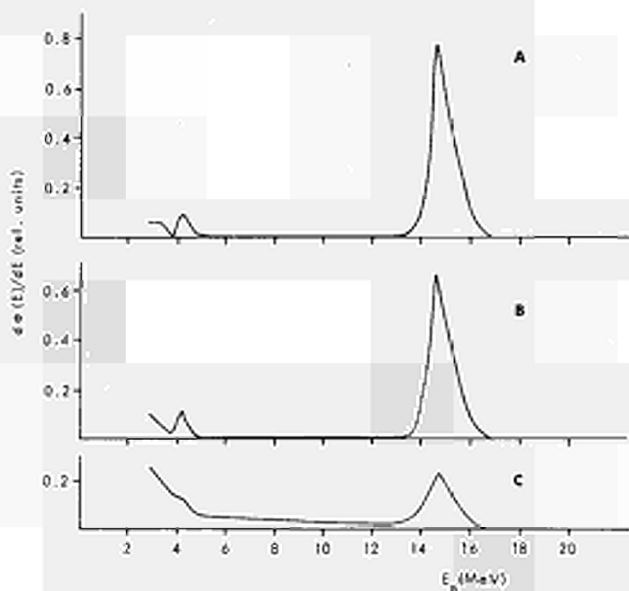


Figure 2. Same for neutrons with primary energy of 14.8 MeV.

inner surface area of the collimator duct. This effect of small angle scattering also causes an increase in the flux density with about 15 per cent relative to the free-in-air situation, as could be derived from these measurements. A similar increase was observed for the dose in collimated beams relative to free-in-air conditions (Broerse, 1967) and could also be deduced from event size distributions as measured with tissue-equivalent proportional counters (Hogeweg, 1975).

The spectra measured behind the shielding clearly show a considerable contribution of low energy scattered neutrons. For the d-D neutron beam, a more or less continuous spectrum is measured between 1 and 4 MeV. For the d-T neutron beam, the peak at an energy of 14.8 MeV is reduced considerably. Further neutron energy measurements at various positions in a phantom are in progress.

The RBE of the neutron component of the radiation field may increase as a function of distance from the central beam axis, because such an increase is generally observed for lower neutron energies and lower dose values. However, the contribution of gamma radiation to the total dose increases considerably outside the penumbral area (Broerse et al., 1974) and this effect will tend to decrease the RBE for the mixed radiation field. Experiments with cultured cells of human kidney origin are presently being carried out in order to determine the biologically effective dose.

#### References

- Broerse, J.J., *Kerntechnik*, 10, (1967), 446.  
Broerse, J.J., Broers-Challiss, J.E., and Maruyama, T., p. 627, In: Proc. First Symposium on Neutron Dosimetry in Biology and Medicine, Commission of the European Communities, Luxembourg, 1972.  
Colvett, R.D., Marino, S.A., and Rodgers, R.C., In: Annual Report on Research Project, 1971, NYO-2740-8.  
Hogeweg, B., 1974, personal communication.  
Hogeweg, B., In: Proc. 5th. Symposium on Microdosimetry, Verbania, 1975. Commission of the European Communities, in press.

#### LIST OF PUBLICATIONS Contract No. 113-72-1 BIOC

- Broerse, J.J., and Barendsen, G.W., Radiobiological and physical characteristics of fast neutron beams.  
In: Proceedings of the IXth International Cancer Congress, Florence, Italy, 1974. Vol. 5, Surgery, Radiotherapy and Chemotherapy of Cancer, Amsterdam Excerpta Medica, pp. 168-173 (1975).
- Broerse, J.J., Broers-Challiss, J.E. and Mijnheer, B.J., Depth-dose measurements of d-T neutrons for radiotherapy applications.  
*Strahlentherapie*, 149 (1975), pp. 585-596.

Contractor:

International Commission on Radiation Units and  
Measurements (ICRU)

Contract Number:

113-72-1 BIOC

Head of research teams:

Harold O. Wyckoff

General subject of contract:

ICRU Neutron Dosimetry Intercomparison Project

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Several unforeseen events have delayed the completion of the report on the Intercomparison Study. However, the analysis is essentially complete although the discussion of the results is still to be prepared. It is expected that this will be completed in 1976.





Contractor: GSF - Projektgruppe Neuherberg  
- Projektgruppe Frankfurt  
MRC Cyclotron Unit  
TNO Radiobiological Institute

Contract No.: 113-72-1 BIOC

General Subject: European Neutron Dosimetry  
Intercomparison Project (ENDIP)

Heads of Project: D.K. BEWLEY, J.J. BROERSE (Chairman),  
G. BURGER, W. POHLIT.

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In the past period the European Neutron Dosimetry Intercomparison Project (ENDIP) has been performed at two locations: GSF-Neuherberg (for institutes, working on neutron radiobiology and related dosimetry studies) and TNO-Rijswijk (for institutes working on fast neutron radiotherapy). The measurements at Neuherberg have been carried out in free air for mono-energetic neutron beams with energies of 15.5, 5.5, 2.1 and 0.67 MeV and for fission neutrons emitted by californium-252. The measurements at Rijswijk have been performed for collimated beams of d-T and d-D neutrons (maximum energies 15,5 and 5.5 MeV, respectively) both free-in-air and at three depths in a phantom.

The mono-energetic neutrons were produced with 3 MeV Van De Graaff accelerators. The neutron beam at Neuherberg has been monitored by a system (Moses, monitor system of the ENDIP service) consisting of TE chamber, fission chamber and moderating counters. The system at Rijswijk (Josua, joint system for neutron dosimetry intercomparison) included two disc-type transmission ionization chambers and a Geiger-Müller counter. Alignment of the participants' dosimeter was performed with the aid of a movable support system; the positioning was checked with two telescopes.

Over the period April to July, 1975, a total of ten groups participated in the ENDIP sessions at Neuherberg, whereas twelve groups performed measurements at Rijswijk over the period October to December, 1975. The first results from the participants have been received, the analysis of all data will be performed in the coming contract period.



VI.

KOORDINIERUNGSTÄTIGKEIT

COORDINATION

ACTIVITES DE COORDINATION



## VI. COORDINATION

The three successive multiannual Biology/Health Protection Programmes have resulted in the building-up of an integrated scientific community which has genuinely worked on a cooperative base and has in this way endeavoured to one of the most important aims of the Commission's intervention in research. In fact, the implementation of the provisions of the Euratom Treaty in the field of Radiation Biology has served to demonstrate from the very start that the coordinating role of the Commission is an absolute prerequisite to the formulation and execution of Community objectives.

A detailed scheme of the different levels of coordination has already been published.<sup>+)</sup>

As an important tool of coordination "Study Groups" have been set up since the second multiannual Biology/Health protection programme.

Their principal role has been to serve as scientific forums bringing together, for each programme sector or objective the parties under contract to the Commission, the Commission itself and also independent experts. Those who met in 1975 have been listed in part A of this chapter.

Part B lists the meetings which the Commission has organized itself or actively supported, within the limits of its financial resources. It is evident from the worldwide resonance that some of these meetings have encountered, that they play an encouraging and stimulating role for the European scientific community.

The Health Protection Directorate also organizes meetings of experts whose work has both the effect of coordinating and stimulating the research in the Commission's Biology/Health Protection programme, especially with a view to practical measures for promoting radiation protection.

Part C lists these groups, particularly the Group of Experts on "Basic Safety Standards" mentioned in Article 31 of the Euratom Treaty.

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<sup>+) Annual Report 1972 "Biology/Health Protection", EUR 4864, chapter "Coordination", pages 747-751.</sup>



A.

Meetings of  
Study Groups in 1975

Study Group on Plant Cell and Plant Tissue Cultures

Grünbach (Germany), 30 January 1975

14 participants from 5 countries and the Commission

Principal subject :

Discussion of the results obtained by the contractants with regard to

- anther and pollen cultures
- protoplasts
- differentiation in vitro.

---

Study Group "European Neutron Dosimetry Intercomparison Project"

Rijswijk, Z.H., 20-21 January 1975

3 participants from 2 countries and the Commission

Principal subject :

Preparation of the intercomparison programme

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Study Group "European Neutron Dosimetry Intercomparison Project"

Neuherberg-München, 17-18 April 1975

3 participants from 2 countries and the Commission

Principal subject :

Discussion on technical aspects of ENDIP

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Study Group "Dosimetry"

Rijswijk, Z.H. 24-25 April 1975

26 participants from 4 countries and the Commission

Principal subject :

Physical and biological data relevant to radiation protection and radiotherapy

---

Study Group "Primary Effects of Ionizing Radiation on Nucleic Acids"

Brussels, 23-24 June 1975

18 participants from 7 countries and the Commission

Principal subject :

Advances in studies of radiation effects in DNA ; properties and behaviour of radicals in nucleic acids in solid and liquid state and in complexes of nucleic acids and dyes or amino acids ; state of the book on "Effects of Ionizing Radiation on DNA ; physical, chemical and biological aspects"

---

Study Group "Radioentomology"

Munich, Germany, 24-25 September 1975

18 participants from 6 countries and the Commission

Principal subject :

Development of the sterile insect technique, of genetic control systems others than sterile insect technique, of nuclear technique in entomology ; studies on behaviour, physiology, histology, mass rearing and population dynamics

---

Study Group "European Neutron Dosimetry Intercomparison Project"

Rijswijk, Z.H., 15-16 October 1975

4 participants from 2 countries and the Commission

Principal subject :

Examination of preliminary results of the intercomparison.

---

Study Group on the Hereditary Effects of Radiations

London, 19-21 October 1975

55 participants from 8 countries and the Commission

Principal subject :

Discussion of the results obtained by the contractants with regard to

- the modifications of dose-effect relations and chromosomal aberrations
  - sensitivity and repair in higher organisms and in their cultured cells
  - sensitivity and repair in microorganisms
-



Study Group "Personal Dosimetry"

Luxembourg, 27 October 1975

23 experts of 8 countries and the Commission

Principal subject :

Discussion of the programme of research on personal dosimetry

---

Study Group "Primary Effects of Ionizing Radiation on Nucleic Acids"

Brussels, 16 December 1975

5 participants from 2 countries and the Commission

Principal subject :

Finalizing of the book on "Effects of Ionizing Radiation on DNA ;  
physical, chemical and biological aspects"

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B.

Meetings organized or co-sponsored by  
the Commission of the European Communities in 1975

Council meeting of the European Organization for Research on Treatment  
of Cancer (EORTC)

Brussels, 25 January 1975  
21 participants from 7 countries

Principal subject :  
Leukemia and hematosarcoma ; proliferation-inhibitory effect of  
leukemic cells ; in-vitro methodologies of hemopoietic stem cells ;  
data-center EORTC

---

Seminar : Exchange of views between the competent authorities of the  
riparian states on the level of radioactive contamination  
in the river Meuse

Namur, 24-25 April 1975  
30 participants from 3 countries and the Commission

---

Group of experts responsible for the radiation protection in nuclear  
power plants

Luxembourg, 6 May 1975  
35 experts from 9 countries and the Commission

Principal subject :  
Radiation protection in nuclear power plants

---

Third European Congress of the International Radiation Protection  
Association

Amsterdam, 13-16 May 1975  
Principal subject :  
Criteria for Radiation Protection

---

Meeting of the co-operative group of the European Organization for  
Research on Treatment of Cancer (EORTC)

Paris, 28 June 1975

17 participants from 8 countries

Principal subject :

Immuno-oncology

---

Mutation Breeding Contact Group

Cadarache, France, 9-12 September 1975

53 participants from 18 countries and the Commission

Principal subject :

- incompatibility and reproductive barriers in higher plants
  - protein improvement
  - disease resistance
  - mutation breeding of vegetatively propagated species
- 

Fifth Symposium on Microdosimetry

Verbania Pallanza, 22-26 September 1975

130 participants from 13 countries and the Commission

Principal subject :

Spectral and spatial distribution of radiation-energy transfer in irradiated tissue and radiation-energy deposition in organs, cells and their components, and biomacromolecules. Physical, chemical and biological aspects of microdosimetry of monoenergetic ions, fast neutrons and their secondaries, and low LET radiation

---

International Conference on Molecular- and Microdistribution of  
Radioisotopes and Biological Consequences

Jülich, 2-4 October 1975

90 participants from 11 countries and the Commission

Principal subject :

Influence of microdistribution of incorporated radioisotopes on biological effects

---

Seminar : Information and Education in radiation protection

Brussels, 7-8 October 1975

40 representatives of the trade unions in the 9 countries and the Commission

---

Symposium on "Radiation and Cellular Central Processes"

Giessen, Germany, 6-11 October 1975

50 participants from 14 countries and the Commission

Principal subjects :

- radiation and metabolic key processes
  - repair and recovery
  - cell division and progression
- 

Point source Committee and workshop of the bone seeking isotope project (European Late Effects Project Group)

Casaccia, 16-17 October 1975

50 participants from 7 countries

Principal subject :

EBONY-Project ; Tritium ; Decorporation of transuranian elements

---

Seminar : Problems posed by the growing use of common goods containing radioactive substances

Luxembourg, 13-14 November 1975

80 participants from the 9 countries and the Commission

---

Workshop : Intercomparison of dosimeters. Calibration with monochromatic X-rays between 10 keV to 300 keV,  $\gamma$ -rays of  $^{60}\text{Co}$  and n- $\gamma$  capture radiation of 9 MeV.

Fontenay-aux-Roses, 19 November - 5 December 1975

40 participants from 8 countries and the Commission organized in common with the Commissariat à l'Energie Atomique

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C.

Meetings of Experts

Chapter III of the Euratom Treaty in 1975

Group of Experts "Article 37" of the Euratom Treaty

Brussels, 30-31 January 1975

28 participants from the 9 countries and the Commission

Principal subject :

Examination of estimates radioactive effluent discharge from nuclear power stations

---

Group of Experts "Article 37" of the Euratom Treaty

Paris, 20-21 February 1975

12 participants from the 9 countries and the Commission

Principal subject :

Comparison of iodine filters

---

Group of Experts "Personal Dosimetry"

Luxembourg, 6 March 1975

14 participants from the 9 countries and the Commission

Principal subject :

Preparation of technical recommendations for radio-photoluminescent Dosimetry

---

Group of Experts "Article 36" of the Euratom Treaty

Luxembourg, 24-25 March 1975

30 participants of the 9 countries and the Commission

Principal subject :

Environmental Radioactivity

---

Group of Experts "Personal Dosimetry"

Luxembourg, 6 May 1975

21 participants of the 9 countries and the Commission

Principal subject :

Neutron dosimetry

---

Group of Experts "Article 31" of the Euratom Treaty

Le Vesinet, 29-30 May 1975

22 experts from the 9 countries and the Commission

Principal subject :

Review of Euratom Basic Safety Standards

---

Group of Experts "Personal Dosimetry"

Luxembourg, 12-13 June 1975

4 experts of 2 countries and the Commission

Principal subject :

Drafting technical recommendations for Radiophotoluminescent  
Dosimetry

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Group of Experts "Personal Dosimetry"

Luxembourg, 1-2 July 1975

3 experts from 2 countries and the Commission

Principal subject :

Drafting technical recommendations for Radiophotoluminescent  
Dosimetry

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Group of Experts "Article 37" of the Euratom Treaty

Brussels, 11 July 1975

28 experts from the 9 countries and the Commission

Principal subject :

Examination of estimates radioactive effluent discharge from nuclear  
power stations

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Group of Experts "Article 37" of the Euratom Treaty

Mol, 2-3 September 1975

12 experts from the 9 countries and the Commission

Principal subject :

Intercomparison of iodine filters

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Group of Experts "Personal Dosimetry"

Luxembourg, 28 October 1975

23 experts from 8 countries and the Commission

Principal subject :

Approval of the draft "technical recommendations for photoluminescent dosimetry". Discussion of the result of the intercomparison of personal doseimeters executed in spring 1975

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Anhang zum Abschnitt III. 5. "Langzeitwirkungen", Bd. I.

Annex to the section III. 5. "Long-term effects", vol. I.

Annexe à la section III. 5. "Effets à long terme", vol. I.



Commission Associate: ENEL - Ente Nazionale Energia Elettrica

Contract number: 062 - 72 - 6 - PST 1

Head of research team: Prof. Antonio Farulla

General subject of the contract: Effects of prolonged exposure to low levels of ionizing radiation; morphological, cytochemical and cytogenetic researches on circulating lymphocytes of subjects professionally exposed to the hazard of ionizing radiation in a nuclear power station of Enel.

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The present report concerns the results of the chromosome analysis carried out during 1975 on workers professionally exposed to ionizing radiation in a nuclear power station of Enel. Karyotype analysis was performed on 52 nuclear workers and on 26 unexposed persons which formed the control group. Also summary data of the cytogenetic controls from 1972 to 1975 are reported.

The research activity in 1975 was directed toward the evaluation of possible metabolic changes in subjects professionally exposed to radiation. Among the various biochemical parameters of cell damage induced by radiation, the first choice was restricted to RNA synthesis, since this metabolic activity is a very sensitive parameter of an initial cellular radiation damage.

#### Publications

- A. Farulla, F.A. Manzoli: Comportamento dei recettori dei linfociti B e T sottoposti ad irradiazione. XI Congr. Naz. Radiobiologia, Rapallo 25-26 aprile 1975
- A. Farulla, F.A. Manzoli: Aspetti dei linfociti B e T sottoposti ad irradiazione. Soc. Ital. Ematologia, Simposio su "La Biologia del Linfocita", Pescara 26 giugno 1975
- A. Farulla, G. Naro e coll.: Radiosensibilità cromosomica in vitro: influenza di una preesistente patologia cromosomica congenita o acquisita. Atti XIX Congr. Ass. Ital. Fisica Sanit. Protez. Radiazioni, Levico Terme 17-19 settembre 1975
- A. Farulla, G. Naro e coll.: Studio cromosomico di soggetti professionalmente esposti alle onde radar. Ibid.
- A. Farulla, F.A. Manzoli: Effect of radiation on membrane receptors of B and T lymphocytes. Radiat. Res. in press
- A. Farulla: L'apporto di nuove metodiche: la dosimetria biologica. Securitas, in corso di stampa.

Results of project n. 1

Head of project and scientific collaborators:

A. Farulla - A. Manzoli - V. Monesi - G. Naro

Project title: Effects of prolonged exposure to low levels of ionizing radiation: morphological, cytochemical and cytogenetic researches on circulating lymphocytes of subjects professionally exposed to the hazard of ionizing radiation in a nuclear power station of Enel.

#### CYTOGENETIC SURVEY

During 1975 a cytogenetic analysis of cultured circulating lymphocytes was carried out on 52 nuclear workers, who in that period absorbed an external radiation dose between 0,6 rem and 4 rem.

Karyotype analysis at the first mitotic division was performed 48-52 hours after culture. A total of 4.420 cells were analyzed in 52 cultures during 1975. On an average, 85 cells from each individual were scored.

During the same period 26 cultures were performed on blood samples from control subjects, of the same age as the studied group; these subjects were not professionally exposed to ionizing radiation and were not submitted to radiological checkups or radiotherapy in a past few years. These cultures were also harvested 48-52 hours after culture and a total of 2.106 cells were analyzed; on an average, 81 cells from each individual were scored.

Out of 4.420 metaphase figures of the group of subjects professionally exposed the incidence of aneuploidy was 6,1 % (236 hypodiploid and 34 hyperdiploid cells); 330 cells (7,4%) displayed chromatid-type aberrations (gaps and breaks); no chromosome-type aberrations were found.

Out of 2.106 cells of the control group the incidence of aneuploidy was 5,1% (99 hypodiploid and 9 hyperdiploid cells) and the incidence of the cells with chromatid-type aberrations was 5,4% (115 cells); in this group no chromosome-type aberrations were found too.

#### Summary data of the cytogenetic controls from 1972 to 1975.

Altogether 208 cultures on blood samples taken from the group of professionally exposed subjects and 61 cultures of the control group were performed.

Dosimetric data. The group of the professionally exposed subjects had absorbed from 1972 to 1975 an external radiation dose varying between 2 rem and 8 rem. Altogether, since the start of the work in a nuclear plant up to the end of 1975, the accumulated dose ranges from 10 rem to 30 rem (in 8-11 years).

Types and frequencies of chromosomal abnormalities. In the 208 cultures of lymphocytes from nuclear workers we have analyzed

16,133 mitotic cells; in the 61 cultures of the control group 4,568 cells. The main characteristics of the chromosome picture may be summarized as follows.

Numerical abnormalities. In nuclear workers we have found an aneuploidy incidence of 4,7% mainly represented by hypodiploidy. In the control group the incidence of aneuploidy (also in this case with clear prevalence of hypodiploidy) was 4,9%.

Chromatid-type structural abnormalities. In the group of subjects professionally exposed 1,234 cells (7,6%) displayed chromatid-type aberrations, almost exclusively represented by gaps and breaks. In the control group the cells carrying this type of aberrations were 247 (5,4%) with the same characteristics.

Chromosome-type structural abnormalities. Chromosome-type aberrations were not observed both in the exposed group and in the control group; only exceptionally any chromosome deletions were scored in the two groups.

#### BIOCHEMICAL STUDIES IN LYMPHOCYTES FROM SUBJECTS PROFESSIONALLY EXPOSED TO RADIATION.

The research activity carried out in 1972-1974 was aimed to characterize several morphological and biochemical parameters of human lymphocytes stimulated in culture with mitogenic agents. These parameters may represent very sensitive tests of the cell damage caused by small doses of radiation such as those absorbed by professionally exposed workers.

In synthesis, the morphological, cytochemical and biochemical studies performed in the last three years were concerned with the following topics.

1) The kinetics of lymphocyte transformation in culture and cytochemical changes of nucleoli, cytoplasm and chromatin after stimulation with PHA. The most significant cytochemical changes observed concern the nucleoli which become more basophilic and prominent, the cytoplasm which enlarges and becomes stuffed with ribosomes, the chromatin which becomes less condensed and stains more deeply with acridin orange. These changes occur very early, before the activation of DNA synthesis, and concern only a percentage of the cell population.

2) The activity of alkaline phosphatase and of some lysosomal enzymes in lymphocytes stimulated and not stimulated with PHA. The enzymatic activity increases significantly in the stimulated population and concerns only 30 to 50% of the cell population.

3) The protein and RNA synthesis in non stimulated and stimulated lymphocytes. The incorporation of labeled precursors into protein and total RNA increases very rapidly after stimulation with PHA indicating an intense activation of transcriptional and metabolic activities. The increase in RNA synthesis is probably mainly related to the accumulation of a great number of ribosomes following activation, which was seen in

electron microscopy. It is however likely that other portions of the genome, besides the ribosomal cistrons, are activated following stimulation with PHA. This conclusion is consistent with the morphological observation that the amount of euchromatin versus heterochromatin increases significantly in the transformed lymphocytes.

4) Modification of cytochemical and biochemical parameters in professionally exposed workers with respect to the control population. Preliminary data indicated that the rate of protein synthesis is not apparently affected in professionally exposed workers with respect to the control population.

The research activity in 1975 was directed toward the evaluation of possible metabolic changes in subjects professionally exposed to radiation. Among the various biochemical parameters of cell damage induced by radiation, the first choice was restricted to RNA synthesis, since this metabolic activity is a very sensitive parameter of an initial cellular radiation damage.

#### Material and methods.

Eight cultures were performed from human subjects who had accumulated in 8-11 years a total absorbed dose ranging from 15 rem to 25 rem. Nine cultures were performed on blood from normal unexposed subjects.

Suspensions of human leukocytes were obtained with the method described earlier.  $2 \times 10^6$  cells, suspended in TC 199 culture medium supplemented with 15% calf serum were seeded in Petri dishes and incubated for various time intervals at 37°C in a 5% CO<sub>2</sub> atmosphere. PHA was added to some cultures 1 hr. after seeding. Two different experimental approaches were used for the labeling procedure. In one type of experiments <sup>3</sup>H-uridine (NEN, specific activity 2Ci/mM) at the dose of 20 uc/ml was added for 30 min to the culture medium at different intervals of cultures. The cultures were then harvested and processed for liquid scintillation counting or for autoradiography. In another series of experiments the labeled precursors at the same concentration was added soon after seeding and left until the time of harvesting (continuous incubation).

The determination of incorporation of the labeled precursor into total RNA was performed with two methods: autoradiography and liquid scintillation counting. For autoradiography, microscopic slides were introduced into the Petri dishes. At the end of the experiment, the slides were washed three times in a non-radioactive medium without serum, fixed in ethanol 70%, and processed for autoradiography using NTB2 liquid emulsion. For the determination of radioactivity by liquid scintillation counting, at the end of each incubation period, the cells were centrifuged, suspended in non-radioactive medium containing an excess of cold uridine to arrest the incorporation, and centrifuged again. The pellet was dissolved in distilled water and treated with TCA. The TCA precipitate was collected on millipore filter and the filtrate was counted with a liquid scintillation counter.



## Results.

In control cultures, as it was reported preliminary in the previous year, the rate of  $^3\text{H}$ -uridine incorporation rises rapidly until 10 hours after addition of PHA. After this time there is a fluctuation of the rate of incorporation around a constant value. In some cases the rate of incorporation keeps increasing slowly, in other decreases somewhat, reflecting probably a different rate of cell degeneration in various cultures. In cultures incubated in the continuous presence of the labeled precursor there was a very rapid increase of RNA synthesis during the first 10 hr. after addition of PHA, followed by a slight decrease at late intervals. It is noteworthy that the activation of RNA synthesis induced by PHA occurs very rapidly. As early as 1 hr. after addition of the drug, a significant increase of the incorporation was noted with respect to the unstimulated cultures.

Preliminary experiments were undertaken to elucidate the nature of the RNA which is synthesized in stimulated lymphocytes. A sucrose gradient analysis has shown that the specific activity of the 18 and 28 S ribosomal increases considerably in the stimulated cultures with respect to the control cultures, indicating that a great proportion of the RNA produced is ribosomal RNA.

The autoradiographic experiments showed that both the percentage of labeled cells and the degree of labeling increase considerably after stimulation. The distribution of silver grains over the nucleus was uniform, suggesting that other portions of the genome besides the nucleolus organizers are involved in the increased rate of RNA transcription.

Both the percentage of labeled cells and the rate of incorporation of the precursor were not significantly different in cultures from exposed people and in cultures from normal subjects. The time when the first significant rise in the rate of incorporation begins is also similar to that observed in control cultures.

Experiments were also undertaken on the activity of catalase detected by the DAB reaction (3-3'-diamino-benzidine) in stimulated lymphocytes from normal and professionally exposed subjects. The results along this line are still preliminary, and do not allow any definitive conclusion. The main difficulty encountered with the cytochemical valuation of this enzymatic activity is that the intensity of the reaction is very variable from cell to cell, which makes it very difficult to detect differences between cultures from normal people and cultures from exposed subjects. Experiments are under progress with the aim to quantitate the data using histophotometric techniques.





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