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ASSOCIATION
European Atomic Energy Community - EURATOM
Instituut voor Toepassing van Atoomenergie in de Landbouw - ITAL

APPLICATION OF ATOMIC ENERGY IN AGRICULTURE

Annual Report 1973

1974

Association No. 094-72-1 BIAN

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Commission of the
European Communities
D.G. XIII - C.I.D.
29, rue Aldringen
L u x e m b o u r g

December 1974

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primary radiation effects,
mutation breeding,
preservation of food by means of radiation,
radiation genetics of insect pests;

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related studies on plantmaterial;

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ABSTRACT

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— Radiation effects:
  primary radiation effects,
  mutation breeding,
  preservation of food by means of radiation,
  radiation genetics of insect pests;

— genetic studies:
  related studies on plantmaterial;

— soil-plant studies:
  uptake of specific elements by plants,
  behaviour of specific elements in the soil and water environment,
  heavy metals in plants and soils;

— methodology:
  related to dosimetric, other physical and instrumental studies,
  related to studies on biological material,
  related to soil-studies;

— practical applications, services, courses:
  mutation breeding of economically important crops,
  food preservation by irradiation,
  services to other institutions, mainly in the Netherlands,
  courses,
  newsletters;

— publications:
  in press,
  internal reports 1973,
  external reports 1973.

C O N T E N T S

SUMMARY ANNUAL REPORT 1973

GENERAL INFORMATION

The Governing Board of ITAL and the Management Committee of the Association EURATOM-ITAL met three times. Important subjects of discussion were :

- the personnel situation and budgetary matters;
- the progress of research within the actual 5-year programme of the Biology Division of the Commission of the European Communities;
- prospects for scientific cooperation within the enlarged European Community;
- preliminary planning of the new 5-year programme (1976-1980) of the Biology Division of the Commission of the European Communities;
- contacts with the Dutch Ministry of Public Health and Environmental Hygiene;
- selection of new members of the International Scientific Advisory Committee (I.S.A.C.);
- contacts with countries outside the European Community;
- collaboration with the Pilot Plant for Food Irradiation at Wageningen;
- evaluation of the reactor BARN.

The members of the International Scientific Advisory Committee (I.S.A.C.) have met two times. The main topics of their discussion were the 1972 annual reports, the 1973 progress reports (July 1973) and the 1974-programme of both the Institute's projects and the projects of the collaboration contracts in France, Germany, Italy, Belgium and the Netherlands. Considerable attention has also been paid to the preliminary planning of the future 5-year programme of the Biology Division of the European Communities. Other topics of interest were : scientific aspects of the evaluation of the reactor BARN, edition of a series books on "Nuclear Science in Agriculture and Biology" by members of the Association.

The organization, representing the personnel of the Institute (R 11), met regularly during 1973. This group functions as an advisory body to the direction on matters of personnel, administration, budget and research. Important topics of discussion were : variable working hours, reorganization of research within the Dutch Ministry of Agriculture and Fishery, safety at the Institute, attendance on foreign guestworkers.

The scientific staff of the Association's Institute met three times for discussion of a first approach towards the planning of their participation in the future 5-year programme of the Biology Division of the European Communities.

A special Commission (Chairman Ir. G. Wansink) held several meetings, in which the usefulness of the reactor BARN was evaluated in relation to the actual and planned future programme.

Also in 1973, scientists from western and eastern European countries, from Japan, from the developing countries, visited the Association's Institute as well as the Institutes of the collaboration contract partners for short or longer periods.

The programme for 1973 has once more been carried out in close cooperation with other scientific institutes and organizations. Examples of this scientific collaboration are:

- on different aspects of the application programme within workinggroups of the European Society of Nuclear methods in Agriculture (ESNA) and with representatives of the new member-countries of the European Community;
- on pollution, radioactive and other, with the Biology Division in Ispra and Institutes in the Netherlands, Belgium and Germany;
- on radiation effects within the European working group for Microdosimetry;
- on standardization of absorbed dose and dose distribution measurements within the European Late Effects Project Group (EULEP);
- on mutation breeding (vegetatively propagated crop, protein improvement, disease resistance) in higher plants in the Mutation Breeding Contact Group. Attempts were made to contact plant breeders in the new member-countries of the European Community;
- on incompatibility in higher plants with several institutes and organizations within the European Community;
- 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, Denmark, Belgium and Germany;
- 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 "Organization Internationale de la lutte Biologique (OILB). Cooperation within projects of the IAEA and of the entomology programme of the Biology Division.

RESEARCH

Radiation effects

Primary effects

Certain consequences arising from the proposals of the molecular theory have been checked.

- the predicted correlation between cell death and chromosome aberration has been found to be statistically highly significant and independent of cell phase and the presence or absence of a radiosensitizer.
- the relationship between intercentromeric distance and fragment length in dicentrics in *Tradescantia paludosa* L. has been found to satisfy that predicted by the molecular hypothesis.
- the LET dependence of cell survival can be explained and estimated on the basis of the LET dependence of the induction of a DNA double strand break in one radiation event.

Further implications of the hypothesis have been derived for radiological protection and mutation breeding

- the radiation biological effect at low doses should be linearly related to dose with no threshold.
- the limiting RBE at low doses should be the same for different biological endpoints in the same cell.
- the maximum mutation frequency should be independent of radiation type or conditions.
- the mutation spectrum should be independent of radiation type but depends on radiation dose.

Some other radiation effects in plants

For better understanding of the basic mechanism of radiation damage leading to the reduction in pollen germination and peroxidase activity after irradiation, and of the relationship between the two effects, studies, using *Tradescantia paludosa* L. have been started.

The radiation dose-fractionation effect, observed in leaves of *Saintpaulia ionantha* H. Wendl. cv. Utrecht, was investigated, using X-rays. Data of experiments investigating the possibility of the existence of a radiation-induced protective agent are still inconclusive. A change was observed in the radiosensitivity of the leaves after storage: with a longer time interval between detaching and irradiation (up to 24 h) radiosensitivity of the leaves was decreasing. Another fact observed was the stimulation of the production of adventitious plantlets at the base of the leaf petiole by low doses of radiation (1 - 3 krad).

Mutation breeding

Using adventitious bud techniques in mutation breeding of vegetatively propagated crops, the following results were obtained:

- Of *Streptocarpus*, by a combined use of radiation, colchicine-treatment and the adventitious bud technique (detached leaves), it was demonstrated that commercial results can be obtained within a relatively short period. An autotetraploid mutant, cv. Albatros, was released to the trade. Of *Achimenes* two mutants of cv. Paul Arnold were released, namely cvs. Cupido (compact) and Orion (large flowers).
- The adventitious bud formation of *Chrysanthemum morifolium* Ram. cultivars Bravo and Super Yellow was investigated *in vivo* and *in vitro*. *In vivo*, using leaves of cv. Bravo, adventitious shoots emerge usually from callus at the base of the petiole 2 - 6 months after leaf excision.
  In vitro, both cultivars regenerated adventitious shoots on explants of the leaf, the flower-stalk and the flower-head (capitulum). On capitulum-explants shoots arise 2 - 3 weeks after incubation. The most elongated shoots were isolated and induced to adventitious root formation and approximately a half year after incubation uniform flowering plants were obtained.
- The adventitious bud technique, using detached leaves, in *Chrysanthemum morifolium* Ram cv. Bravo, proved to be less useful. The majority of the mutants were chimeric, probably because the adventitious buds

do not originate from epidermal tissue but are formed on callus, developed at the base of the petiole.

In mutation research in potato (*Solanum tuberosum* L.) special attention was paid to establishing further correlations between morphological and histological observations by studying tuber resp. flower characteristics and microscopic sections from irradiated shoot apices of periclinal chimeras. Previous ideas about perforations, reduplications and chimeric structures were partly confirmed and extended. Work on the production of a new diploid testclone progressed slowly. A suggested method for the production of adventitious sprouts (Jørgensen method) was tried and rejected. Some practical experiments concerning yield and disease resistance were continued on a limited scale.

The protein production of some pea (*Pisum sativum* L.) recombinants and their parental mutants was analysed considering not only the seed protein content but also the seed yield per plant and the protein quality, i.e. the amino acid spectra of the respective genotypes. Some of the most promising pea mutants have been tested in Europe, India and Ghana in order to study the dependence of their selection value on climatic factors. In these various regions, some of the genotypes studied show a completely different reaction concerning their protein production as related to the initial line.

With respect to the genetic variation of protein and lysine content in barley (*Hordeum vulgare* L.), the variability of both characters was tested in a number of mutants originating from different commercial varieties. The induced mutants were divided into macro- and micro-mutants according to preselection for yield, mildew resistance, earliness, fertility and some morphological traits, like culm length, spike density and tillering. To make selection more efficient a scheme for simultaneous and/or separate selection of "% protein in dry matter", "% lysine in dry matter", and "% lysine in protein" was used in screening for superior mutants in both mutant groups. The pre-screening for high protein and high lysine content was based on the DBC-method. The 10% best lines in each group were checked by Kjeldahltests for protein and by an enzymatic-colorimetric test for lysine content.

In the first year of activity in this project on protein improvement in opaque maize (*Zea Mays* L.), 2500 seeds of the B 37 $\overline{02}$ (opaque 2) have been X-ray treated (4000 and 8000 rad). In the spring of 1973 the irradiated seeds were sown at Fiorenzuola (PC). At flowering time the plants were self fertilized by hand pollination. In the 1973 autumn the obtained ears have been collected, dried and shelled.

In work concerning mutation breeding for disease resistance new sources of resistance have been found among the wild species of tomato against *Fusarium oxysporum* Schl ex Fr. races 1 and 2 and against race 1 of *Verticillium albo-atrum* Rke et Berth. No resistance was found against race 2 of the latter fungus. Two plants of *Lycopersicon hirsutum* H.B. & K. were found to be partially resistant to *Phytophthora infestans* (Mont) De By race T_1. Screening of M_2 seedlings for the *V. albo-atrum* race 2 resistance was started, but no resistant mutant plant was found so far.

New mutant lines of *Triticum durum* (Desf.) from cv. Cappelli have been isolated as to higher and lower resistance to a race of bunt particularly virulent to this species.

In the first year of the work on *Pisum sativum* L., seeds of the varieties Sprinter (S) and Freezer 69 (F) have been treated with physical mutagens (X-rays; doses: 10 and 15 krad; symbols R_1 and R_2, respectively) and with chemical agents (diethilsulphate; doses 1 °/oo and 1,5%; symbols C_1 and C_2, respectively). From approx. 6000 adult plants, seeds have been collected keeping separately seeds produced on the stem and on the secondary branches. M_1 progenies have also been classified according to seed setting. Greenhouse trials have been performed in order to set up a screening technique that could provide an efficient and quick means for selecting resistant pea plants. A screening method was devised for large-scale inoculation. With this technique a large number of plants can be evaluated, in a very short time, for powdery mildew resistance. Symptoms appear 4 days from inoculation and the final disease ratings are taken 6 days from inoculation.

A collection of mildew resistant barley (*Hordeum vulgare* L.) mutants was built up. It consists of plants with vertical as well as horizontal resistance. Furthermore, genetic analysis of mildew resistant mutants were carried out. Both, the mode of inheritance and the number of resistance genes were studied. In some of the mutants mildew resistance belongs to a pleiotropic character complex. Is this the case, the mutants were crossed with other varieties in order to demonstrate the possibility of separating mildew resistance from the undesired features controlled by the same gene. Such undesired characters are a.o. reduced grain yield, sterility, low tillering and chlorophyll deficiencies.

Finally in mutation breeding for dwarfrust (*Puccinia hordei* Otth.) resistance in barley, seed of the cv. Minerva was mutagenically treated with X-rays (15 krad) and EMS (0, 25, 50, 75 m mol). The M_1 has been grown in the field in 1973.

Irradiation in food preservation

Irradiated glucose inhibits or retards growth of sensitive micro-organisms. However, gradual adaptation occurs after several passages through glucose, irradiated at doses, which are sub-lethal to the micro-organisms considered. Sensitive and adapted strains are being compared physiologically (growth) and cytologically (metabolism).

Wateractivity being one of the main factors influencing radiation-resistance of micro-organisms, radiation-inert models were tested on their suitability for maintaining a high survival for micro-organisms at different wateractivities. Present results indicate that the range of wateractivities to be tested cannot be covered by one and the same model. Furthermore, it was demonstrated that the growthphase of the microorganisms influences the numerical recovery.

The metal content of *Bacillus subtilis* spores is affected by the cultural conditions during sporulation and alterations in this content may influence the resistance to radiation and heat treatments. Increasing the calcium concentration in the sporulation medium reduced

the manganese content of the spores. There was an optimum concentration for maximum calcium accumulation and for heat resistance of the spores. The radiation resistance was higher for spores formed in media containing minimum levels of calcium. The incorporation of manganese was greater at higher manganese concentration in the sporulation medium; spores showing high accumulation of manganese were more resistant to irradiation. Radiation sensitization by potassium iodate was only effective with spores containing low levels of calcium.

In general the keeping quality of peeled potatoes and prepacked cut chicory remained better in non-perforated bags than in the perforated ones. An irradiation of only 50 krad frequently resulted in a considerable reduction of the total viable count and therefore in an increase of keeping period by almost 100%. Colour improvement by cystein was only observed in peeled potatoes. In relation to the discolouration problem in cut vegetables, different extraction methods for estimation of phenols and quinons were tried out; the observed absorption spectrum, however, did not permit further analysis. At doses of 100 krad, the activities of polyphenolase and peroxidase were reduced, but again no explicit correlation between discolouration and such enzyme-activities could be found.
For estimation of the D-value of yeasts in irradiated deep-frozen strawberries a method adding an extract of yeast and dextrose to the strawberries in an incubator, gave satisfactory results. The radiation resistance of the natural yeast flora, as compared to an enriched one, is higher.

In cooperation with the Dutch Institute of Public Health, in the field of food irradiation a unique wholesomeness programme was started during 1973. Autoclaved, radappertized and control diets are fed to sows during their entire gestation and lactation periods and offsprings up to the fourth generation will be maintained on the mentioned dietary regimes. Furthermore, in the past year a fattening test with 90 hogs was carried out. The meat of these hogs is preserved by different technological processes including irradiation, and consequently fed to rats during a chronic feeding test. Results of the F_1-generation did not show any significant difference with data from practical rat breeding.

Genetic control of insect pests by irradiation

Using X-irradiated 7 day-old *Hylemya antiqua* Meigen males as test insects, dose response curves were established for dominant lethality and late embryonic lethality. For the induction and isolation of chromosome aberrations for insect control, a dose of 500 rads of X-rays was given to males in the parental generation. The F_1 progeny were outcrossed to normal insects and the fertility of these matings was measured. Matings which showed reduced fertility were checked cytologically for the presence of aberrations. Two translocations and one inversion have now been identified and characterized. Inbreeding programmes are underway to attempt to make the aberrations homozygous.

The programme on genetical control of *Adoxophyes orana* F.v.R., on the one hand, aimed at gaining better insight in the influence of the moment of irradiation, in relation with the stage at which meiotic divisions mainly occur. On the other hand in a more practical approach (in collaboration with G.W. Ankersmit, Agricultural University, Wageningen) it was shown that the released semisterile moths mated with the wild moths

and transmitted chromosomal rearrangements to their progeny.

Furthermore the effect of respectively X-rays and fast neutrons on males or females of *Tetranychus urticae* Koch, for the induction of dominant recessive lethals and translocations was studied. Dominant lethality is induced in sperm, at all dosages by a mechanism based on a single radiation event with an R.B.E. of 1, whereas in eggs two radiation events are involved with an R.B.E. of 2,5.
Comparing the mortality of haploid and diploid eggs, produced by irradiated females does not permit a correct measurement of the number of recessive lethals. The improved F_2-method shows that, both in eggs and sperm, the induction of translocations by X-rays is linear with dose; with neutrons the relationship between dose and translocation frequency is more complex. Apparently the optimum dose for induction of useful translocations in sperm is 0,5 krad X-rays; at higher dosages translocations are frequently associated with a recessive lethal (20% $\pm$ 6% at 1,5 krad) and obtaining a homozygous form of the translocation is difficult.

Genetic studies

With respect to the problem of the localization of the unstable chlorophyll C_6 and C_{12} genes, in tomato (*Lycopersicon esculentum* Mill.) a number of trisomic plants have been selected.

In a crossing between recessive Moneymaker and Moneymaker subsequent to $^{14}N/^{15}N$ and $^{10}B/^{11}B$ treatment of the plants during cultivation and followed by thermal neutron irradiation, 2 parthenogenetic haploids have been obtained.

Self-incompatibility in higher plants

In the first part of this programme the induction of self-compatibility was considered.
The results obtained, after γ-irradiation of a dihaploid *Solanum tuberosum* L. seem to indicate that the self-compatibility character is radio-induced and of a genetic nature. There is also evidence that self-compatibility in dihaploid *S. tuberosum* originates from a duplication of the S-locus. Dihaploids then appear heterozygous for this duplication. Selfing such dihaploids should produce duplication homozygotes. Test-cross results suggest such homozygotes to be non-viable. It is still ambiguous whether in the style, pollen with the duplicated segment is at a certain disadvantage in competition with normal pollen.
Pollen mothercells (P.M.C.), seeds and leaves of *Nicotiana alata* Link and Otto have been treated with different mutagens (X-rays, fast neutrons, γ-rays, EMS). The results show that P.M.C. treatment with X-rays and fast neutrons are very effective for inducing self-compatibility mutations. Similar mutation spectra are observed after X-rays and fast neutrons.
In Italy, induction by irradiation and selection for selfcompatibility in populations of *Prunus avium* L. and *Olea europea* L. is in good progress.

With respect to research on the nature and type of incompatibility, its linkage relationship with the S-locus and the identity of the S-bearing chromosome, the following results were obtained:

Two genetic models are presented to explain unilateral non-crossability of *Solarium verrucosum* Schlecht and diploid *Solanum tuberosum* L. Pollen tube growth inhibition is different when either incompatibility or unilateral non-crossability is involved. The discovery of *S. tuberosum* clones accepting *S. verrucosum* pollen enabled reciprocal crosses between the species. Only F_1's with *S. verrucosum* as a female exhibited characteristic male sterility types.
As the relevance of the (co)dominance relationships of the S-alleles in *Lobularia maritima* (L.) Desv., based on Haruta's method, was not unambigous, the analysis has been supplemented by Fabig & Nowak's I_1 diallel analysis.

At the Casaccia (Italy), and in collaboration with the group of Prof. Sarfatti in Siena, a major attempt was made for differentiating, in an embryo-cultured hybrid between selfcompatible *Lycopersicon esculentum* Mill and self-incompatible *Lycopersicon peruvianum* Mill, the genetic control of self-incompatibility (transmitted via *L. peruvianum*) from that of unilateral interspecific incompatibility (transmitted via *L. esculentum*). The investigations showed that self-incompatibility and unilateral interspecific incompatibility segregated in the pollen of the hybrid as alleles of a same locus or as different genes from a same linkage group. Other analyses of the hybrid and of an unstable clone of *Nicotiana alata* Link and Otto indicated furthermore that one of the first manifestations of the incompatibility reactions involved a general cessation of protein synthesis which does not occur in the self-compatible pollen mutants suspected to carry, on a centric fragment, a duplication of the nucleolar region for chromosome 3.

At further attempts to identify the S-bearing chromosome in *Nicotiana alata* at Wageningen two plants, which were slightly self-compatible, have been found in the progeny of the cross triploid x diploid. These plants may also have an "additional chromosome" on which the S-locus is located.

Further research on the biochemistry of the incompatibility reaction showed that:
Pollination and sybsequent pollen tube growth through styles of *Nicotiana alata* Link and Otto cause an increase of the total peroxidase activity and of several peroxidase isoenzymes of the style. This increase is more pronounced in cross- than in self-pollinated styles, especially after four days when many compatible pollen tubes have reached the ovules. During the progamic phase only one difference was found between the isoenzyme patterns of self- and cross-pollinated styles: one of the new peroxidases found in pollinated styles only appeared after cross-pollination.

At Nijmegen (the Netherlands) much attention has been paid to the effect of X-rays on the biochemistry of pollen growth in *Petunia* and *Pseudotsuga*, and to the synthesis of RNA and proteins in relation to the interaction between pollentube and style.

More than 120 portions of pollen of different plant species have been collected and tested for their germination ability and respiratory capacity in humid air. The insensivity to X- and γ-irradiation of both bi- and trinucleate types of pollen, suggests the lack of denovo synthesis of enzymes for the respiration process. No difference in rate

of uptake of water vapour was found between the two types. Therefore, the problem of the origin of the considerable differences in respiration rate between bi- and trinucleate types of pollen needs a more biochemical approach.

Soil-Plant relationships

In this chapter is grouped all information on the behaviour of different elements and compounds in the plant and soil environment.

Uptake of specific elements by plants

In very dilute solutions, the influence of optimum concentrations of P, K, Ca, Mg on uptake of N by rice (*Oryza sativa* L., cv. I.R. 8) and soybean (*Glycine max* L. Merr.) plants was studied at Louvain (Belgium).

Kinetic studies showed that in a ternary H-P-K nutrient system, the ionic charge balance is merely securated by the K^+-uptake and that, as a result the uptake rates of phosphates and protons increase. The nitrate uptake is accompanied by a high stoichiometric consumption of H^+ ions.
At pH lower than 4, the uptake of SO_4^{--} ions produces a further reduction of the pH, due to a shift of the equilibrium in the ionic dissociation of HSO_4^- ions.
Uptake of chloride changes the behaviour of the ternary H-P-K system in the nutrient medium to that of a binary H-P system

The study of an adapted root growth medium composed of an anionic and a cationic synthetic resin was continued. Much attention was paid to their physico-chemical properties, exchange capacity, saturation, density and loading curves were determined. A growth experiment with barley (*Hordeum vulgare* L. cv. Aramir) using strong ion exchangers mixed with sand gave no satisfactory results. Substitution of the sand by coarse perlite improved the oxygen and water management of the medium. Other types of resin are actually tried out. Nutritional balances of four plant species in gravel culture with a Hoagland-Arnon nutrient solution were determined. An experimental set up for control of the most important growth factors as well as the ionic concentrations of the equilibrium solution and the diffusion constants of the main nutrient ions in function of the moisture content of the root medium has been worked out.

Furthermore double labelling experiments using two isotopes of respectively calcium and cesium were carried out. Tomato plants (*Lycopersicon esculentum* Mill. cv. Marette VF) were grown on a diluted Hoagland-Arnon I nutrient solution (3,02 me/l). Four labelling ratios (ratio between microcuries per litre of both isotopes) were used. The ratio of both isotopes in the root, stem and leaves was determined and the immobilization of both cesium isotopes in different plant organs was studied. The counting of the samples is actually in progress.
Finally the interaction between Ca^{++} and Mg^{++} at adsorption sites of isolated spinach chloroplasts (*Spinacea oleracea* L.) was studied. Attention was also paid to the relation between cell metabolism and the transport process of Ca^{++} in these organelles and to the molecular components of the transport system.

Behaviour of specific elements in soils and water

At Louvain (Belgium), most of the work concerned the improvement of the submodel describing the movement of cations through the soil under saturated conditions taking into account ion exchange isotherms and solubility reactions.

A new start was given to the improvement of the mathematical model for movement of N in soils by a thorough evaluation of all actually available models.

Sampling for verification of the model concerning the behaviour of Sr and Cs in soils of Western Europe proceeded according to planning.

The adsorption properties and the movement in soil columns of two compounds, one insecticide (Trichloronate) and one herbicide (Bam) were studied and the results were compared with simulation models. A first model taking into account an instantaneous equilibrium between solid and liquid phase and a rate factor of 2.10^{-3} for decomposition, moderately agrees with the experimental values for leaching of Trichloronate in a sand column.
The result of the application of a model with a non-instantaneous equilibrium, considering an irreversible adsorption phenomenon, is in good agreement with the leaching of Bam through a sand column.

As blue-green algae (*Nostoc* species) can utilize meta- and poly-phosphate as sole source of phosphorus under nitrogen-fixing conditions, they may contribute to the eutrophication of natural surface water. The algae responded to extreme low levels of phosphate (0.1 - 0.3 mg P per litre) and, therefore, they can be used as a bioassay for the determination of available phosphate in natural water.
During the eutrophication in general the green algae (*Scenedesmus* species), initially present, are replaced by blue-green algae (*Aphanizomenon*, *Anabaena* and *Nostoc* species, and *Oscillatoria agardhii* Gomont). In extreme poluted water *Oscillatoria agardhii* is the dominant organism. It apparently mainly grows at the expense of complex organic compounds released by lysis of previous algal associations.

Heavy metals in soils and plants

A mercury monitoring programme has been carried out in soils in situ in Western Europe and the distribution over the profile was determined up to a depth of 1 meter. Migration studies have also been done on undisturbed soil columns, applying different mercury compounds. None of the compounds studied moved, neither under aerobic nor anaerobic conditions. A computer simulation model has been developed for the behaviour of mercury in the ecosystem.

Migration studies have also been carried out on undisturbed soil columns for chromium and zinc. Chromium apparently does not move, neither under aerobic nor under anaerobic conditions. On the contrary zinc moves. The distribution of chromium over the profile of some soils in situ has been determined up to a depth of one meter.

The absorption of Cr^{+++} ions by intact rice plants (*Oryza sativa* L. cv Arborio) occurs in 3 phases, the last of them apparently being the only one requiring some interference of the general metabolism of the plants. The metabolic phase is characterized by two absorption

isotherms both fitting a saturation kinetics. The plant mineral nutrition cannot be considered as a significant vector of the pollution by chromium, whatever its chemical form.

The roots of tomato plant (*Lycopersicon esculentum* Mill, cv. Marette) accumulate the major part of cadmium and its transport rate decreases with increasing contamination of a Hoagland solution when iron is supplied as FeEDTA. The possibilities to study the effect of Cd^{++} over a long growing period were considered taking into account its equilibrium with EDDHA, the other cations, and a solid phase of ferric hydroxide. In the vessels of the stem, cadmium can be removed by calcium and probably also by other ions.

Finally, chloroplasts isolated from spinach leaves (*Spinacea oleracea* L.) are shown to absorb and to transform CrO_4^{2-} concentration in darkness. A reduction of CrO_4^{2-} accompanied by an O_2 evolution occurs in illuminated chloroplasts. The maximum CrO_4^{2-} concentration, which is still acceptable to chloroplasts, depends on the concurrency of this compound with a terminal acceptor of the photosynthesis. However, this CrO_4^{2-} concentration is independent of the rate of photosynthesis.
The cadmium ion is strongly complexed by ligands like Cl^- and TES, which are present in high concentrations in the chloroplast medium. Cadmium is an inhibitor of the electron transport chain, and shows some influence on the energy metabolism of the chloroplasts.

Methodology

A further aspect of the Association's programme is the development of methods using radiation or radioactive isotopes as tracers.

At Strasbourg (France), the study of the development of the corpuscular latent image has been continued. The activation of the development centers and sub-centers was investigated using gold and other heavy metals in the activation solutions. An interesting result has been obtained with iridium salts, which seem to favorise an "inactivation" of the latent image.

Different original "compact grain" developers have been formulated. According to some preliminary results the kinetics of development of nuclear emulsions are considerably improved at higher temperature in the range from 10 up to 20 ^{o}C.

The "activated" autoradiographic methods have been applied in a study of the nucleolar DNA synthesis in the liver of adult rats and to the problem of the differentiation of isolated nerve cells in culture.

A thin cell layer with a very high accumulation of calcium was found to be present in the fruits of beanplants (*Phaseolus vulgaris* L. cv. Saxa), both at the dorsal and the ventral side. An investigation of the nature and functioning of this accumulation layer was started by a combined application of *in vivo* and *in vitro* determination methods: semiconductor detector measurements (countrate and analysis of the energy spectra), microautoradiography and X-ray fluorescence in the scanning electron microscope. The cell layer ("bundle sheath") contained large amounts of small calciumoxalate crystals. Important differences in redistribution were observed between the dorsal and the ventral side of the fruit.

At Hannover (Germany) efforts were concentrated on experiments to develop the equipment for mass determination of plantations. In connection with a study on the diagnosis and therapy of dry rot, *Fomes annosus* was grown in nutrient solutions of different concentrations of radioactive manganese (^{54}Mn). Experiments showed an influence on the growth rate of the fungus.
Rubidium-86 injections into different parts of trees and auto-radiographic methods were applied to investigate the root system of trees in the soil.
Studies on the evaporation and condensation of water vapor in desert loess were continued. The experiments yielded results concerning depth and speed of penetration of water vapor originating from the atmosphere under the condition of daily temperature inversions.
After the application of the equipment for measuring low moisture contents in thin soil layers in the laboratory during an IAEA training course, experiments were carried out in the Negev-Desert in cooperation with the Institute of Botany of the Hebrew University, Jerusalem (Israel).

Practical applications, services and courses

In this part of the report, some information is given concerning the practical application of nuclear methods and techniques, worked out within the Association. These applications are in the fields of dosimetry, mutation breeding of vegetatively propagated crops, food irradiation, combined heat-irradiation treatments, food hygiene, sprout inhibition, determination of heavy metals in fish and fishery products, measurement of density variation and velocity in flowing granular material, measurement of the residence time of different liquids in an evaporator, determination of water-oil ratios in soils.

Finally the services of the Association's Institute for radiation protection at Wageningen and its surroundings and the different courses have to be mentioned.

RADIATION EFFECTS

PRIMARY RADIATION EFFECTS

Primary radiation effects in inert material - K.H. CHADWICK, K.J. PUITE.

TL measurements on BeO at low temperature

Ceramic BeO material offers very good possibilities as TL dose meter compared to routine systems as LiF and CaF_2. BeO discs have been used successfully, together with LiF powder, in the second EULEP inter-comparison project. However, the supralinearity of the TL response of BeO starting at about 30 rad may present difficulties for routine use. More insight in the mechanism of supralinearity is gained exposing BeO at liquid nitrogen temperature (LNT) and measuring the spectral emission of the TL glow curves in the temperature range from LNT-300°C.

Spectral measurements have been carried out using X-ray doses of 5 krad for the -98, -116, +185 and 284 °C glow peaks. These experiments have not yet been completed but the data obtained indicate that the various peaks all exhibit a similar emission spectrum. This may point to a simple mechanism of luminescence. The measurements will be repeated at doses of 50 krad.

Radiochromic Dye Systems

New thin foils are now commercially available using the radiochromic dye system which are satisfactory in use. One foil with a UV inhibitor can be handled normally in the laboratory without light induced effects. This foil, with nylon base, can be used from $\sim$ 100 krad and will be specially suitable for electron dosimetry in food irradiation. Measurements are being made together with perspex dosimeters to set up a calibration curve and to study the reproducibility of these dosimeters.

Perspex dosimeters

General use of the perspex dosimeters has been made for food irradiation in the Pilot Plant for Food Irradiation, radiobiological experiments with microorganisms and to check the dose and dose distribution in the Gamma-ster plant for radiation sterilization of medical products following an additional load of ^{60}Co.

Statistics in Radiation Processing

Some thought has been given to the statistics and dosimetry measurements necessary in the commissioning of an irradiation process in an automatic continuously operating irradiation plant. It has been concluded that the plant operator can never be absolutely certain that all the product receives a necessary dose but can only make a statement of statistical confidence on the probability that the product is properly irradiated. The conclusions of this work will be written up in an IAEA booklet "Dosimetry for the Radiation Processing of Food" and in a paper which will be presented at a symposium on Radiation sterilization of medical products to be held under the auspices of Johnson and Johnson in Vienna in April 1974.

Energy dependence of TL dosimeters in a phantom

Intercomparison studies of dose and dose distribution in animal radiobiology using TL dosimeters have indicated the need to determine the TL response/rad in muscle within an animal phantom. To this purpose a mouse-sized test phantom containing TL dosimeters has been exposed to X-rays having a HVL value of 1.4 - 2.8 mm Cu. Dependence of the response of ^{7}LiF dosimeters on HVL of the incident beam has resulted in an increase of TL response/rad in muscle for dosimeters positioned at the central axis of the phantom compared with the response of similar dosimeters exposed free-in-air. An increase of 7-3% in TL response/rad in muscle was found over the HVL range. The spread in effective energy inside the phantom was small and the dose distribution may be estimated directly from the TL response of ^{7}LiF dosimeters at the entrance, central and exit position in the phantom. It seems to be difficult to determine unique HVL values in a phantom using TL dosimeters (figure 1 and 2).

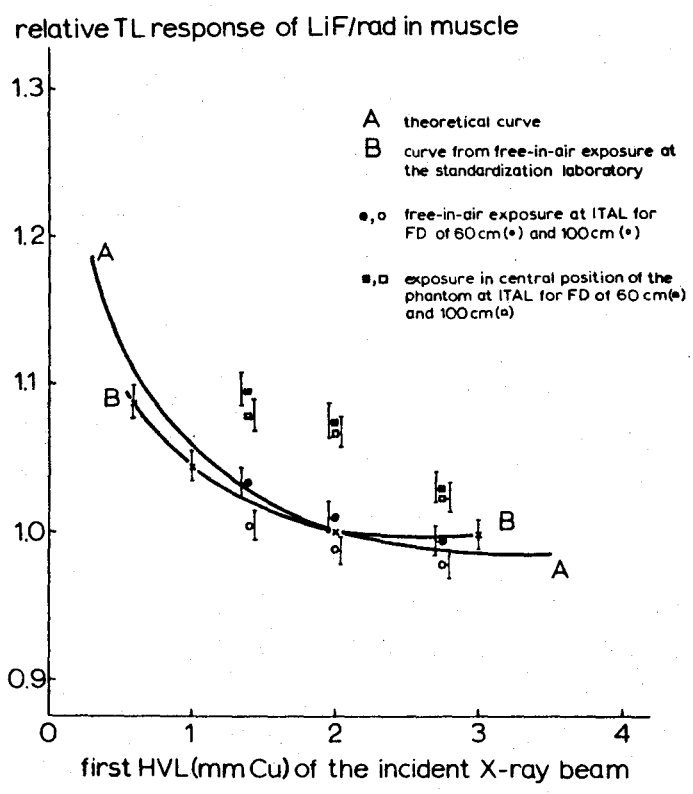


Figure 1 - Relative TL response of LiF/rad in muscle. Normalization at 2 mm Cu HVL.

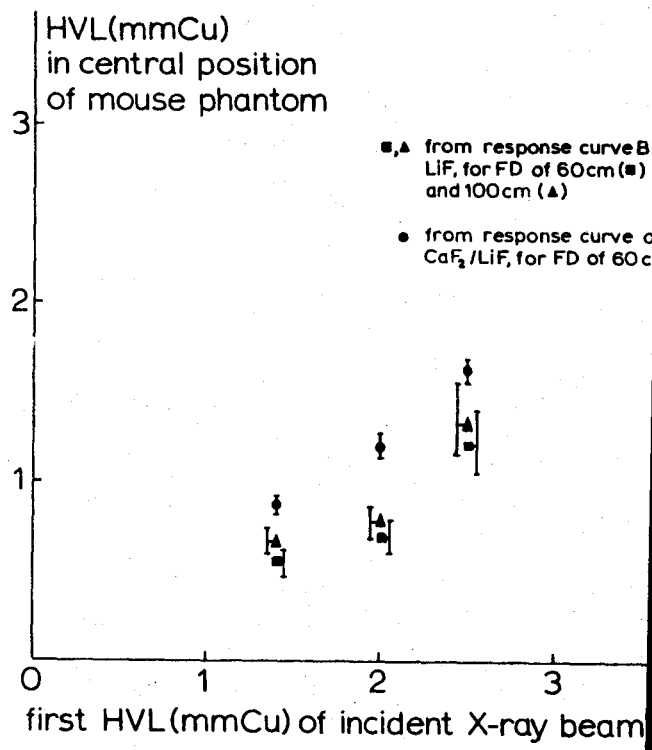


Figure 2 - HVL values in the mouse phantom compared to the HVL of the incident beam.

Primary radiation effects in biological material - K.H. CHADWICK, H.P. LEENHOUTS, K.J. PUITE, W.F. OOSTERHEERT - A. SPANJERS, a student at Nijmegen University, who worked for 6 months on the *Tradescantia* dicentrics.

A theoretical approach

In 1973 the theoretical approach to explain the effect of radiation on biological cells has been further developed.
The model is based on the assumption that double strand breaks in the DNA helix are the most critical lesions which lead to cell death in eukaryotic cells. It is further assumed that the induction of double strand breaks by radiation is quadratically related to dose. It has also been proposed that DNA double strand breaks could give rise to chromosome aberrations on the basis of the unineme concept of chromosome structure. This proposal brings with it the consequence that the rejoining of a normal chromosome end to a broken chromosome end should be possible.
One of the consequences of these proposals is that a direct relationship between cell death and chromosome aberrations should exist, not because the one necessarily causes the other, but because both arise from the same origin. The direct relationship can be derived as follows: if $N = \alpha D + \beta D^2$ is the number of DNA double strand breaks and cell survival is given by

$$S = e^{-\rho(\alpha D - \beta D^2)}$$

and the yield of chromosome aberrations is given by $Y = k(\alpha D + \beta D^2)$ then

$$\ln S = -\frac{\rho Y}{k}$$

This correlation has been found to exist independent of cell phase and the use of a radiation sensitizer. The correlation was statistically highly significant (figure 3).
A second consequence of these proposals is that if a chromosome aberration can be formed from one break in the chromosome backbone plus rejoining between the broken end and a normal telomere the distance between the two centromeres of a dicentric must have a minimum length equal to at least the length of one chromosome arm. The intercentromeric distance and associated fragment length have been measured in *Tradescantia paludosa* L. after irradiation with 300 rad X rays. Using measurements on normal unirradiated mitoses an area on a dicentric versus fragment graph could be defined within which the measurements should fall. The results, shown in figure 4, fell within the defined area and are compatible with the theoretical proposals. The results do not rigourously exclude the alternative proposals of the exchange and classical theories of chromosome aberration formation.
A third consequence of the proposals is that the induction of DNA double strand breaks in one radiation event is LET dependent and should be reflected in the LET dependence of the "α" coefficient which can be derived from cell survival curves. Calculations using a single track structure model and the fixed geometric structure of the DNA helix have been made and compared with the LET dependence of the induction of DNA double strand breaks and the "α" coefficient determined from cell survival studies published in the literature. The results shown in figures 5 and 6 show that the calculations agree well with the experimental results,

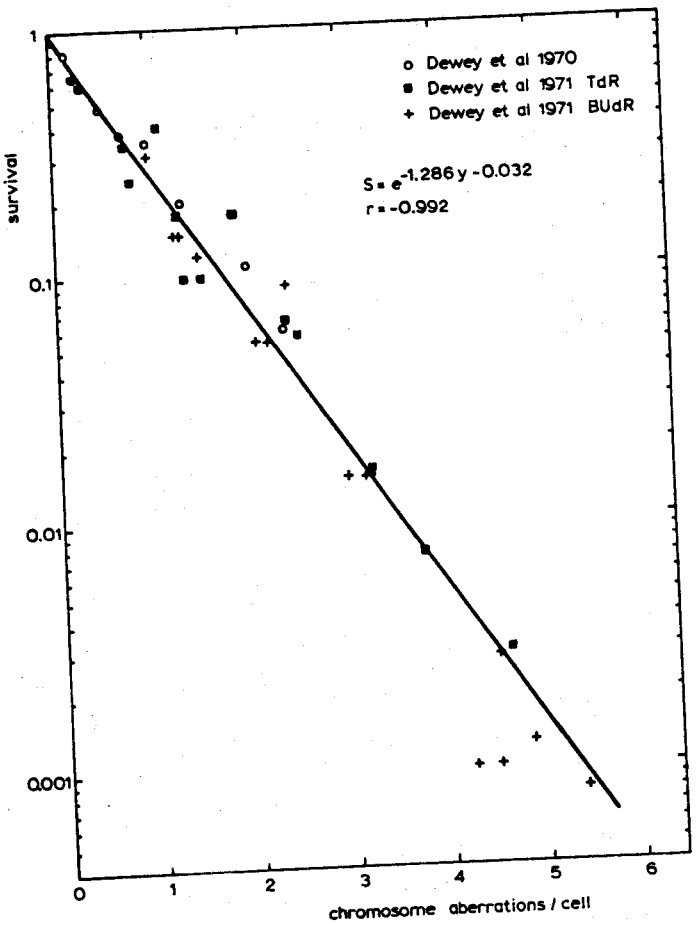
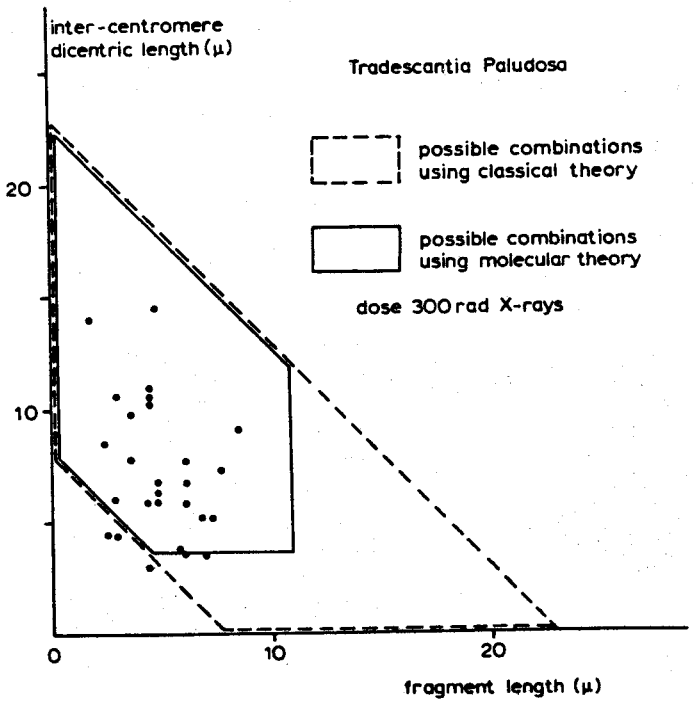


Figure 3

The direct correlation between cell death and chromosome aberrations (data from Dewey *et al* Radiation Research 43, 1971). The correlation is the same independent of cell phase and the presence or absence of a radiosensitizer

Figure 4

The dependence of inter-centromeric distance and fragment length in dicentrics found in *Tradescantia paludosa*. The continuous line defines the area expected on the basis of the new proposals. The dotted line defines the area expected on the basis of the classical and exchange theories.



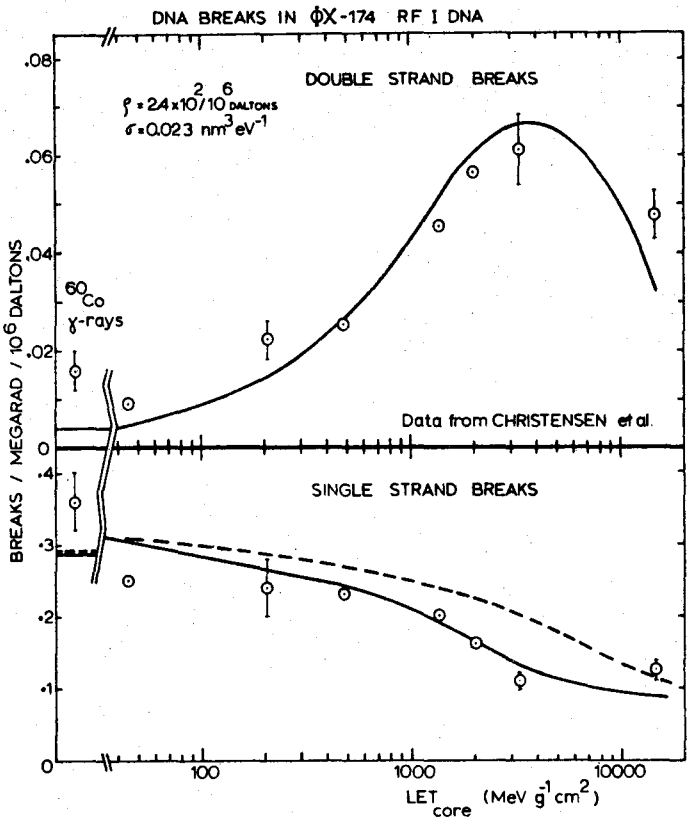


Figure 5

Comparison of the calculations, (line) and experimental results on the LET dependence of the induction of double strand breaks in DNA *in vitro*. (data from Christensen *et al.* International Journal of Radiation Biology 22, 1972).

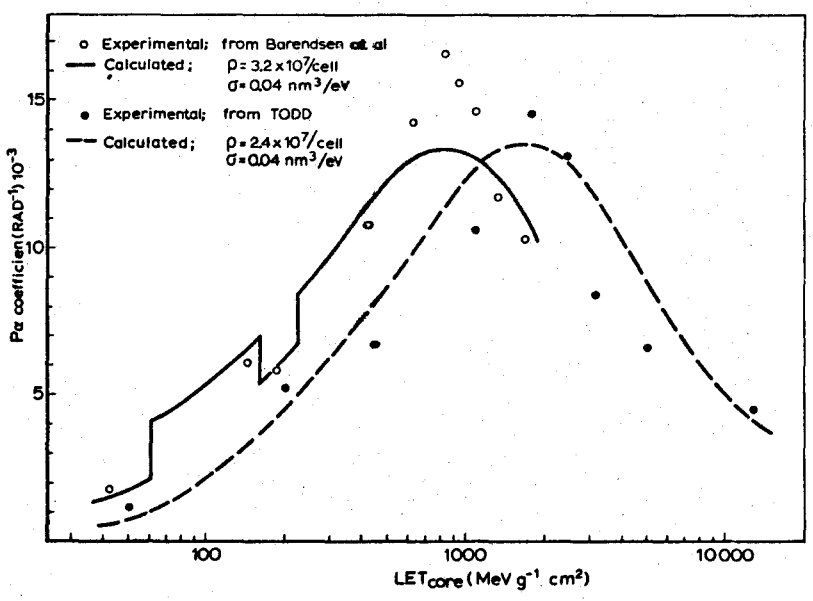


Figure 6 - Comparison of the calculations (line) and experimental results on the LET dependence of cell survival in T 1 kidney cells (data from Todd and Barendsen).

both for cell survival and DNA double strand breaks *in vitro*.
The apparent difference between the LET dependence of cell killing of T 1 kidney cells as measured by Todd and Barendsen was resolved in the calculations and could be explained as resulting from different track structure parameters coupled to a fixed geometry of a double target.
An attempt was made to broaden the field of the theory and the implications of the model for radiological protection were considered. The important conclusions arising from this work was that at very low doses the radiobiological effect should be linearly related to dose with no threshold at the cellular level and that the limiting relative biological effectiveness applicable at low doses will be the same for different effects such as mutations or cell death in the same cell.
Further thought was given to the genetic consequence of a double strand break in the DNA and it was suggested that the break, or the repair of the break might lead to the induction of mutations. An equation was derived to describe the induction of mutations with dose assuming that only a double strand break could lead to a mutation or to cell death and that a mutation could only be expressed if the cell divided. The equation is

$$M = k(1-e^{-q(\alpha D+\beta D^2)})\, e^{-\rho(\alpha D+\beta D^2)}$$

The implications of this equation for practical applications are that the maximum number of mutations is independent of the type of radiation and that the mutation spectrum will be dependent on radiation dose but not on the type of radiation used.

Further biological control of primary radiation effects - K.J. PUITE, P.A.T.J. WERRY.

Pollen germination studies using *Tradescantia paludosa* L.

Tradescantia plants are well suited for the fundamental radiobiological research, which is carried out in direct relationship with the theoretical model studies of the molecular mechanism of radiation on biological tissue. The period of interphase of 5 days before first pollen mitosis is known to have a constant radiosensitivity; chromosomal aberrations can easily be scored at the metaphase of pollen mitosis, while the germinating ability of mature pollen is considered to be related to the intactness of the vegetative nucleus arising from the mitotic division. When the percentage of germinating pollen is plotted as a function of dose, curves can be obtained comparable with survival curves of mammalian cells.
Firstly, a method for germination of Tradescantia pollen *in vitro* has been developed, starting from data available in literature. The results of these experiments (in collaboration with Miss G.S. Bokelmann) are collected in the ITAL External Report No. 9.
Each inflorescence contains a series of buds which are all in a different stage of development. Each bud contains a synchronous cell population. Irradiation of an inflorescence results in exposure of these cell populations which are also at different developmental stage. Under optimal climatic conditions one flower will open each day. Therefore, the effect of radiation can be studied over a whole range of pollen grain development. In figure 7 data are collected after exposure to different X-ray doses (2 mm Cu HVL, exposure rate 90 rad/min). During the first 1-4 days after irradiation no influence of the doses given can be seen. These data correspond to pollen grains exposed after they have passed mitosis. Pollen exposed during interphase before their mitotic division (days 6-10) show a reduced germination percentage. A further reduction in germination takes place at day 11, 12 when the pollen have been exposed in their meiotic phase.
The proportion of pollen germinating after exposure to X-rays, during interphase is plotted in figure 8. The curve shows some similarity with cell survival curves.

Peroxidase activity of irradiated pollen of *Tradescantia paludosa* L.

Also another type of radiation damage caused during exposure may be scored with mature pollen, e.g. the lack of peroxidase activity in a pollen grain. The peroxidase activity can be detected by transferring mature pollen to 15 ml of a saturated benzidine solution to which 0.2 ml of a 3% H_2O_2 solution has been added. Pollen grains containing peroxidase become enlarged and are coloured deeply blue. After about two hours the blue colour is turned into brown.
A reduced peroxidase activity is found when the exposure has taken place during the interphase of pollen grain development. The results of an exposure during this stage is shown in figure 8. The porportion of pollen grains with peroxidase activity seems to follow a straight line when the data are plotted on a semi-logarithmic scale. Further experiments are directed towards an understanding of the basic mechanism of this effect and the relationship with germination curves.

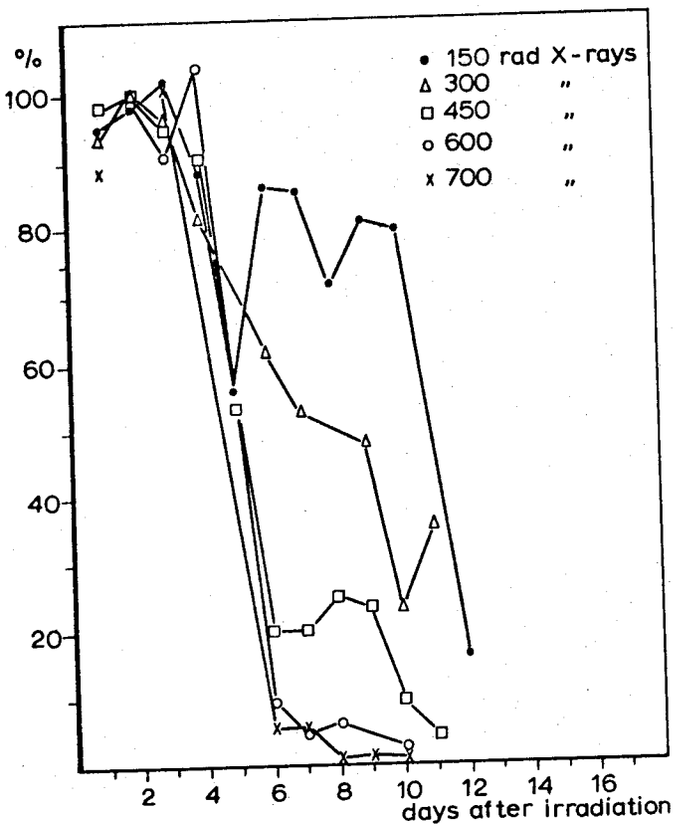


Figure 7

Percentage of pollen grains of *Tradescantia paludosa* L. germinating as a function of time after different X-ray doses (2 min Cu HVL, exposure rate 90 rad/min).

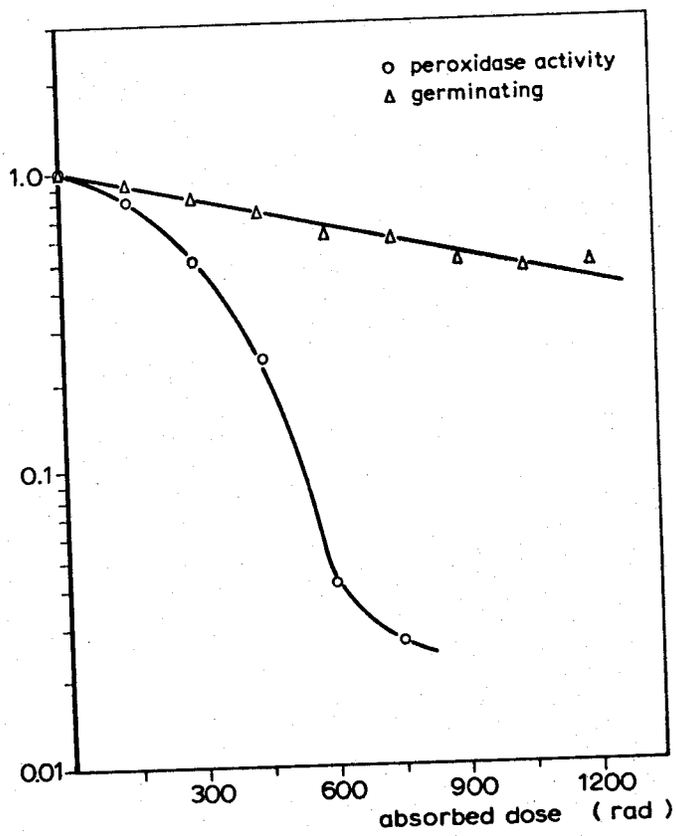


Figure 8

Proportion of pollen grains of *Tradescantia paludosa* L. germinating or showing peroxidase activity, when exposed to different X-ray doses (2 mm Cu HVL, exposure rate 90 rad/min; data taken 7, 8 and 9 days after irradiation).

The radiation dose-fractionation effect in *Saintpaulia* - H.M.G. GROOT, C. BROERTJES, K.H. CHADWICK, H.P. LEENHOUTS, K.J. PUITE.

Leaves of *Saintpaulia ionantha* H. Wendl. c.v. Utrecht show, when exposed to a low initial irradiation dose, a higher radioresistance against a second dose, given 8 to 24 hours after the first dose, in comparison to unpretreated leaves. This effect has been observed with X-rays as well as with fast neutrons. A few possibilities about the origin of this dose-fractionation effect, such as changes in metabolic activity of the leaves, transport of a radiation-induced protective agent, were investigated. So far, only X-rays have been used.
Concerning the metabolic activity of the leaves special attention was paid to the influence of temperature. Different temperature-treatments (6, 13, 20 and 29 °C) have been applied to detached leaves in sealed polyethylene bags, 24 hours before the first irradiation, during the 8-h time interval between irradiations and 24 hours after the second irradiation. In addition, similar treatments were applied exclusively during the 24-h time interval between the two irradiations. No fractionation effect was observed at 6 °C, this temperature proved to be too low, as there were (some) symptoms of cold damage. Differences in reaction at the temperature-treatments of 13, 20 and 29 °C were observed, especially in the second experiment (temperature-treatment during time interval only), in which the greatest fractionation effect was obtained at 13 °C. These differences might, however, be related to time interval: it is possible that the time interval of 24 hours was optimum for 13 °C and not for 20 and 29 °C. An experiment was carried out in which a range of intervals (1 - 4 - 7 - 10 days) at two temperatures (10 and 20 °C was investigated. The dose-fractionation effect was highest at the 24-h interval and decreased with longer time intervals for both temperatures; decrease, however, was faster at 20 °C (no fractionation effect at 7-days interval) than at 10 °C (still a small fractionation effect at 7-days interval). Noteworthy was the relatively higher dose-fractionation effect at almost lethal doses (10% survival) in comparison to doses resulting in 70% survival. Considering the results of this experiment it might well be, that in the range of 0 - 4 days not enough data were present. It was impossible to conclude from the data available, whether or not the optimum time interval (interval resulting in the highest dose-fractionation effect) was different for the two temperature-treatments. Therefore, an experiment was carried out in which time intervals of 24-48-72-96 hours were chosen for the treatment at 10 °C and of 8-16-24-96 hours for the treatment at 20 °C. Results of this experiment are not yet available.
Attention was paid as well to the influence of the gas environment upon the dose-fractionation effect. An experiment, investigating the influence of the gas environment inside sealed or open polyethylene bags, in which the leaves normally are irradiated and stored during the time interval between irradiations, resulted in a somewhat higher dose-fractionation effect for sealed than for open bags.
Experiments in 1972 suggested that leaves, irradiated with 500 rad X-rays showed a somewhat higher respiratory rate (measured with the Infra Red Gas Analyser) during the first 24 hours after detaching, as compared to un-irradiated leaves. To see whether or not respiration is correlated in some way with the dose-fractionation effect, a preliminary experiment was

carried out, in which respiration was partly blocked, during the 8-h time interval between the two irradiations, by a CO/air mixture (in the dark). Results of this experiment cannot be presented yet.
In order to investigate whether or not transport is involved in the development of the dose-fractionation effect, petioles of leaves were irradiated with high X-ray doses, with pretreatment (8 or 24 h before) or without pretreatment of either the leaf blade or the petiole. As this experiment did not give clear results, a more detailed experiment was carried out, of which no data are available yet.
Concerning the radiosensitivity of the leaves it was demonstrated that the time interval between detaching and irradiation was of importance: a change in radiosensitivity of the leaves after storage was observed. Leaves, irradiated 8 hours after detaching and stored during this period in sealed polyethylene bags in the dark, were less radiosensitive than leaves, irradiated immediately after detaching, whereas leaves, irradiated on the plant and detached 1 hour after irradiation, were the least radiosensitive. These results might have consequences for other experiments carried out formerly (acutely irradiated leaves were mostly irradiated directly after detaching, while leaves, exposed to fractionated irradiation, generally received the final high dose after 8 hours).
This "time-effect" has been confirmed by several other experiments. There are indications that the radiosensitivity of the leaves decreases with a longer time interval between detaching and irradiation up to a 24-h interval. Data, giving the radiosensitivity at longer time intervals are inconclusive. Experiments have been started to investigate this "time-effect" more thoroughly.
Another fact, demonstrated by several experiments, is the stimulation of the production of adventitious plantlets at the base of the leaf petiole by low doses of radiation (1-3 krad). Whether this stimulation is correlated in some way to the dose-fractionation effect is unknown. This idea, however, was not supported by a low-temperature treatment (6 °C during a 24-h time interval), resulting in a stimulation without a dose-fractionation effect. The stimulation as such seems to be a fact, it seems to be independent of wounding-effects (caused by detaching the leaves of the plant), since the stimulation was observed both when the leaves were irradiated after detaching and when the leaves were irradiated on the plant and detached several hours (up to 24 hours) later.
Finally it is being investigated whether or not the determination of adventitious plantlets at the base of the leaf petiole at an earlier developmental stage gives results comparable to the normal production data (obtained 6 months after planting).

MUTATION BREEDING

Adventitious bud technique - C. BROERTJES, S. ROEST, G.S. BOKELMANN.

The use of the adventitious bud technique, viz. 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 are obtained. This has been demonstrated in a number of crops such as *Achimenes*, *Streptocarpus* and others. This aspect of the project (a) is being continued, though on a somewhat smaller scale, as a demonstration for (potential) users, whereby the combined use of radiation, colchicine-treatment and so-called conventional breeding methods is emphasized. During this work at times mutants with commercial value are produced (see below).
The second and increasingly important part of the project (b) 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 also look promising, either as a tool in plant breeding or as a (fast) propagation method. As soon as part (b) has produced results, it will be investigated whether or not artificially induced adventitious shoots of the method(s) in question develop from one cell and consequently are or are not of interest for mutation breeding (c).
a. In 1971 a number of mutants were produced of the white flowering *Streptocarpus* cv. Maassen's White (a spontaneous mutant of "Constant Nymph"). Besides, a number of autotetraploids were produced by colchicine-treatment (using the adventitious bud technique) of "Maassen's White" as well as of mutant 7111 (a mutant of "Maassen's White" with longer flower stalks and larger number of flowers). All were propagated clonally and compared with each other in 1972. During a final test in June 1973 none of the mutants proved to be of commercial value. One of the autotetraploid forms of mutant 7111, having large white flowers and very sturdy flower stalks, however, was awarded a Certificate of Merit and has been released to the trade under the name "Albatros". This demonstrates that a combination of radiation, colchicine-treatment and the use of the adventitious bud technique can produce commercial results within a relatively short period.
In *Achimenes* a few mutants, produced earlier, were selected in 1972 for final judgement in 1973. Two of them were awarded a Certificate of Merit, namely a mutant with a very compact growth habit, even under sub-optimal greenhouse conditions. It is, moreover, extremely free flowering. This very promising mutant will be commercialized as "Cupido". A second mutant has large flowers and a less condensed growth habit; its name is "Orion". The pink-flowering *Achimenes* cultivars are increasingly attracting interest, partly because better forms have been introduced recently (more condensed growth habit, free flowering, also under less optimal conditions). The flower size, however, is not sufficient. A combined use of colchicine-treatment and radiation, together with the adventitious bud technique, seems to be the appropriate way to improve these cultivars. To this end leaves of three of the best cultivars, namely "Tarantella", "Repelsteeltje" and "Little Beauty" have been subjected to colchicine-treatment. A number of (very attractive) autotetraploids have been

produced of all three cultivars. They are being propagated clonally and will be or are already irradiated to produce mutants at the tetraploid level (with large(r) flowers and more intense flower colours). This again is to demonstrate the usefulness of combining the above mentioned techniques.
Two of the many *Kalanchoë* mutants, obtained after irradiation of leaves, were selected early 1973, to be propagated for a final test. One of the mutants had an extremely bright red flower colour, even when grown during December and January (see publication by Broertjes and Leffring).
Muscari, *Endymion* and *Scilla*, three species belonging to the *Liliaceae* can be reproduced very readily via the adventitious bud technique. Thousands of adventitious plantlets of *Muscari* flowered and many mutants were observed. Although most mutants were only slightly different from the control, we got the impression that none of them showed chimerism. Twenty of the most striking mutants will be propagated to finally test whether or not adventitious plantlets also ultimately originate from one cell. At the same time cytological-histological observations are being carried out. *Scilla* did, so far, not produce mutants; *Endymion* did not yet flower.

b. The adventitious bud formation of *Chrysanthemum morifolium* Ram. cultivars Bravo and Super Yellow was investigated *in vivo* and *in vitro*. For that purpose vegetative and generative stockplants were cultivated under respectively long and short day conditions.
In vivo, leaves of both cultivars (using 9 leaves per shoot and designating the top leaf as position 1) were excised and distributed over the treatments. Adventitious roots arise directly from the base of the petiole; the first roots appear after 9 days and 3 weeks, after leaf detachment; all the leaves are rooted.
Adventitious shoots so far exclusively develop on leaves of cv. Bravo; usually emerging from callus at the base of the petiole within a period of 2 - 6 months after leaf excision.
It was observed that leaves of some plants regenerated more adventitious shoots and more readily than leaves of other plants. Leaves of the positions 1, 2, 8 and 9 showed a higher regeneration ability than leaves of the intermediate positions. Adventitious shoot formation was stimulated by a combined treatment with 1% β-indolebutyric acid (on talc basis) after 0 weeks and 6-benzylamino-purine (BA, on lanolin basis) 10^{-5} g/ml after 3 weeks.
Exposure to light of the base of the petiole and the upper part of a few roots appeared to be favourable for adventitious shoot formation compared to continuous darkness (the normal procedure). In the former situation, occasionally, the shoots did not originate from the base of the petiole but from roots.
In vitro, both cultivars regenerated adventitious shoots on explants of the leaf, the flower-stalk and the flower-head (capitulum). The cv. Super Yellow always initiated more shoots than comparable explants of the cv. Bravo. The application of the medium of Murashige and Skoog considerably improved the formation of adventitious shoots. The addition of a cytokinin (BA) and a sugar (sucrose) proved to be of essential significance and furthermore the initiation of adventitious shoots was

enhanced by using explants as young as possible.
Leaf-explants regenerate shoots via callus, usually at the base of the petiole, 3 weeks after incubation,and explants of the flower-stalk yield a direct regeneration over the whole length of the segment and the first adventitious shoots emerge 10 days after incubation.
Shoots arise on capitulum-explants directly from the receptacle or indirectly via callus 2 - 3 weeks after incubation. The development of the shoots was increased when the disc and ray florets and upper involucral bracts were nearly completely cut off. Shoots with a length of at least 1 cm were isolated from the capitulum and the basal end of the shoot was treated with 1% β-indoleacetic acid (on talc basis). After a subsequent transfer of the shoot to unsterilized soil (a mixture of leaf mould and sand) adventitious root formation was induced and complete plantlets were produced a few months after incubation. Approximately a half year after incubation uniform flowering plants were obtained.

c. The usefulness of the adventitious bud technique, using detached leaves, was tested with *Chrysanthemum morifolium* Ram. cv. Bravo. Usually a very restricted percentage of the leaves produce adventitious shoots, but in one experiment, for reasons unknown, over 50% of the leaves produced 1 - 3 shoots. Since the leaves were irradiated with 500 rad X-rays it was possible to analyse whether or not solid mutants can be obtained by this method. The preliminary results are, in short, that the majority of the plants, carrying a visible mutation, are chimeric of structure and that, consequently, the production of adventitious shoots via callus tissue (formed at the base of the petioles) is less useful than the formation of adventitious buds from epidermal tissue (as is demonstrated with *Achimenes*, *Begonia*, *Streptocarpus*, etc.).

Mutation research in potato (*Solanum tuberosum* L.) - A.M. VAN HARTEN, H. BOUTER.

Regeneration of X-irradiated potato material

To study the behaviour of 3 histogenic layers after irradiating potato meristems, the stable monecto-chimeric mutant EM52 (tuber constitution L_1 genetically yellow, L_2 + L_3 genetically red) with red splashed tubers and white flowers was selected from the homohistic red-tubered and lilac-flowered cv. Desirée.
Two additional trials were performed to complete last year's information. In the first trial single eyepieces of tubers of this mutant were irradiated with 0, 500, 1000, 2000 and 4000 rad (dose rate 250 rad/min) and studied both morphologically in the greenhouse and microscopically from microtome sections of irradiated shoot apices.
In the second experiment the phenomenon of layer replacement was studied morphologically on 2 related clones: EM52 (red splashed monecto-chimera) and E52 (a fully red clone, derived from L_2 and L_3 tissue of EM52).
Four dosages of X-rays (400, 800, 1600, 2400 rad + control), 2 dose-rates (20 and 200 rad/min) and 2 temperatures (12 and 20 °C) were used.
Results are given in tables 1 and 2.

Table 1 - Frequencies of tuber colour changes to (sectored-) red and yellow vM_1 tubers of X-rayed, red-splashed, tubered EM52.

Objects	No of studied vM_1		% sectored-/red in		% yellow in	
	plants	tubers	plants	tubers	plants	tubers
Control	35	107	0	0	0	0
500 rad	37	121	0	0	0	0
1000 rad	33	95	3	1	0	0
2000 rad	35	123	31	16	1	6
4000 rad	21	92	57	50	19	12

Table 2 - Frequencies of tubercolour changes from EM52 (red-splashed to red and yellow) and E52 (red to red-splashed and yellow).

Objects	% surviving vM_1 plants		% tubercolour changes in plants		% tubercolour changes in tubers		% yellow tubers in plants		% yellow tubers in tubers	
	EM52	E52	EM52	E52	EM52	E52	EM52	E52	EM52	E52
Control	96	97	9	0,7	3	0,7	0	0	0	0
400 rad	99	89	17	0,7	9	0,6	0,3	0	0,2	0
800 rad	81	88	19	1,9	9	1,1	1,4	0	0,6	0
1600 rad	83	87	33	2,3	16	1,8	1,1	0	0,4	0
2400 rad	86	81	50	5,8	25	2,8	2,1	0	0,5	0
24 rad/min	89	77	33	1,8	16	1,8	2,2	0	0,8	0
240 rad/min	85	96	27	1,7	13	1,7	0,2	0	0,1	0

The above results highly corroborate the assumption of layer perforations, reduplications and re-differentiation to prove that L_1 is genetically yellow.
Histological observations suggest differences in mutability between histogenic layers in potato, or perhaps between red and yellow tissue.

In a small experiment with the clone EM52 the effects of "dry" and "wet" irradiation were compared. Irradiation under water gave a lower radiation sensitivity and an increase of perforations. This point will be re-investigated.
The new diploid basic clone containing (very probably) 6 monofactorial marker genes in heterozygous condition has been subjected to some preliminary irradiation experiments. Results showed a high radiation sensitivity but rather low mutation frequencies. Unfortunately the clone proves to be very susceptible to spider-mite attacks.
Adventitious sprout formation from detached leaves and leaflets has been studied now for three years, using more than 700.000 leaflets. Results were disencouraging. *In vitro*-work with epidermal leaf tissue did not start yet, because of lack of accomodation. Another method of producing (adventitious?) plantlets from detached rooted sprouts (the so-called Jørgensen method) was tried, but had to be rejected as sprouts (and consecutively tubers) did not originate from L_1 but mainly

reproduced the three-layered structure of the monecto-chimeric base clone EM52 (see before) on which experiments were performed.

Screening for resistance to diseases

A rather significant increase of resistance against leaf-roll virus was found in several irradiated series during several years. Results will soon be worked out and published.
Y-virus investigations showed greatly deviating results for 1973 as compared with previous years. The experiments will be continued.

Yield experiments

The 1972 results showed some promising correlations with former trials. Experiments will be continued.

Maintenance and multiplication of clones

About 1200 sub-clones derived from irradiated plantmaterial of the cultivars Bintje, Burmania and Desirée and of some dihaploid and diploid material are maintained in the new polders under favourable conditions to serve as basic material for further experiments at Wageningen and for demonstration purposes.

Generative programme

Investigations to produce a vigorous fertile testclone, heterozygous for several characters are continued. Progress is slow.

Protein improvement

Protein production of Pisum (*Pisum sativum* L.) mutants and recombinants in relation to protein quality - W. GOTTSCHALK, H.P. MÜLLER, D. MÜLLER.

The protein content of the seed meal is a theoretical value which can easily be obtained; therefore, it is possible to compare a great number of mutants with one another. More important, however, is the protein production per plant because the seed production of the plants is considered in this value. The method used can be demonstrated comparing the data obtained from two mutants and their recombinant (figure 9). Mutant 68C shows a seed production nearly similar to that of the initial line in a long years average, while mutant 1201A is essentially better. The recombinant R 350, homozygous for both the mutated genes, behaves as mutant 68C, that means as the worst parent. The three genotypes agree with one another and with the initial line with regard to the protein content in equal amounts of seed meal. Also with regard to the total amount of the essential amino acids nearly no differences could be found. The content of methionine - the limiting amino acid in legume proteins - of both the mutants is higher as compared with that of the control. This is also valid with regard to the recombinant, but in this case the recombinant agrees with the better of the two parents.

A completely different situation is obtained if also the seed production of the genotypes is considered. The productivity of mutant 1201A is obvious because the increased number of seeds per plant is not influenced by any negative factors. The recombinant, however, has not only a reduced seed

number per plant, but also a reduced seed size. Therefore, its mean value for the character "seed weight per plant" is only about 90% of the corresponding mean of the initial line. A similar relation is found with regard to the protein production per plant. Mutant 1201A shows a particularly favourable situation as far as
- the seed protein content per plant,
- the total amount of essential amino acids per plant,
- and the content of methionine

is concerned.
Mutant 68C shows a different situation for these parameters, but in general, it can likewise be regarded as positive in comparison to the initial line. The recombinant, however, does not reach the favourable properties of its parents. It has to be regarded negatively with the exception of its methionine production. Similar results were obtained for all the amino acids of some mutants and their recombinants.

A few mutants have been analysed with regard to the quantitative and qualitative situation of the sugars present in their seeds. They show a similar genetically conditioned diversity as reported for the proteins. There are certain indications that the germination behaviour of the mutant depends to some extent on their glucose content.

The influence of climatic factors upon seed production and protein in *Pisum* - W. GOTTSCHALK, H.P. MÜLLER, D. MÜLLER.

Some of the most promising mutants of our collection show a very distinguishable response to different climatic conditions. This is not only generally valid with regard to the seed production, but also with regard to the quantitative and qualitative protein situation. This becomes clear by comparing the material grown at Bonn/Germany and at Udaipur/India. Mutants 46C (earliness) and 1201A (stem bifurcation) do not show any significant difference with regard to their total protein content under the moderate and subtropical conditions (figure 10). The fasciated mutant 251A, however, shows striking differences. The protein content of its seed meal in Bonn is about equal to that of the initial line. In Udaipur, however, it is about 30 percent higher. Thus, the mutant is obviously able to utilize the Indian soil and/or the climatic conditions for its protein synthesis essentially better than the initial line.

In 1973, the evaluation of our mutants in India was not possible because the plants dried as a consequence of the lack of monsoon rainfalls. An early ripening mutant, however, was not only able to survive, but even to produce seeds. Thus, it was superior to its initial line as well as to the Indian local varieties. This is one of the very rare examples for the positive selection value of an experimentally produced mutant under extreme climatic conditions. The highest yielding mutant of our assortment - a fasciated genotype, which does not flower in India - was able to produce seeds under the tropical conditions of Ghana.

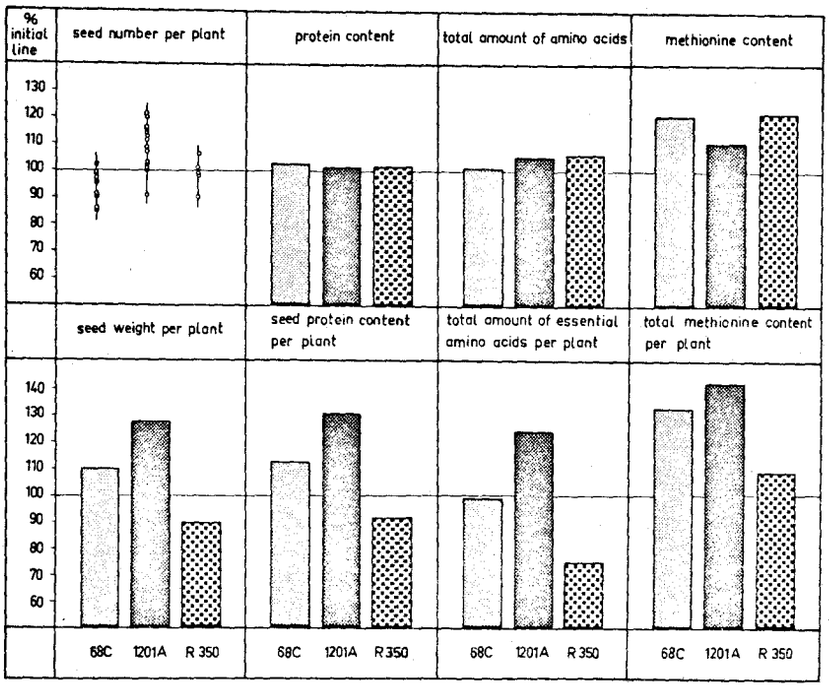


Figure 9 - Comparison of some yield characters of the useful mutants 68C (increased ovule number), 1201A (stem bifurcation) and its recombinant R 350 (= 68C/1201A) as related to the corresponding mean values of the initial line.

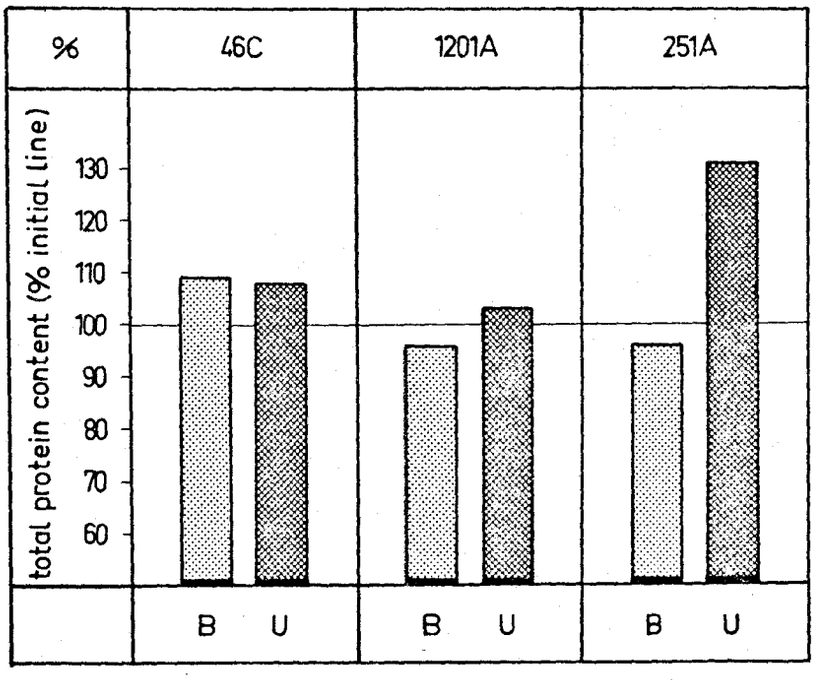


Figure 10 - The protein content of the seed meal of mutants 46C (earliness), 1201A (stem bifurcation) and 251A (stem fasciation) cultivated at Bonn/Germany and at Udaipur/India.

Cytogenetic investigations concerning pea mutants - W. GOTTSCHALK, H.P. MÜLLER, D. MÜLLER.

The meiotic behaviour and fertility of seven radiation induced translocation lines was analysed. In six of them, two non-homologous chromosomes are involved. In the seventh mutant, three non-homologous chromosomes have been altered structurally forming a ring of 6 chromosomes in the first meiotic metaphase. The identification of the translocated chromosomes and the localization of the translocation point was possible in one of these lines analyzing its karyotype of mitotic metaphase chromosomes. Moreover, two tertiary trisomics were isolated derived from translocated plants. Four lines homozygous for the translocated chromosomes were developed from translocation-heterozygous strains.

Utilization of natural and mutagen-induced protein variability in diploid barley (*Hordeum vulgare* L.) - H. GAUL, H. WALTHER, V. LIND.

In 1973, the induced variability from 594 micro-mutants and 756 macro-mutants was tested for "% protein in dry matter" and "% lysine in dry matter". As a criterion for the biological value of the protein "% lysine in protein" was used. All macro- and micro-mutants originated from the commercial varieties "Matura", "Haisa II" and "Amsel". All mutants were preselected for economical characters other than protein quantity and quality, during at least 6 generations. Comparisons among the two groups of mutants and between mutants and the original varieties were made, using all three selection criteria simultaneously. The results can be summarized as follows:

- The induced total variability of protein and lysine content is comparable to the spontaneous variation, known from conventional breeding. In addition, however, some extremely deviating lines were selected in the upper and the lower ranges of variation for all three selection criteria.
- In macro-mutants as well as in micro-mutants the variation of protein and lysine content is normally distributed with mean values close to their mother varieties. This is rather unexpected and possibly due to changes of other morphological characters related to protein quantity and quality.
- The negative correlation between "% lysine in protein" and "% protein in dry matter" has been reported in the last years' results for a limited number of mutants. This is also confirmed for a large population of mutants with different sub-groups. For the total population the relation found was $r = -0.72^{**}$. If protein and lysine measures are given on dry matter basis, the relation is positive with $r = 0.67^{**}$.
- Since the variation of the induced mutants exceeds the mean values of their mother varieties, selection of valuable mutants seems possible in both mutant groups. A selection rate of 10% is obviously sufficient in pre-screening and detection of superior mutant lines improved in both, protein and lysine content.

- According to the type of distribution the protein content is thought to be inherited quantitatively and in a polygenic way, the lysine content being mono- or oligogenic. The environmental modification of these characters cannot be separated as yet from the genotypical variation on the basis of these results.
- The changes in protein and lysine content due to mutagenic treatment seem to occur more often in micro-steps than in macro-steps.

Utilization of the protein variability of diploidized tetraploid barley (*Hordeum vulgare* L.) - H. GAUL, W. FRIEDT, H. WALTHER.

From previous results tetraploid barley strains are known to contain higher amounts of protein and lysine in dry matter. For a population of 120 tetraploid mutants these results were confirmed. The average difference in protein content between diploid and tetraploid mutants amounts to 4%. For lysine content the average difference amounts to 0.1%.
As for micro- and macro-mutants, the mean of the tetraploid mutant population is close to the average value for the original varieties. It seems, therefore, quite possible to select either for high protein or high lysine mutants, or for mutants high in protein and lysine.

Since the amount of lysine in protein is used as a measure of the biological value of protein, this selection criterion was checked in the diploid and tetraploid mutant groups. On account of the negative correlation between "% lysine in protein" and "% protein in dry matter", superior mutants with high amounts of protein and lysine in dry matter indicate a low content of "% lysine in protein". The tetraploid strains, therefore, produce in general protein with the lowest biological value.

Using a three-dimensional selection scheme, fields of covariation were differentiated to make selection possible including the biological value of the protein. According to this scheme, several superior lines were selected in all three mutant groups. The values of the best line in each group are presented for comparison:

Micro-mutants:	% protein in dry matter	14.3
	% lysine in dry matter	0.68
	% lysine in protein	4.75
Macro-mutants:	% protein in dry matter	20.8
	% lysine in dry matter	0.73
	% lysine in protein	3.50
Tetraploid mutants:	% protein in dry matter	19.1
	% lysine in dry matter	0.71
	% lysine in protein	3.70

Qualitative genetic improvement of plant protein in opague maize (*Zea Mays* L.) - A. BIANCHI, C. LORENZONI, M. STANCA, F. SALAMINI.

During the first year of activitv no results of scientific meaning were expected from this project, taking into account the time needed for induction of genetic variability affecting the behaviour of the

opaque-2 gene (o_2).

Some results, however, about the behaviour and the number of the plants obtained from the irradiated seeds are available. The 4000 rad dose yielded a result comparable to the D L 50, either for the B 37 o_2 and its normal counterpart. The 8000 rad dose reduced heavily the seed germination and the fertility of the plants. The number of progenies collected for each treatment was:

4000 rad (B 37 o_2):	1079 ears
4000 rad (B 37 normal):	290 ears
8000 rad (B 37 o_2):	163 ears
8000 rad (B 37 normal):	50 ears

In each progeny, a number of seeds varying from 8 tot 150 was found.

Disease resistance

Selection and analysis of spontaneous and induced mutations for disease resistance in various *Lycopersicon* species and in *Triticum durum* (Desf.)

The project on disease resistance in tomato started in 1973 with the purpose to find or to induce resistance against two pathogenic agents for which no resistance is so far available.

The pathogenic agents (*Verticillium albo-atrum* (Rke. & Berth.) race 2 and *Phytophtora infestans* (Mont.) De By, race T_1) have been obtained and the methods for their storage and propagation and the techniques for the infection and screening analysis between healthy and diseased plants have been set up (Tomarchio, in press).

Irradiation of Italian tomato cvs. was performed by gamma treatments of both gametes following a procedure already set up in this Laboratory during a previous contract: this kind of a treatment allows the absence of a chimeric situation in the M_1 plants, so that a higher number of M_2 plants for each M_1 plant is available for the analysis. Several M_1 plants are now growing in the greenhouse. In the same time, about 8.000 M_2 seedlings coming from previous irradiations of the gametes of "S. Marzano" cv., were scored for *V. albo-atrum* Rke. & Berth.) (race 2) resistance, but no resistant plant was found.

Eight wild species of tomato were analyzed for vertical resistance to the two above mentioned parasites and to the other agents of the tracheomicosis in tomato (race 1 of *V. albo-atrum* (Rke. & Berth.); races 1 and 2 of *Fusarium oxysporum f. lycopersyci* (Schl. ex Fr.)). The results of the experiments (Monti, Saccardo and Tomarchio, in press) are reported in table 3: new sources of resistance have been found against the pathogenic agents for which resistance is already available; for instance, three species (*Lycopersicon chilense* (Dun.), *Lycopersicon hirsutum* (H.B. & K.) and *Solanum pennellii* (Correll.)) were found to be resistant to race 2 of *F. oxysporum*, besides *Lycopersicon peruvianum* Mill., already known to be resistant to such disease. No resistance has been found against race 2 of *V. albo-atrum*. As *P. infestans* race T_1 is concerned, two plants of *L. hirsutum* were found to be partially resistant to the fungus and studies have been started for a better evaluation of such a reaction.

Table 3 - Reaction to *Verticillium albo-atrem* (Rke. & Berth.), *Fusarium oxysporum f. lycopersici* (Schl.ex Fr.) and to *Phytophthora infestans* ((Mont.) De By) in different species of *Lycopersicon* and in *Solanum pennellii* (Correll.).

SPECIES \ PATHOGEN	*V. albo-atrum*		*F. oxysporum f. Lyc.*		*P. infestans*
	race 1	race 2	race 1	race 2	race T_1
L. esculentum (S. Marzano)	S	S	S	S	S
L. pimpinellifolium	S	S	R	S	S
F_1 (*L. exculentum* x *L. pimpinel.*)	S	S	R	S	S
L. peruvianum v. *humifusum* (*L. pissisi*)	S	S	R	S	S
L. peruvianum - line 1	R	S	S	S	S
L. peruvianum - line 2	R	S	R	R	S
L. chilense	R	S	R	R	S
L. hirsutum	S	S	R	R	S*
F_1 (*L. esculentum* x *L. hirsutum*)	S	S	R	R	S
F_1 (*L. esculentum* x *L. Cheesmanii*)	S	S	R	S	S
Solanum pennellii	S	S	R	R	S
F_1 (*L. esculentum* x *S. pennellii*)	S	S	R	R	S

* two plants partially resistant

Crosses between some of the wild species and *Lycopersicon esculentum* Mill. have been performed also and the analysis of the F_1 plants confirmed the results obtained on the wild species themselves and the dominant behaviour of such resistances.

Analysis for bunt (*Tilletia triticoides* (Savul.))resistance in *Triticum durum* (Desf.) has been performed using 162 mutant lines isolated from cv. Cappelli. Mutant lines were isolated after treatment with X-rays, fast and thermal neutrons, EMS. All mutants have been purified and their distinct morphological or physiological traits described in several generations. Cappelli control showed a moderate resistance (20% of attack) to the mixture of the extremely virulent races of *Tilletia*. About ten mutant lines showed no trace of infection, while about 40 lines are below 7% of infected plants. Some lines, however, showed a much higher level of susceptibility, which is reaching more than 40% of diseased plants.
All set of mutants will be retested in 1973-74 in order to confirm the previous year's results. Results obtained so far seem to confirm the previous findings as to the possibility of modifying the bunt resistant reaction after treatment with mutagenic agents.

Induction of mutations for resistance to *Erisiphe polygoni* and to *Ascochyta* blight in commercial varieties of *Pisum sativum* L. -

G.T. SCARASCIA-MUGNOZZA, A. CICCARONE, A. GRANITI, M. CIRULLI, G. PACUCCI, V. DELLACECCA, C. DE PACE, A. BLANCO, A. FILIPPETTI, A. MONTEMURRO.

In the first year of the contract, seeds of varieties Sprinter (S) and Freezer 69 (F) have been treated with physical mutagens (X rays; dosis: 10 and 15 krad; symbols: R_1 and R_2, respectively) and with chemical agents (diethylsulphate; doses: 1 °/oo and 1,5 °/oo; symbols C_1 and C_2, respectively). The germination rate ascertained in greenhouse test was around 25 - 30%. Thus, 5900 seedlings from treated seeds have been transplanted in the field, together with 1215 seedlings from untreated seeds.

Altogether, 5208 adult plants have been harvested (table 4). Seeds have been collected keeping separately seed produced on the stem and on the series of secondary branches, named from the bottom: B-C-D. Moreover, stem-progenies and branch-progenies have been discriminated according to the size of seed-setting.
Table 5 summarizes the amount and characteristics of the M_1-progenies following the above mentioned criteria.

Observations of the presence of infertile or atrophic pods in M_1 adult plants have been carried out as an indication of the effectiveness of the mutagenic treatments. Table 6 shows that the phenomena of sterility and semisterility are directly correlated with increasing doses, both in chemical as well as in physical mutagenic treatments.

During 1973, greenhouse tests have been carried out to determine the host range of certain isolates of *E. polygoni* obtained from pea and other host plants collected from different areas of southern Italy. Also, greenhouse trials have been performed in order to set up a screening technique that could provide an efficient and quick means for selecting resistant pea plants.

Table 4 - Number of plants in the M_1 generation of varieties Sprinter (S) and Freezer 69 (F) after treatment with different X-ray (R) and diethylsulfate (C) doses.

Treatment	Adult plants
SC_1	591
SC_2	850
SR_1	510
SR_2	368
FC_1	868
FC_2	737
FR_1	509
FR_2	775
	5208

Table 5 - Number of seeds in stem and branch M_1-progenies

M_1 progenies from	Number of seeds for progeny < 15	15-30	30-50	> 50	Total
Stem	322	641	904	3288	5155
Branch B	302	732	1017	1287	3338
Branch C	153	395	626	634	1808
Branch D	68	193	267	294	822

Table 6 - Percentage of semisterile and sterile plants in treatments as specified in Table 4.

Treatment	Semisterile and sterile plants
FC_1	23,54%
FC_2	40,14%
FR_1	35,16%
FR_2	39,00%
SC_1	18,52%
SC_2	45,41%
SR_1	42,25%
SR_2	55,22%
F test	0,19%
S test	1,16%

The preliminary results of cross inoculation tests seem to indicate that populations of *E. polygoni* isolated from pea plant possess a restricted range of host plant species. However, some pea isolates of the fungus exhibited a phenomenon of adaptation when inoculated in sweetpea (*Lathyrus odoratus* L.).
A screening method was devised for large-scale inoculation; with this technique large numbers of plants can be evaluated, in a very short time, for powdery mildew resistance. Symptoms appear 4 days from inoculation and the final disease ratings are taken 6 days from inoculation.

With the purpose of promoting in the M_2 generation the great expression of the variability induced by the treatments, all M_1-progenies with less than 40 seeds (i.e. those in which it should be presumed that the mutagenic effect was more drastic have been sown in the field in December 1973).
From the remaining M_1-progenies (i.e. those with 40 or >40 seeds) 20 seeds per progeny will be allotted for phytopathological screenings in the greenhouse. The remaining seeds of those progenies, showing cases of disease resistance, will be sown again in greenhouses and in the field, for: confirmation of the resistance, isolation of resistant or tolerant individuals, ascertainment of other morphological and/or physiological mutations, and for seed multiplication.

Selection of mildew resistant barley (*Hordeum vulgare* L.) mutants following mutation induction - V. LIND, H. GAUL.

Our collection of mildew resistant barley mutants includes 91 strains induced in eight varieties. In 1973, 59 mutants proved to be resistant against the mildew population to be found in our region. These mutants comprised infection types from 0 to 1, the scale of infection types running from 0 to 4 (0 = highly resistant, 4 = susceptible). Twelve highly resistant mutants had necrotic and chlorotic flecking on the leaves. Similar leaf-fleckings are found for mutants having the ml-o gene on the chromosome 4. We have not yet investigated whether or not the genes in our mutants are identical to the ml-o gene. The relatively high frequency of resistant mutants with such a phenotype suggests that mutations mainly occur at one locus.
Thirty-two mutant strains were more or less susceptible; they comprised infection types from 2 to 4. In earlier tests these mutants were resistant only against the mildew race C_2.
By treating seeds with various X-ray doses and/or EMS-concentrations, new mildew resistant mutants will be induced. In 1973 three M_1-generations were grown. The original varieties "Villa", "Bido", and "Carina" have very good brewing quality; "Villa" has, in addition, a high yielding capacity. All of these varieties, however, are susceptible to mildew.
The M_2-plants were subjected to the mildew population mentioned above in a greenhouse experiment. By inocculating the plants with a mixture of various mildew races, only those mutants could be clearly recognized that have developed resistance against several of the mildew races or at least against the most aggressive ones.
Uniform mutagenic treatments are being applied every year. Thus, our experiments will provide information on the frequency of induced mildew resistant mutants. This frequency may depend on the mutagens used, their dose and concentration. In addition, we shall also investigate whether

or not the response of loci to different mutagens is the same. Studies with erectoides-mutants indicate the existance of interactions between mutagens and mutability of loci.

Influence of the genetic background on the expression of mildew resistance - H. GAUL, V. LIND.

In order to use mildew resistant mutants in breeding programmes effectively, information on the genes conditioning the resistance is necessary. In 1972 we have carried out a series of crossing experiments. In 1973, the F_1-generations of these experiments were sown in the field. Since all of the attempted cross combinations could not be accomplished in 1972 some crossings were only made this winter.
In backcrosses between mutants and their mother varieties the mode of inheritance of the genes conditioning the resistance reaction was studied. In all of the F_1-generations the susceptibility of the mother varieties dominated, i.e. the F_1-plants were mildew infested.

Twenty-four strains of our collection were crossed with each other in a diallel system. Twelve mutants induced in the varieties "Gerda", "Haisa II", and "Matura" have the chlorotic leaf-flecking mentioned previously. Crossing these mutants with each other results in the F_1-plants having the same phenotype as the parents. We assume that the same gene mutated in all of the mutants.

Besides this recessively inherited gene, another gene was found among the "Matura" mutants. The latter also affects complete resistance but in addition, the plants have no chlorophyll deficiencies.

Results of the F_1-analyses indicate that in crosses between several "Haisa II"-mutants three different recessively inherited resistance genes may be distinguished. Thus, three groups of mutants can be established. When, on the one hand, we were crossing the plants within one group, the F_1-generations obtained were resistant. On the other hand, when mutants of different groups were crossed with one another, the F_1-plants turned out to be susceptible.

We assume that in some of the mutants with chlorotic spots the leaf-flecking and the mildew resistance belong to one pleiotropic character complex. Therefore, we have initiated crossing experiments with the aim of producing mildew resistant strains without any of the undesirable features. The F_1-generations were susceptible to mildew and had no chlorophyll deficiencies. Selection will be started in the F_2-generation.

Resistance to dwarfrust (*Puccini hordei* Otth.) in barley (*Hordeum vulgare* L.) - J.E. PARLEVLIET, A. VAN OMMEREN.

To investigate the possibilities of mutation induction of partial resistance against dwarfrust, *Puccinia hordei* Otth. in barley seed, the cultivar "Minerva" has been treated in 1973. Dry seeds received 0 or 15 krad X-rays. Seeds soaked for 20 hours in running water at 12-13°C were treated during 6 hours at 24°C with 25, 50 or 75 m mol E.M.S. after which the seeds were washed and planted. The M_1 plants showed the usual reduction in vigour of growth and fertility. The irradiated plants showed hardly a decrease in emergence percentage; the growth, however, was clearly less vigorous. The E.M.S. treatments were sown in jiffy-pots and planted in the field after 3 leaves had been formed. Table 7 shows the reduction in growth and fertility.

Table 7 - Percentage of emergence, seedling height 20 days after sowing, and number of seeds per ear of EMS and X-rays-treated "Minerva" plants.

Treatment	% of emergence	seedling height	seeds per ear
0 m mol EMS	93	21.5	15.0
25 m mol EMS	90	18.5	12.0
50 m mol EMS	79	14.0	4.2
75 m mol EMS	70	8.5	5.6
0 krad X-rays	-	-	15.3
15 krad X-rays	-	-	6.1

Although the 75 m mol EMS treatment had slightly more seeds per ear than the 50 m mol EMS treatment it should be realized that in the field considerably more plants of the latter treatment survived than in the former. The real level of sterility therefore might be somewhat lower especially in the 75 m mol EMS treatment.

PRESERVATION OF FOOD BY MEANS OF RADIATION

Inhibitory effect of irradiated media on microorganisms - S.C.E. ROMKES.

Irradiated glucose is inhibitory to growth and in some cases even cytotoxic to microorganisms. The mechanism of this cytotoxic effect is not known. Insight into this mechanism could prove to be valuable in the field of food preservation by irradiation.
In the preceding year some hundreds of bacterial strains have been isolated from fish, and have been exposed to an irradiated synthetic medium, or to a synthetic medium containing irradiated glucose. Irradiated-medium sensitive strains have been selected. This year several more sensitive strains have been isolated. The selected strains were grown in chemically defined liquid media under conditions of controlled pH, temperature and aeration in a fermentor.
By passing sensitive strains through irradiated medium and repeating the process with the surviving cells at higher doses of irradiation, derivative strains were obtained, that were far less sensitive to irradiated medium, although their growthrate in irradiated medium would be slightly less than that of the original strain in the non-irradiated medium (figure 11). Growth curves for sensitive and non-sensitive strains were recorded for several media.
In a few instances a reduced uptake of glucose has been measured, but it has not been established whether this was a consequence of inhibited growth or of blocked uptake mechanisms.
In contrast to earlier findings, irradiated glucose solution could without subsequent autoclaving be sufficient to retard or stop growth in the most sensitive of the selected strains of *Micrococci*. In several cases it has been found that irradiation of the complete synthetic medium would inhibit growth, whereas separate irradiation of glucose alone did not. In two cases, both using yeasts, this has been shown to be due to radiation breakdown of vitamins. In one case addition of riboflavin could reverse this inhibition of growth.
Cells of one sensitive strain and its adapted counterpart are being grown in larger amounts in an orbital incubator in order to accumulate starting material for biochemical analysis.
It was noted, however, that the adapted strain sometimes reverted to the original sensitive state.
Ribosomal protein compositions for both strains are being compared by means of the Two-Dimensional electrophoresis method of Kaltschmidt. Preliminary results seem to show a difference in these protein patterns.

The influence of environmental factors on the radiation resistance of micro-organisms - J.G. VAN KOOIJ.

All the experimental work was devoted to the development of a model, which is suitable for the study of the influence of wateractivity on the radiation-resistance of microorganisms. The model was tested primarily on its suitability for maintaining a high survival for microorganisms to be cultivated on it during the experimental period. In order to minimalize possible effects of the model per se on the microorganisms as the result of irradiation, we elaborated solely on models made up from solids, which show a high degree of radiation-inertness.

The following models were used:
(1) Cellulose as strips of filterpaper to which bacteria are added.
(2) Lyophilized powder of a 3% Carboxy-methyl-cellulose-solution (CMC) in M/15 Phosphatebuffer or in minimum medium, to which the tested bacteria are added.
(3) Precipitation of bacteria on cellulose solution in M/15 Phosphate-buffer or in minimum medium.
(4) Vacuum dried powder of a cellulose solution in M/15 Phosphatebuffer, to which the bacteria are added.

Upon preparation the wateractivity of the models is lower than 0.55. The models were conditioned to wateractivities of 0.55, 0.75 and 0.90 resp. by means of saturated salt solutions. The time needed for reaching equilibrium, and the rate of inactivation of the test-microorganism incorporated in the models were determined during conditioning. The microorganisms used throughout the experiments belonged to the *Enterobacteriaceae*, and they were cultured in nutrient broth, and harvested at two stages of their development viz. log. and stationary phases.
Results so far obtained indicate:
- a poor recovery of bacteria from cellulose-strips, which makes the model unsuitable;
- lyophilized powder of CMC is only suitable for the lower water-activities, because of the fact that the equilibrium-time becomes unduly long for high water-activities with the result of complete inactivation of the micro-organisms used;
- the model based on precipitation of bacteria on cellulose offers some prospects; although time of conditioning is still rather long, the rate of inactivation seems acceptable.

A clear result of all experiments was that the recovery from the different models after conditioning to various water-activities was much better for microorganisms, which were added to the models in the stationary phase of their growing cycle than for those in the logarithmic phase.

The radiation and heat resistance of bacterial spores - H. STEGEMAN.

Metal content and radiation resistance

Metals play important roles in formation and in final properties of bacterial spores. The aim of this study is to get more basic information about the role of metals in relation to the radiation resistance. The experiments concerned this year the influence of calcium and manganese concentration in the sporulation medium on the calcium and manganese content of spores of a mesophilic strain and their effect on the resistance to a radiation and heat treatment.
Spores of *Bacillus subtilis* ATCC 6633 were cultivated in a chemically defined and in a complex liquid sporulation medium with different calcium/manganese contents. The manganese content of spores was determined by neutron activation analysis and the calcium content with atomic absorption analysis (cooperation with P. Poelstra and N.v.d.Klugt). Radiation was from a ^{60}Co source which irradiated spores in water at a dose rate of approx. 0,9 Mrad/h at about 20°C.

Table 8 shows the influence of the calcium level in a synthetic sporulation medium containing 2,5 ppm manganese, on the metal content of the spores and on the radiation resistance. Increasing the calcium concentration in the sporulation medium reduced the manganese content of the spores. There was an optimum concentration for maximum calcium accumulation. The radiation resistance was significantly ($P = 0,05$) higher for spores formed in media containing minimum levels of calcium. The heat resistance increased by addition of calcium up to a level of 50 ppm calcium and decreased at higher calcium concentration.
Tables 9 and 10 show that the incorporation of manganese was greater at higher manganese concentration in the synthetic and complex sporulation medium. According to table 8 spores with high accumulation of manganese were more resistant to irradiation than spores with low or normal manganese level. The spores produced in media with different manganese concentrations varied slightly in heat sensitivity. Spores formed in the synthetic medium were more heat resistant than spores formed in the complex medium.

Role of metal content in radiosensitization

Spores of *Bacillus subtilis* ATCC 6633, cultivated in a synthetic sporulation medium with 1 ppm Ca were inactivated by γ-irradiation more effectively in the presence of 1 mM KJO_3 than in the absence of KJO_3. The radiation of spores cultivated in the same sporulation medium with 50 ppm Ca was unaffected by potassiumiodate.
These results appear to be in accordance with the suggested inactivation mechanism in the literature by γ-irradiation in the presence of iodate.

Activation of irradiated spores

Heating of spore suspensions of some bacterial species result in an increased viable colony count. This process of "conditioning the spore to germination" has been called "activation". The activated spores retain most of their porperties and the process of activation is in most cases reversible. Heat activation is the simplest method of activation, but other treatments can replace the heat effect (e.g. exposure to low pH (1 to 3) or to Na-DPA). The aim of these experiments was to study the activating effect on irradiated spores. Spores of a rough variant of *Bacillus stearothermophilus* strain 1518, obtained from M.L. Fields (University of Missouri, U.S.A.) were produced by indubation for two days at 55°C on nutrient agar containing 1 ppm manganese and irradiated. Microscopic examination with a counting chamber showed that 5% or less of the unheated spores germinated and gave colonies. Optimal heat activation and activation by exposure to low pH resulted approximately in a 8-fold colony count; exposure to Na-DPA in about 18-fold colony count. Figure 12 also shows that activation of irradiated spores by exposure to low pH is possible.
Similar results were obtained by spores activated by exposure to Na-DPA. Heat activation of irradiated spores resulted first in an increased viable colony count, but at higher irradiation doses the inactivation of heated spores was greater than that of non-activated spores (see figure 13).

Table 8 - Radiation resistance and metal content of spores formed in a chemically defined sporulation medium with 2,5 ppm manganese and varied calcium levels.

Ca^{2+} in medium (ppm)	Ca^{2+} in spores (% dry wt)	Mn^{2+} in spores (% dry wt)	D_{10}-value (krad) (95% confidence limits)
1	0.51	1.15	187 ± 10
5	0.91	0.43	170 ± 4
10	1.52	0.32	162 ± 10
25	2.42	0.19	141 ± 5
50	5.77	0.23	140 ± 4
100	5.55	0.23	144 ± 7
150	3.58	0.08	143 ± 3

Table 9 - Radiation resistance and metal content of spores formed in a chemically defined sporulation medium with 50 ppm calcium and varied manganese levels.

Mn^{2+} in medium (ppm)	Ca^{2+} in spores (% dry wt)	Mn^{2+} in spores (% dry wt)	D_{10}-value (krad) (95% confidence limits)
0.05	6.73	0.009	130 ± 4
0.1	5.34	0.01	137 ± 4
1	4.18	0.08	140 ± 4
2.5	5.77	0.23	140 ± 4
10	5.32	1.29	150 ± 4

Table 10 - Radiation resistance and metal content of spores formed in a complex sporulation medium with 60 ppm calcium and varied manganese levels.

Mn^{2+} in medium (ppm)	Ca^{2+} in spores (% dry wt)	Mn^{2+} in spores (% dry wt)	D_{10}-value (krad) (95% confidence limits)
0.05	3.88	0.008	146 ± 7
0.1	2.87	0.007	152 ± 6
1	2.45	0.09	153 ± 6
10	2.99	0.63	173 ± 7

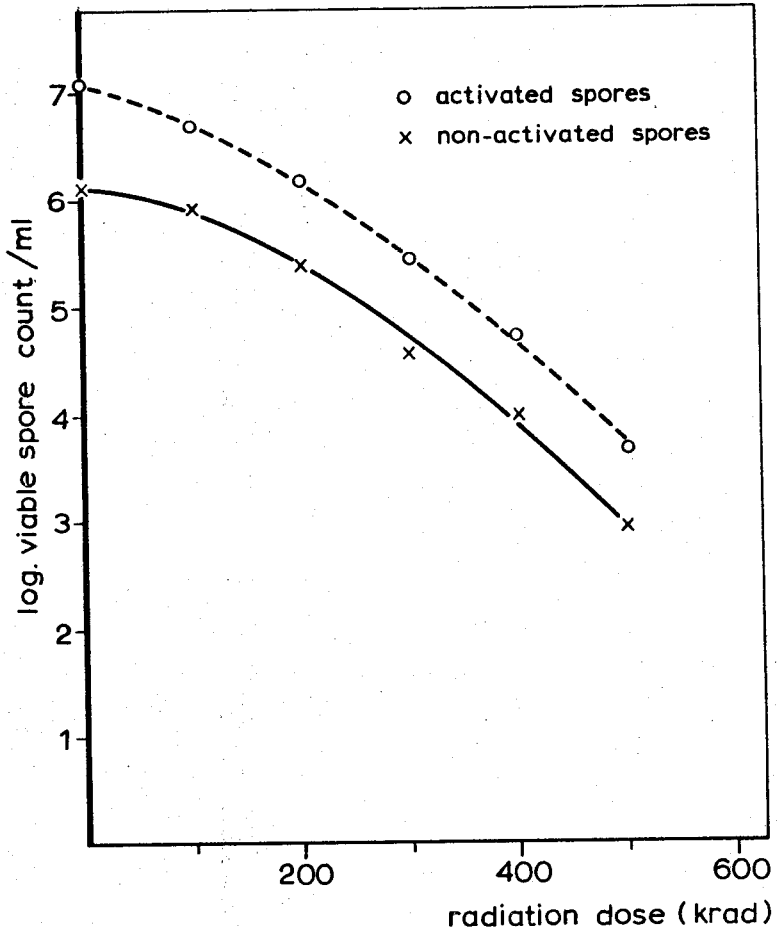


Figure 12 - Effect of low pH activation on the survival of irradiated spores.

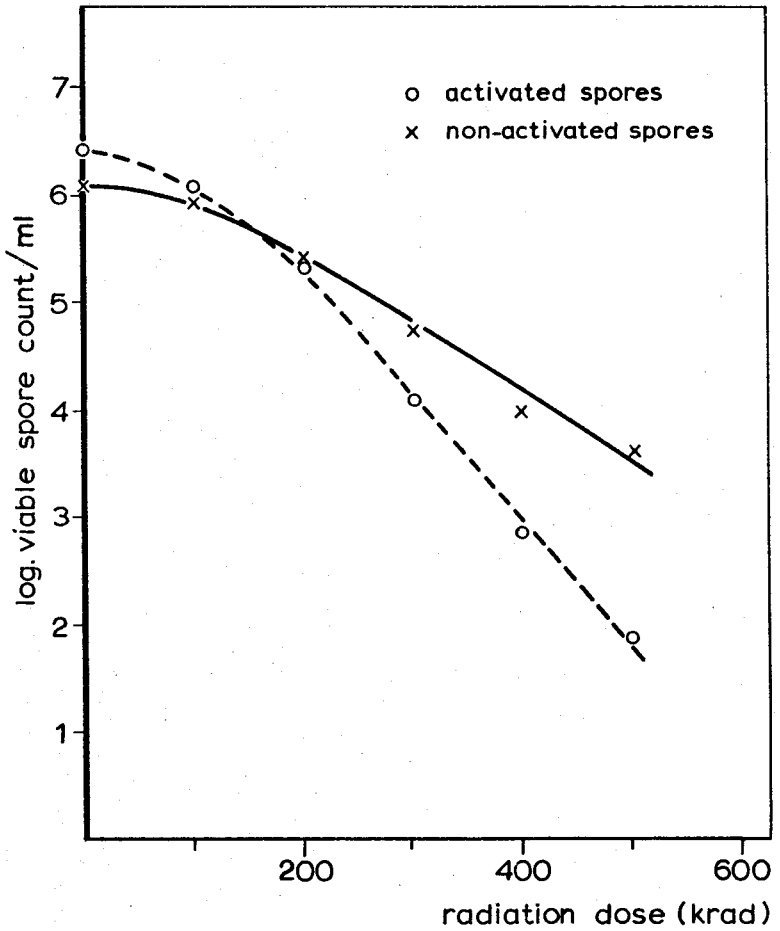


Figure 13 - Effect of heat activation on the survival of irradiated spores.

Wholesomeness of irradiated food - J.G. VAN KOOIJ.

Experiments with mini-pigs

A final evaluation of the results of a wholesomeness study, using different diets with minipigs has indicated that the animals fed the γ-irradiated diet during two years have performed as well as the group which received the control diet.
A study of the toxicological parameters was carried out by the National Institute of Public Health and supervised by Dr. M.J. van Logten. The effect of an irradiated diet on growth of the minipigs could not be evaluated, because the animals were 11 months old at the start of the experiment. Other parameters such as heamatologic aspects, weight of organs and histopathologic data did not demonstrate differences between animals fed irradiated diet and those given the control diet, with the exception that the minipigs fed with an irradiated diet showed a slight increase in the number of neutrophiles. This phenomenon demands further research to clarify its possible relationship with the feeding of irradiated food to minipigs.

Experiments with Groot Yorkshire sows

- Production of first generation.

The initial foundation stock consisting of 39 sows and 4 boars was obtained from a number of farms known for their good breeding results. In addition a group of 6 sows was kept as a reserve. The sows were upon mating divided into 3 experimental groups of 13 each, which were fed non-treated, autoclaved and 5 Mrad irradiated feed resp. During the gestation period the sows were maintained on a dietary regime of 2 kg per day of a standard commercially prepared and pelleted gestation-feed, and water ad lib. During lactation the ration was increased to 3 kg/day. Three months after mating a final selection of the experimental groups was carried out. Four sows were identified nonpregnant; one sow died, and 6 sows were disposed of, because time of farrow was too late to fit properly in the schedule set for the production of F_1-generation.
Table 11 shows some performance data of the first offspring of the first generation raised under normal farm environment.
The results presented in table 11 are comparable to those obtained in farm practice. Postnatal and preweaning losses of the group of sows fed with irradiated feed, although the losses are comparable to the group of sows fed nontreated feed, could have been less, if the number of piglets produced by one of the sows had been in the normal range: 3 of them were born dead and 6 died a few days later because of lack of viability. On the whole, litter size fluctuated in all experimental groups. This is also normal for sows farrowing for the first time.

- Feeding test with fattening hogs.

Three groups of 30 hogs each, and corresponding with the 3 diets to be tested, were selected out of the first offspring and maintained on an ad libitum dietry hog-feed regime during the entire period of fattening, which lasted about 100 days.
Results of this experiment are not yet available.

Table 11 - Some performance data of F_1-generation raised under normal farm environment.

Experimental group	Number of sows			Number of litters	Number born alive	Number born dead	Number of pigs weaned	% loss up to weaning
	bred	disposed of	nonpregnant					
nontreated feed	13	2	1	9	76	2	63	17.1
irradiated feed	14	3	1	9*	90	10	75	16.6
autoclaved feed	12	1	2	9	74	5	67	9.5

* one sow produced 18 piglets.

- Production of second offspring of the first generation.

Postweaning piglets of the first offspring of the 3 test-rations were selected, and housed in separate holding pens.
At the age of 7 months 3 groups consisting of 12 hogs and 8 boars each, were selected out of available material. These 3 groups will be used for the production of a second generation, of which the first offspring is expected medio 1974.

Experiments with diets containing irradiated compounds on rats

A preliminary test with irradiated spices has been started to evaluate the amount of spice that can be added to the standard diet of rats without causing toxic effects. The information to be obtained from this test will provide data to set up the final protocol of a semi-chronic rat feeding test with irradiated spices.

Ionizing radiation in food technology - D.IS. LANGERAK.

In 1973 a number of experiments have been carried out with peeled potatoes, prepacked chicory, fresh and deepfrozen strawberries and further attention was also paid to the problems of discolouration caused by oxidation of polyphenols.

Peeled potatoes

Previous research in 1971 and 1972 showed that the keeping quality of peeled potatoes treated with $Na_2S_2O_5$ (sulphite) was considerably improved by irradiation (synergistic effect). However, a disadvantage of this treatment was that the taste was affected at a dipping concentration > 0,5% and an irradiation dose > 50 krad.
In collaboration with the Netherlands Institute for Applied Home Economics Research (NITHO) the following aspects were investigated: the optimum packaging; the minimum dipping concentration; the maximum irradiation dose.

- Sensory tests

This investigation has been carried out on the basis of sensory tests, by a trained panel and a consumer panel using the variety Bintje. The product was dipped in a $Na_2S_2O_5$ concentration of 0.25% and 0.5% and packed in polythene bags of 0.02 mm thickness, without or with 2 perforations of 2 mm Ø. The irradiation dose was either 0 or 50 krad $\pm$ 10%. The prepacked product was stored for 4 days at 10°C.
The keeping quality of the peeled potatoes was better in the non-perforated bags than in the perforated bags. However, the taste was better in the perforated bags.
The results of the sensory test by the trained panel showed that the object 0.5% $Na_2S_2O_5$ + 50 krad received the highest score after a storage time longer than 3 days (see figure 14).
The consumer panel did not notice a significant difference in taste between fresh and treated potatoes (0.5% $Na_2S_2O_5$ + 50 krad).

- Anti-oxydants

The influence of cystein instead of $Na_2S_2O_5$, in combination with irradiation, on colour and keeping quality of prepacked peeled potatoes was also investigated.

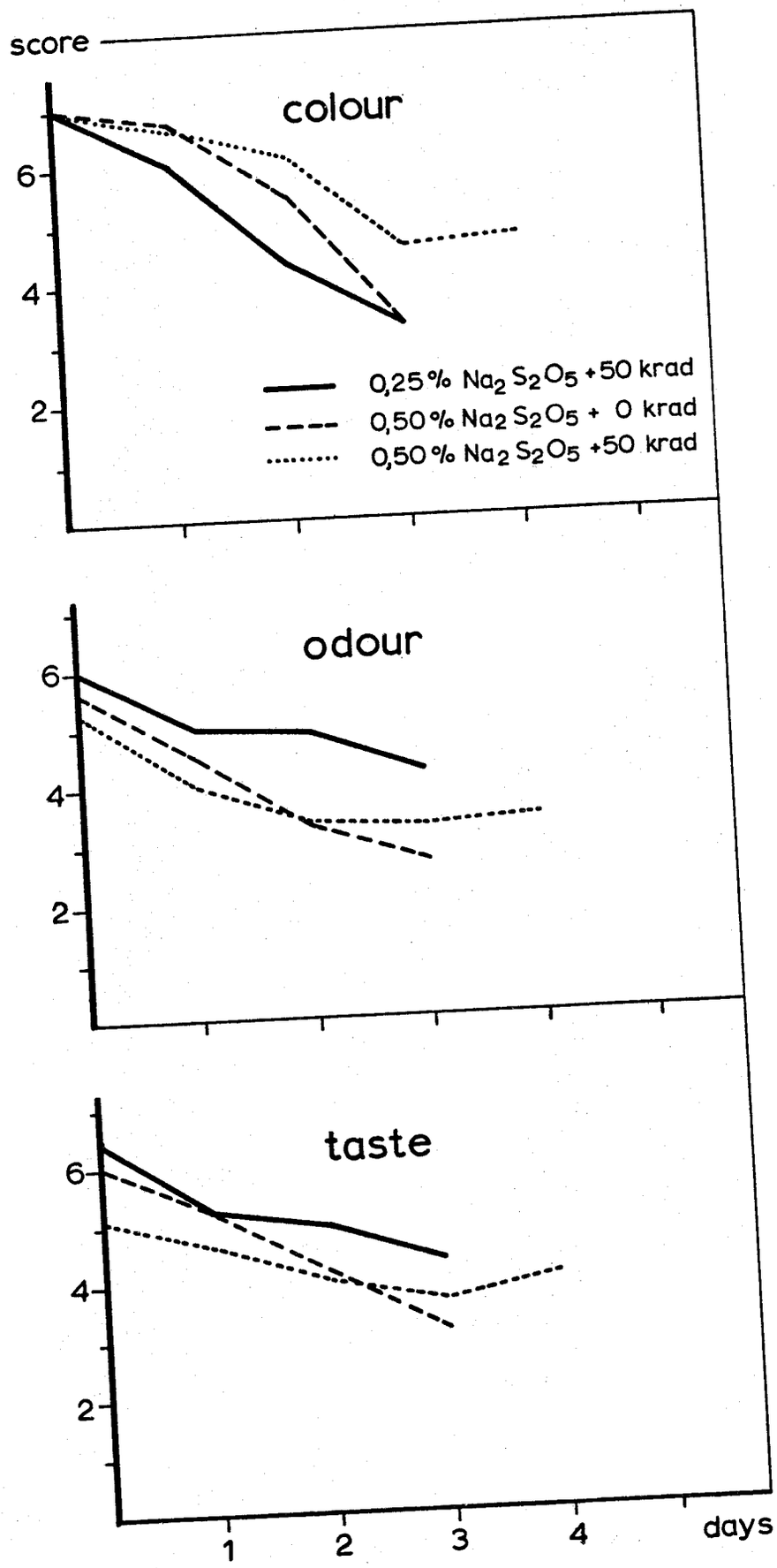


Figure 14 - Sensory test by trained panel. The peeled potatoes were treated with $Na_2S_2O_5$ in combination with irradiation and stored at 10°C. The data are the average of 2 experiments.

The product was dipped in cystein concentrations of 0.01%, 0.5% and 1% and packed in non-perforated polythene bags of 0.2 mm thickness. The irradiation doses amounted to 0 and 50 krad γ-rays.
The results showed that cystein in combination with irradiation synergistically improved keeping quality, which, however, lasted for a shorter period than with $Na_2S_2O_5$. The best object was 1% cystein + 50 krad. No clear off-flavour was noticed.

Prepacked Chicory (*Cichorum intybus* L.)

Prepacked chicory discolours and spoils quickly after processing. The aim of this investigation was to study the influence of packaging, irradiation and anti-oxydants on colour, keeping quality and micro-biological contamination.
Seven experiments have been carried out. All experiments were processed in the same way, only the spin-drier times were varied from $\frac{1}{4}$ up to 1 min. As an anti-oxydant was used cystein in concentrations of 10^{-4}M, 5×10^{-4}M, 10^{-3}M and 5×10^{-3}M. The product was packed in either polythene 0.02 mm without or with 2 perforations or polythene 0.05 mm without perforation. The irradiation doses amounted to respectively 0, 50 and 100 krad γ-rays. Storage temperature: 10°C.

The cut chicory discoloured considerably faster in the perforated bags than in the non-perforated bags. A foil of 0.02 mm was more satisfactory than a foil of 0.05 mm thickness, because with the last foil the CO_2 content in the bags rose to a level above 10%.
Packing in non-perforated bags was only possible in combination with irradiation. In the non-irradiated bags fermentation and decay occurred in consequence of a high microbial count (10^8/gram) and an unfavourable gas composition.
In the beginning of the storage time, an irradiation intensified the pink discolouration. However, after 1 day this discolouration was stabilized, while in the non-irradiated objects discolouration increased.
An irradiation dose of 50 krad or more reduced the total viable count with 3 to 4 decimals and consequently the keeping quality was lengthened by approx. a factor of 100%.
The influence of a cystein treatment on the colour was inconstant. The best results were obtained when the chicory was packed in non-perforated bags and irradiated with 50 krad.

Experiments on the storability of strawberry yoghurt using irradiated strawberries

The aim of these experiments was to find out the decimal reduction dose of the yeast flora and the maximum dose with regard to the sensory acceptability.
Because the count of yeast cells is too low for the determination of the D_{10}-value, different techniques for enhancing the number of yeast cells in strawberries have been studied.
Concerning this study the following enrichment techniques were applied to complete fruits, strawberrypulp with and without Ca-proprionate (fungicide), strawberrypulp + yeast and dextrose extract in an incubator at 30°C.

Results of this investigation showed that only with the last mentioned technique the number of yeast cells was sufficient and increased to 10^8 gram in 2 days. The growth of the yeast flora with the help of the other techniques was too slow.

The fresh strawberries were dipped in a solution contaminated with the above mentioned enriched yeast flora and afterwards packed in polythene bags and deepfrozen at -30°C. The strawberries were irradiated with doses of 0 - 400, 600 and 800 krad γ-rays at -30°C and -70°C.
Results of the determination of the D_{10}-value showed that the natural yeast flora was more radiation resistant than the enriched flora. Furthermore the D_{10}-value depended on the deepfreezing temperature and the storage time.
Sensory tests of irradiated deep-frozen strawberries were carried out in collaboration with the Netherlands Institute for Applied Home Economics Research (NITHO).
The results of these tests indicated that doses <600 krad were acceptable.

Discolouration of cut vegetables

The discolouration of cut fruits and vegetables is probably due to the oxydation of phenols. In this oxydation reaction enzymes play an important role. Dependent on the environment (O_2 and CO_2 level) irradiation can accelerate or delay this discolouration.
To get more insight in the above mentioned problems the following aspects are investigated:
(1) the correlation between discolouration and the ratio phenols and quinones (oxydation product of phenols);
(2) the effect of irradiation on enzyme-activities.

ad (1). The experiments have been carried out on cut chicory. For the measurements of the phenols and the quinones the following extraction methods were compared:
(a) 1% NaCl solvent 2°C,
(b) ethanol + 70°C,
(c) ethanol - 30°C,
(d) hexane - 30°C.

The extracts were centrifuged at 100.000 g in the Omega 70.000 at 0°C and afterwards measured in the spectrophotometer in the visible light and U.V. area of the spectrum.
Extraction with NaCl and ethanol + 70°C did not give any information. At ethanol - 30°C and hexane - 30°C peaks were found between 260 - 290 nm (figure 15).
However, this spectrum was too broad for further analysis. Further research with other extraction methods e.q. sephadex column separation is desirable.

ad (2). Effects of irradiation on enzyme-activities in prepacked cut chicory (collaboration Y. Tanaka)
It is accepted that the activity of some enzymes, especially peroxidase, polyphenolase (tyrosinase) increase in injured tissue and that they contribute to browning due to the lignine formation.
Starting from this premise, it was supposed that similar enzymes may also affect the shelflife of prepacked cut vegetables after irradiation.
As a model prepacked cut chicory was chosen and the activities of peroxidase and polyphenolase during storage were controlled. Therefore, extraction is done with a cooled citrate-phosphate buffer (pH 6,5) and enzymes are measured spectrophotometrically by a modified A.C.Machie and B.Chance method.
Results show that the peroxidase activity increases immeciately after cutting but that this increase is reduced by irradiation (doses 0, 100, 300 krad γ-rays). A temporary increase is also observed for the polyphenolase

activity and this increase is delayed for approx. 2 days by irradiation (same doses).
The brown colour appears immediately after irradiation on the edge of the cut samples but no change in the polyphenol content (acetone soluble) as a result of irradiation is observed. These preliminary results suggest that irradiation affects some enzyme activities but these activities could not directly be related to the observed browning.

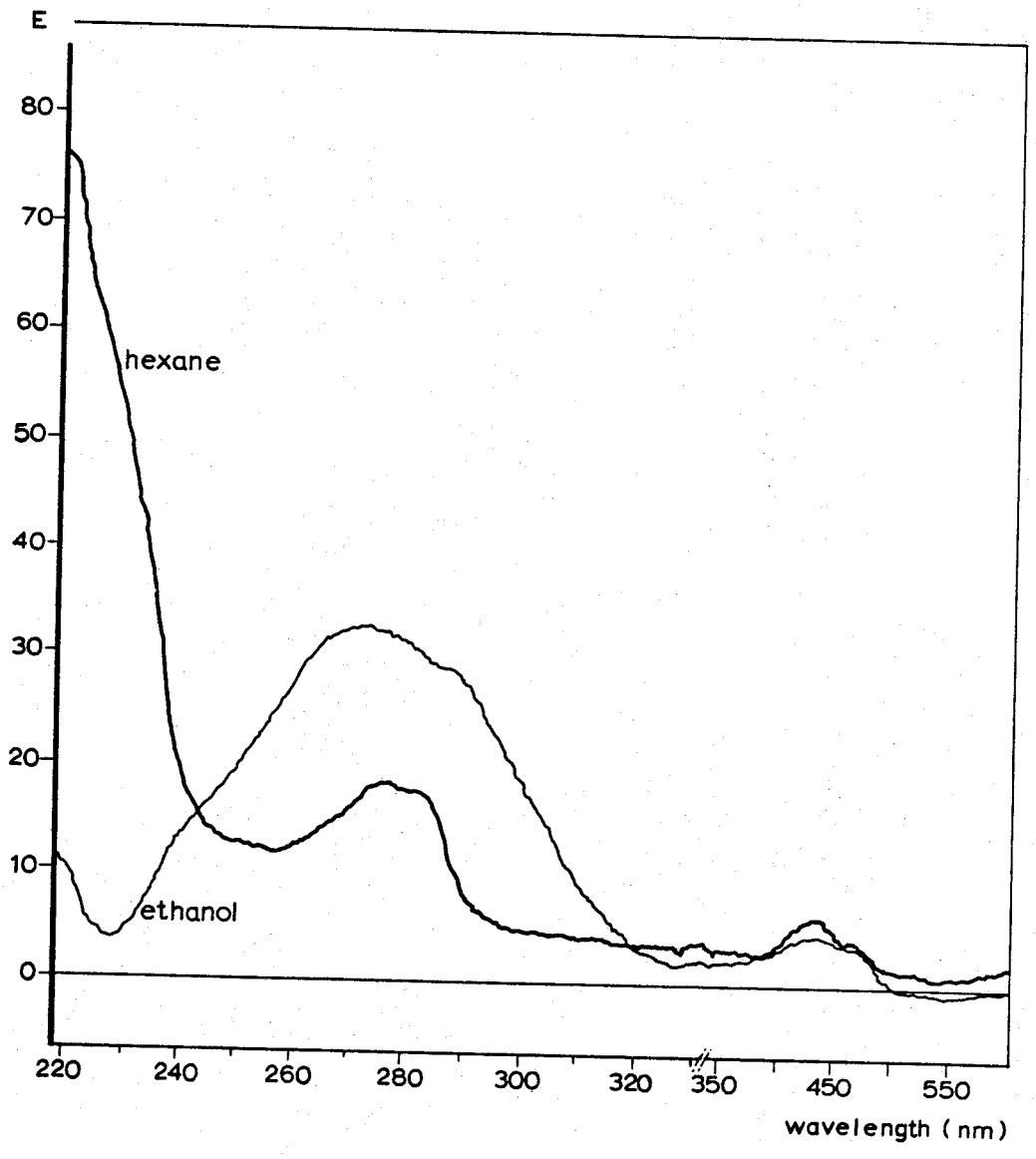


Figure 15 - Absorption spectra of phenols and quinones measured in spectrophotometer. Solvent: Hexane-30°C, Ethanol-30°C.

RADIATION GENETICS OF INSECT PESTS

Study of genetical processes induced by irradiation in the onionfly *Hylemya antiqua* Meigen) - A.S. ROBINSON.

The isolation of radiation induced viable chromosome mutations necessitates the establishment of a dose response curve for dominant lethality.

Dose response curve for dominant lethality

As mature sperm provides a homogeneous cell sample and is sensitive to the induction of translocations, 7 days old adult male *Hylemya antiqua* Meigen were used as test insects. They were treated with various doses of X-rays and mated in mass to virgin females for 3 days. The males were then discarded and the females were placed in individual cages where two biological end-points were assessed in the eggs produced by the mated females; % egg hatch and the % of "brown" eggs. Brown eggs are eggs which do not hatch but in which there has been recognisable development i.e. this provides a measure of late embryonic lethality. The results are shown in figure 16. The male *H. antiqua* is remarkably radiosensitive for Diptera, a dose of 3 krad induces almost 100% dominant lethality. Contrast this with the sterilizing dose for other Diptera, *Glossina morsitans* Westwood,12 krad; *Culex pipiens* L., 8 krad and *Drosophila melanogaster* Meigen, 9 krad.
The top part of figure 16 shows the percentage of brown eggs as a function of dose. There were significant differences between these percentages (F: 5.7*** d.f. 9 and 40). There was a peak in the observed percentage of brown eggs at 1000 rad. The subsequent lowering of the point at higher doses is postulated to occur as the result of increased radiation damage leading to progressively earlier egg death.
In general, the higher the radiation dose administered to the parental generation the more translocations will be recovered in the F_1 offspring. However, if translocations are to be used as homozygotes in an insect control programme it is desirable to use as low a dose as is practically feasible in order to minimise other genetic damage. A dose was chosen which gave 50% dominant lethality i.e. 500 rad.

The isolation of chromosome aberrations in the F_1 generation using egg hatch data and confirmatory cytological evidence

Individual pair matings between F_1's, of both sexes and control insects were made and the % egg hatch and the % of brown eggs was assessed in each case. It has been previously found that translocation heterozygotes produce these sterility effects by acting as late embryonic lethals i.e. they generate brown eggs. Therefore by a combination of these two assessments, tentative aberration stocks were isolated. The presence or absence of aberrations could be verified cytologically. This part of the work was done in collaboration with C. van Heemert, Dept. Genetics, Wageningen.

To the time of writing 3 new aberrations have been isolated, two translocations and a pericentric inversion. The fertility of these aberrations in test crosses to control insects can be seen in Table 12. Both the translocations exhibited a fertility of approximately 50% calculated from the small amount of data so far available. The reduced

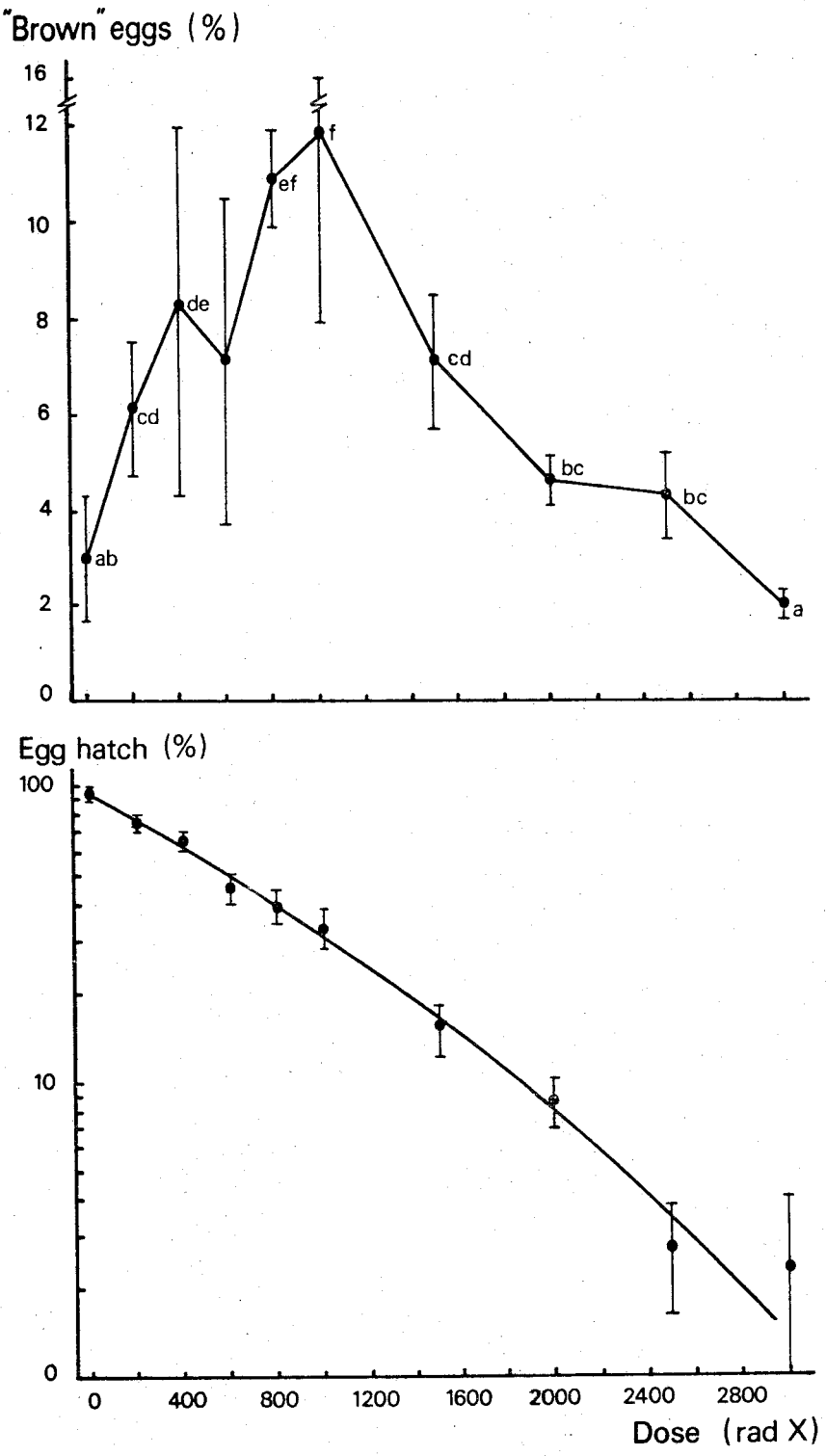


Figure 16 - Dose response curves of % egg hatch (bottom graph) and % brown eggs, late embryonic lethals (top graph) in X-irradiated *Hylemya antiqua* Meigen males mated to control females.*

*Means followed by the same letter are not significantly different from each other at the 5% level as determined by Duncan's multiple range test.

fertility of the inversion provides evidence of genetic crossing over within the inverted segment in the female of *Hylemya antiqua* Meigen. It is postulated that if crossing over is absent in the male (as in most Diptera) then the fertility of the inversion heterozygote will not be reduced.
Inbreeding programmes for the homozygosing of these aberrations have been initiated.

Table 12 - The mean % egg hatch in single pair crosses to control insects of three chromosome aberrations in *Hylemya antiqua*.

	% Egg Hatch	
	♀ tested	♂ tested
Translocation 1	*	48.9
Translocation 2	55.9	62.9
Inversion 1	58.5	*

* data not yet available.

Genetical and radiation biological research on the summerfruit tortrix moth (*Adoxophyes orana* F.R.) - D. SNIEDER.

For the comparison of the effect of fast neutrons and X-rays, adult moths of *A. orana* (up to 24 h old) have been irradiated with a dosage of 3 krad fast neutrons. The resulting progeny was outcrossed with untreated moths in single-pair matings during succeeding generations. In figure 17 the course of the fertility in those succeeding generations is depicted. As for irradiation with X-rays (see Annual report 1972), we can conclude that the F_1 from irradiated females has an average percentage egg hatch that equals or exceeds that of the parent generation, while the F_1 from irradiated males has an average percentage egg hatch which is obviously lower than that of the P-generation. It can also be concluded that within a very few generations (2-3) most of the induced sterility disappears. In other words, in these respects no qualitative but only quantitative differences between X-rays and fast neutrons are found.
Experiments have been carried out to get evidence concerning the hypothesis that the difference found between the heridity of chromosomal aberrations in irradiated male and female moths can be explained by the moment of meiosis in relation to the moment of irradiation. Female moths were therefore irradiated with 2 krad X-rays, 4 resp. 16 h after emergence; their F_1 progeny was outcrossed with untreated moths in single-pair matings. However, no differences in percentages of egg hatching could be detected between both groups of B_1-progeny. Later on, Suomalainen (personal communication) stated that this was acceptable, because of the fact that at any time after emergence, the eggs in the ovaries are only in meiotic metaphase I or in phases preceding it. In other words, this experiment could not give the proper answer because the amount of meiosis at both intervals before irradiation was exactly the same. Concerning the same problem, a study is now in progress after irradiation of third and fifth instar larvae with 2 and 5 krad ^{60}Co γ-rays. Third instar

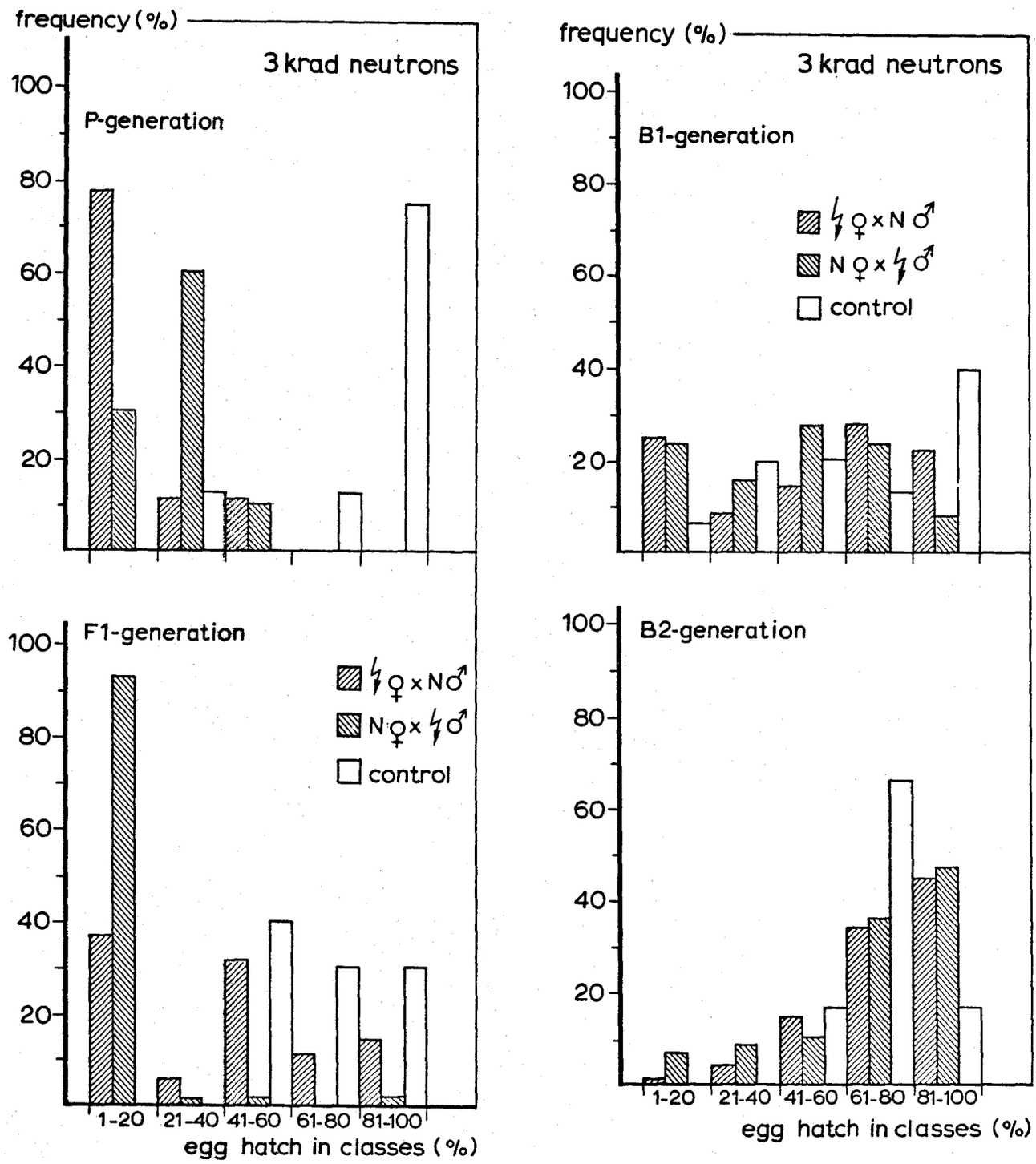


Figure 17 - The course of sterility in succeeding generations after irradiation with 3 krad fast neutrons.

larvae, as well as fifth instar female larvae, all have premeiotic egg and sperm stages, while fifth instar male larvae have partly premeiotic, partly postmeiotic stages.

For a theoretical evaluation of the effects of the release of moths with certain degrees of sterility, a computerprogramme has been worked out. In the original model only percentage sterility, mortality between first instar and adulthood and shifts in the ratio males to females were variable. To trace also the influence of other factors, data on competitiveness,developmental time and multiple mating are being sampled. It was established that untreated females, mated first with untreated males, mate for 23% a second time, while untreated females, mated first with irradiated (25 krad X-rays) males, mate for 55% another time. Figure 18 can be explained in such a way that their irradiated sperm is less competitive than untreated sperm: untreated sperm from the first or second mating induces almost the same distribution of egg hatch classes. Irradiated sperm, however, does not lower after the second mating the fertility to the same degree as does the irradiated sperm of the first mating. Because of the big variation in % of egg hatching, fertilized by untreated sperm, this explanation remains hypothetical.

Finally practical assistance was given to the sterile male release programme of dr. G.W. Ankersmit (Dept. of Entomology, Wageningen). It was established that in fact not sterile but semisterile moths were released. By sampling male larvae from the experimental orchard and searching their testes for visible chromosomal rearrangements, we were able to establish the occurrence of larvae probably originating from a mating between a wild female and an irradiated male. In July 4% of the checked larvae possessed chromosomal aberrations, in September 24%.

Genetical and radiation biological studies on the two-spotted spidermite (*Tetranychus urticae* C.L. Koch) - A.M. Feldmann.

Before translocation homozygous stocks, for genetic control purpose can be efficiently isolated, it is of ultimate importance, that enough basic radiation genetical data concerning the species studied are available. To achieve this aim, the main subjects of the experiments executed in 1973 are the refining and the improvement of the initial experiments in order to establish the dose-response relationship for dominant lethals, recessive lethals and structural chromosome mutations, induced by irradiation of either males or females of *Tetranychus urticae* Koch with X-rays or fast neutrons.

Dominant lethals

T. urticae is an arrhenotokous reproducing arthropod i.e. unfertilized females produce only haploid eggs, that develop into haploid males, fertilized females produce two types of eggs: unfertilized ones, that develop into males and fertilized ones, that develop into diploid females. From this way of reproduction it follows that a simple determination of the hatch of a heterogeneous sample, consisting of haploid- and diploid F_1-eggs is not a reflection of the expression of dominant lethality as is the case in most insects, which produce only diploid eggs. In the F_1-diploids (eggs and developmental stages up to adulthood) only dominant lethals will express themselves by mortality, but in the haploid F_1-eggs and F_1-haploid developmental stages, dominant lethals as well as recessive lethals will do so. As diploid- and haploid eggs and early developmental stages cannot

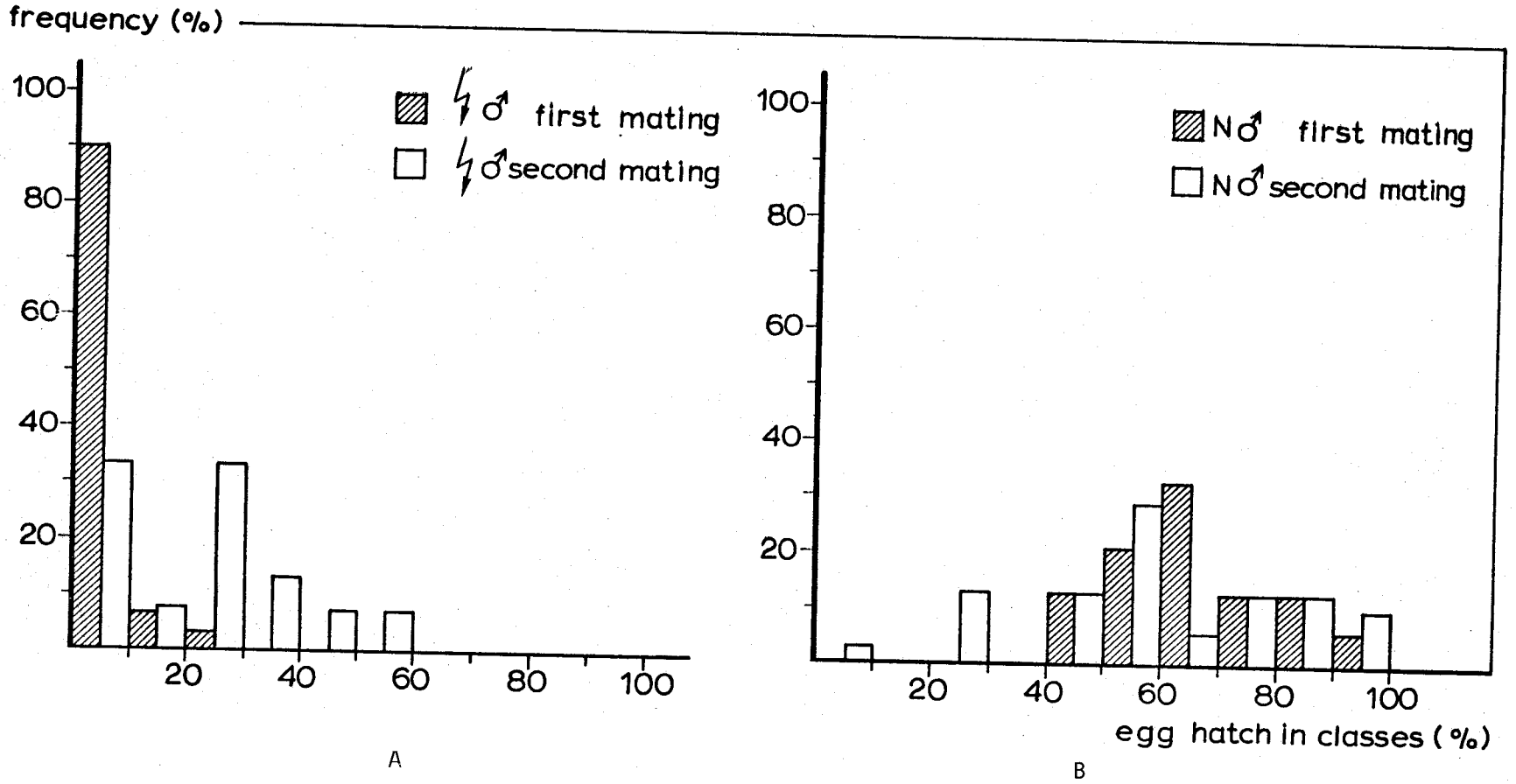


Figure 18 - Frequency distribution of % egg hatching for the following four categories:
1. Eggs laid by untreated females after a first mating with irradiated males (A).
2. Eggs laid by the same females after a subsequent mating with irradiated males (B).
3. Eggs laid by other untreated females after a first mating with untreated males (B).
4. Eggs laid by these females after a subsequent mating with irradiated males (A).

be distinguished morphologically from each other, a method is developed by which the number of diploids in a heterogeneous sample of haploids and diploids can be determined. The estimated mortality frequency of F_1-diploids is then corresponding with the frequency of dominant lethals (assuming at least one dominant lethal per dead F_1-diploid). For calculation of the dominant lethal frequency, two formulas are used, depending on whether males or females are irradiated. When males are irradiated and then mated to virgin females, the radiation effects are only found in eggs with a parental male genome complement (diploid progeny).
Irradiation of virgin females, mated afterwards, produces effects in both types of eggs.

The formulas are:

For male irradiation:
$$D = 1 - \frac{ft\left(e_{mc} - \frac{m_{mc} \times e_{uc}}{m_{uc}}\right)}{fc\left(e_{mt} - \frac{m_{mc} \times e_{uc}}{m_{uc}}\right)}$$

For female irradiation:
$$D = 1 - \frac{ft\ \left(e_{mc} - \frac{m_{mc} \times e_{uc}}{m_{uc}}\right)}{fc\ \left(e_{mt} - \frac{m_{mt} \times e_{ut}}{m_{ut}}\right)}$$

where:

ft = number of females in the test (= irradiation experiment);

e_{mc} = number of F_1-eggs produced in the control in which parental females are mated with untreated males;

m_{mc} = number of F_1-males, produced by the mated females of the control;

e_{uc} = number of F_1-eggs, produced by unmated control females;

m_{uc} = number of adult F_1-males produced by the unmated control females;

fc = number of adult F_1-females counted in the control of the mated females;

e_{mt} = number of F_1-eggs produced by the females of the irradiation experiments;

m_{mt} = number of adult F_1-males produced by the females of the irradiation experiment;

e_{ut} = number of F_1-eggs produced by irradiated virgin females;

m_{ut} = number of F_1-males produced by the irradiated virgin females.

The results obtained by the described method are in agreement with the literature on induction of dominant lethals in insects, in contrary with the results obtained before by using the formula of Atwood et al. (1956). The most probable reason is that Atwood et al. did not incorporate in their calculations the mortality of diploid F_1-eggs, estimated in a control experiment.
The dose-response relationships with dominant lethality for X-rays and

fast neutrons (dose-rate for both is 100 rad/min, γ-contamination of fast neutron irradiation is 264 rad/h), applied to males or females of *T. urticae*, are presented in figures 19, 20 and 21.

The mean value and standard deviation for each dose is calculated from ten replicates, each existing of five parental females mated to five males. The egg-samples from which dominant lethality is calculated, are collected for each treatment in the same period after irradiation of the one day old adult virgin males or one day old adult virgin females i.e. 24-28 h after the irradiation treatment. In order to standardize the results, the survival of F_1-diploids (= 1- dominant lethality frequency) is on the vertical axis and the applied dose is on the horizontal axis of figures 19 and 20. From figure 19a and 20a is concluded that the dose response relationship for dominant lethality induced in sperm is a "one radiation event" mechanism, with an R.B.E. = 1, for the survival of the F_1-zygotes, for all dosages.
From figures 19b and 20b is concluded that the dose response relationship for dominant lethality induced in eggs, is a "two radiation events" mechanism with an R.B.E., for survival of the F_1-zygotes, of 2.5 for all dosages.

Recessive lethals

In figure 21 is presented the survival of F_1 haploids progenerated by irradiated 1 day old adult virgin females, not mated afterwards. The graph of figure 21a is essentially not different from the graph of figure 19b and the same accounts for figures 21b and 20b. Also the R.B.E. at all dosages for the survival of F_1-haploids, produced by X-ray or fast neutron irradiated parental virgin females, is 2.5.
It is demonstrated by these experiments, that in the eggs of *T. urticae* no significant induction of recessive lethals by irradiation of parental virgin females by either X-rays or neutrons is found. In case of such induction the survival of F_1-haploids would have been significantly lower, at the different dosages, than that of F_1-diploids.

Induction of structural chromosome mutations or translocations

In the 1972 annual report, "fertility-patterns" of F_1-females, descending from irradiated male parents, were presented. This classification, however, was found to be too subjective and a more satisfying method was developed in 1973.
In the first method, the mortality of the progeny of the unfertilized F_1-female was compared with the mortality of the haplo-diploid progeny of the same female, produced after mating. It is evident that the magnitude of the possible shift of the mortality depends on the ratio of diploid eggs to the total number of the mixture of haploid- and diploid eggs. (A mortality-shift is expected when the F_1-female is heterozygous for a recessive lethal. When the F_1- female is heterozygous for a translocation, the mortality of the haploids is the same as for the diploids, because of the production of unbalanced gametes).
In the new method, also the sexratio in the progeny of the fertilized F_1-female is established, in order to calculate the mortality of the diploid eggs. So, the mortality of the haploid eggs is compared with the mortality of the diploid eggs, produced by the same F_1-female.
Up till now this new method is applied to male and female irradiation experiments with X-rays as well as with neutrons. These results will be presented in the next annual report.

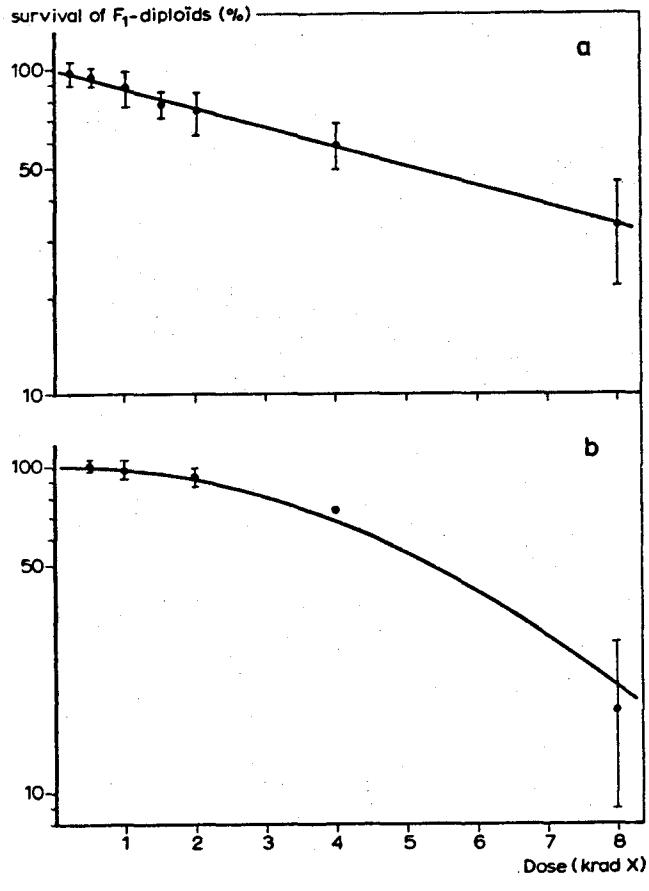


Figure 19
Dose-survival curves for F_1-diploids of *Tetranychus urticae* produced after irradiation with X-rays of:
a) 1 day old adult parental virgin males
b) 1 day old adult parental virgin females.

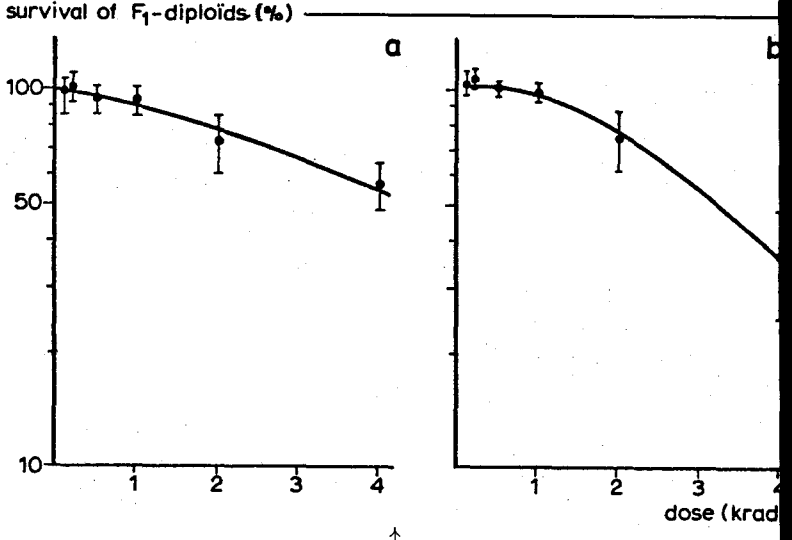


Figure 20
Dose-survival curves for F_1-diploids of *Tetranychus urticae* produced after irradiation with fast neutrons of:
a) 1 day old adult parental virgin males
b) 1 day old adult parental virgin females.

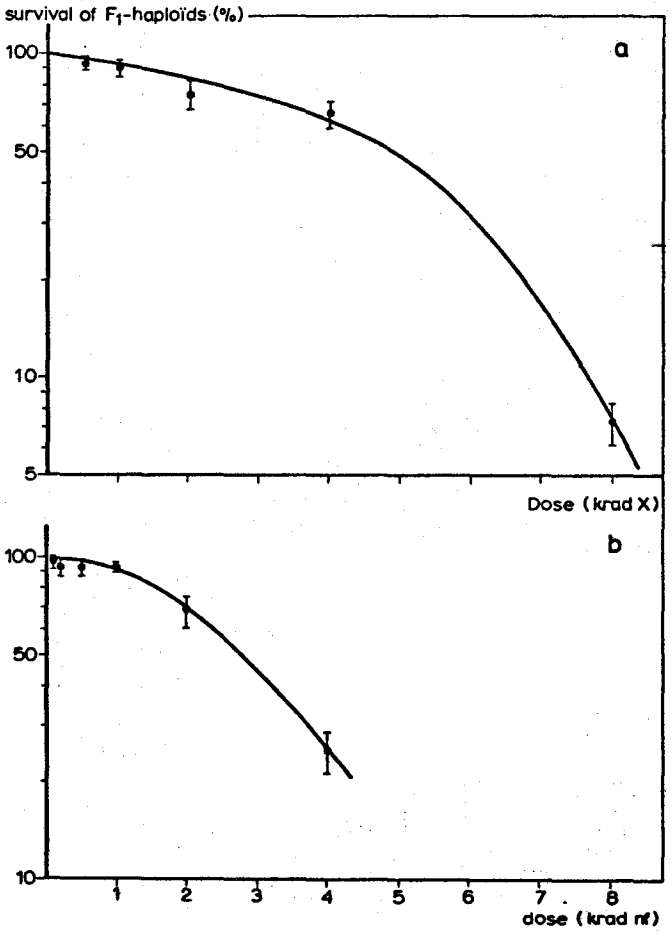


Figure 21
Dose-survival curves for F_1-haploids of *Tetranychus urticae* produced after irradiation of:
a) 1 day old adult parental virgin female with X-rays
b) 1 day old adult parental virgin female with fast neutrons.

GENETIC STUDIES

Further studies on gene instability of chlorophyll mutants in tomato (*Lycopersicon esculentum* Mill) plants - R. ECOCHARD, G. MERKX, A. RINGOET.

In view of the localization of mutants C_6 and C_{12}, the phenotype, seed and fruit production in the F_1 of crosses between these mutants and a series of different trisomics have been analysed. A certain number of plants with aberrant numbers of chromosomes have been selected for further observation.

In a study on the localization of mutant C_{11} using marker lines in general last year's results were confirmed. It was shown, however, that Butler 16 "marker bu/bu" (bushy) is not homozygous for this characteristic.

Induction of a primary monosomic for chromosome 5 had been realized in *Lycopersicon esculentum* Mill by pollen-irradiation with thermal neutrons. This experiment has been repeated using Butler 17 "marker wt" (wilty leaves), which line presents the advantage that point-mutations and monosomics are phenotypically detectable. Twenty-four plants among 772, resulting from 1086 seeds of a crossing Butler 17 ♀ x irradiated pollen of Moneymaker, were selected for cytological observation. No monosomics were found, but 4 plants probably present point-mutations.

Induction of haploidy in tomato (*Lycopersicon esculentum* Mill) - R. ECOCHARD, G. MERKX, A. RINGOET.

Recessive Moneymaker plants (characteristics: anthocyaninless, hairless) were cultivated on ^{15}N, Moneymaker on ^{14}N, in normal Hoagland solutions. ^{14}N-containing material is very sensitive to thermal neutrons, (see earlier reports) and therefore ^{14}N-pollen grains and flowers should be destroyed by irradiation. ^{15}N-pollen grains and flowers should resist this treatment. In the present experiment the following treatments were applied:

- crossed pollinations, 12 hours before irradiation, to allow the growth of the pollen tube in the pistil.
- irradiation in the Barn reactor, (power 100 KW, 24 hours), of the pollinated plants.

We observed a big difference between induction of androgenesis (crossing M x Rec.M) and parthenogenesis (crossing Rec.M x M). In the first case, the percentage of germination is very low, and no haploids were obtained. In the second case, a few (2 haploid on 768 seeds) were found, with a much better germination percentage. These are the results of just one experiment. It will be repeated with emphasis on induction of parthenogenesis.

Self-compatibility in higher crops.

- Induction of self-compatibility in allogamous crops.

Induction of self-compatibility in dihaploid *Solanum tuberosum* L. - A.J.G. VAN GASTEL.

After γ-irradiation and self-pollination of a self-incompatible dihaploid *Solanum tuberosum* L. clone a number of berries with seeds were obtained. In the I_1-progenies of the control no self-compatibility was present.

Half of the offspring of the treated plants displayed the self-compatibility character. However, the self-compatibility character was not expressed in all the flowers of a plant and showed a large variation throughout the year.
In the summer of 1973 the I_1 and some I_2 progenies have been tested. They displayed a similar pattern as was found in 1972. The results obtained, seem to indicate that the self-compatibility character is radiation-induced (no self-compatibility in the control-series) and of a genetic nature (self-compatibility present in the I_1 and I_2). No permanent changes on the S-locus have been induced (no pollen and stylar part mutations are segregating in the progenies studied). Probably only minor genes are involved.

Origin of self-compatibility in dihaploids of *Solanum tuberosum* L. - J.G.TH. HERMSEN.

According to the 1972 report, an interchromosomal duplication of a mutated S-allele is supposed to cause self-compatibility in the dihaploids G254 and B16. Pollen carrying the duplication tended to grow more slowly in the styles than normal pollen (certation). On the basis of the supposed S-genotype of cv. Gineke it was expected that 50% of the Gineke dihaploids should be self-compatible.

In 1973 we first tried to identify plants which are homozygous for the duplication carrying S_1^1. This was done by testing the ratio self-incompatible (s.i.) to self-compatible (s.c.) in the progenies of the cross G254-selfed x G609. In G254-selfed 50% of the plants should be duplication homozygotes ($S_1^1S_1^1$) and produce only s.c. plants after crossing with the s.i. dihaploid G609. The remaining 50% should be duplication heterozygotes, which after crossing with G609 are expected to segregate 1 s.i. : 1 s.c. As only 1 : 1 ratios were found, it was hypothesized that duplication homozygotes either are not formed or are lethal.
In order to collect additional information about this question 500 seeds of G254-selfed were sown in petri-dishes; 488 seeds germinated and were transferred into soil. Only 140 (= 29%) normal plants were obtained, for 305 died at the cotyledonstage and 43 were virescent. These numbers may be explained in two ways. Firstly: four independent recessive (sub)-lethal genes segregate ($X^2 = 4.67$; $P = 0.10\text{-}0.05$); Secondly: the duplication homozygotes (expected number 244) are lethal. The remaining 244 plants then comprise 140 normals, 61 lethals and 43 virescent plants. With one recessive lethal gene and one for virescense the expected numbers are 137.1 normal, 60.9 lethal and 43 virescent respectively ($X^2 = 0.22$; $P = 0.90$).
These results support the view that duplication homozygotes are lethal.

Certation between pollen with and without the duplication was re-investigated by determining the ratios s.c. : s.i. in six F_1 hybrids mentioned in table 13.
The ratios obtained in 1972 are included for comparison.

Table 13 - Numbers of self-compatible (s.c.) and self-incompatible (s.i.) plants in six F_1-populations in two consecutive years.

CROSS	1972		1973	
	s.c.	s.i.	s.c.	s.i.
G254 x B16	22	7	26	8
B16 x G254	26	3	24	11
G254 x G609	17	21	18	21
G609 x G254	10	28	19	21
B16 x G609	20	17	20	20
G609 x B16	14	21	18	21

Two crosses, viz. those with G254 as the male parent, B16 x G254 and G609 x G254, show results which are different in 1972 and 1973. For an explanation of the 1973 ratios there is no need to assume certation, if duplication homozygotes are lethal. However, the ratios 26 : 3 and $1\frac{3}{4}$: 28 obtained in 1972 cannot be explained on this basis. Only when both certation and viability of duplication homozygotes are assumed, the ratios of 1972 fit in with those of 1973. However, the assumption of viable duplication homozygotes cannot be reconciled with the above evidence for lethality of these homozygotes. Obviously this experiment has to be repeated using larger populations.
The test of self-(in)compatibility in Gineke-dihaploids has been hampered by the general occurrence of male sterility and poor flowering in 1973.

Nature and inheritance of unilateral incongruity and unilateral male sterility in interspecific *Solanum* crosses - J.G.TH. HERMSEN, M.S. RAMANNA.

The results of recent investigations in *Solanum* and *Lycopersicon* suggest the absence of direct genetic and physiological relations between incompatibility as such and interspecific incompatibility. Consequently the term "incongruity" has been introduced to replace the term "interspecific incompatibility".
Unilateral incongruity (non-crossability in one direction only) and unilateral male sterility occur when crossing *Solanum verrucosum* Schlecht with diploid *Solanum tuberosum* L. Owing to unilateral incongruity this cross only succeeds when *S. verrucosum* is the female parent. The F_1's obtained in this way exhibit plasmon-genic male sterility. With *S. verrucosum* as the male parent the pollen tubes are inhibited in the *S. tuberosum* styles. This inhibition is genetically determined. Therefore a search was made for diploid *S. tuberosum* genotypes which accept *S. verrucosum* pollen (no inhibition). Such genotypes would enable the production of male fertile F_1 hybrids *S. tuberosum* x *S. verrucosum*. Three such so-called acceptor genotypes were detected recently and mentioned in the 1972 report. In order to study the genetic basis of acceptance several crosses were made among acceptors (A x A), between acceptors and non-acceptors (A x NA) and among non-acceptors (NA x NA), and the progenies tested for acceptance of *S. verrucosum* pollen. Pollen tube growth was investigated in different cross combinations. Finally one set of reciprocal hybrids was studied carefully for occurrence and type of male sterility.

Table 14 - Two genetic models to explain the observed ratios acceptor (A): non-acceptor (NA). Models explained in text.

CROSS	Type parents	Observed		Expected			
		A	NA	Model 1		Model 2	
				A : NA	P	A : NA	P
GB39 selfed	A	6	0	1 : 0	1	1 : 0	1
GB53 selfed	A	14	5	1 : 0	0	3 : 1	>0.95
BG30 selfed	A?	4	13	1 : 3	>0.70	1 : 3	>0.70
BG41 selfed	NA	0	52	0 : 1	1	0 : 1	1
GB47 selfed	NA	3	11	1 : 3	>0.90	3 : 13	>0.90
G254 selfed	NA	1	12	1 : 15	>0.90	3 : 13	>0.35
B16 selfed	NA	7	27	1 : 3	>0.50	3 : 13	>0.65
GB47 x BG41	NA x NA	0	11	0 : 1	1	0 : 1	1
G254 x B16	NA x NA	10	51	1 : 7	>0.35	3 : 13	>0.65
GB53 x G254	A x NA	16	23	1 : 3	<0.05	3 : 5	>0.65
GB39 x B16	A x NA	9	11	1 : 1	>0.65	1 : 1	>0.65
GB53 x B16	A x NA	8	9	1 : 1	>0.90	3 : 5	>0.40
GB39 x BG41	A x NA	0	25	0 : 1	1	0 : 1	1
GB47 x GB39	NA x A	12	15	1 : 1	>0.55	1 : 1	>0.55

Model 1 : Non-acceptance is based on two independent dominant genes.

Model 2 : Acceptance is based on one dominant gene whose activity is suppressed by a dominant inhibitor gene (see table 14). The relatively small populations are due to segregating lethal genes. In order to further elucidate the genetics of acceptance additional crosses have been made, including also one newly found acceptor from *S. tuberosum*, spp. *andigena*. As a consequence of our finding of nearly 100 acceptor genotypes in 1973, many fertile F_1 hybrids with *S. verrucosum* are now available as starting material for resistance breeding.
Pollen tube growth appeared normal in compatible and in congruous crosses, whereas inhibition was observed after incompatible pollinations (gradual thinning bundle of pollen tubes) and when incongruity was involved (mostly a sudden growth stop of all pollen tubes just below the stigma).

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

- Mutation spectrum analysis at the S-locus of *Nicotiana alata* Link and Otto - A.J.G. VAN GASTEL, D. DE NETTANCOURT.

The aim of the experiments is the comparison of self-compatibility mutations induced by mutagenic treatments.

(1) Pollenmother cell (P.M.C.) treatment.

P.M.C.'s of *Nicotiana alata* Link and Otto have been treated with γ-rays, X-rays, fast neutrons and with EMS. A publication of the results obtained after chronic γ-rays is in press and a publication on the results of X-ray and fast neutron-treatments is in preparation. Some general conclusions are: (1) chronic γ-irradiation is very effective for inducing seed-set upon selfing; (2) chronic γ-irradiation and EMS-treatment do not induce self-compatibility mutations; (3) both X-rays (acute) and fast neutrons (acute) are able to induce self-compatibility mutations; (4) the mutation spectrum after X-rays is similar to the one found after fast neutron treatment; (5) fast neutrons induce a higher number of self-compatibility mutations than X-rays do; (6) chronic γ-rays, X-rays, fast neutrons and EMS did not induce constructive mutations.

(2) Seed treatment.

Seeds of *Nicotiana alata* Link and Otto have been treated with EMS. The results have been published in the Incompatibility Newsletter (no. 2). Neither constructive nor negative mutations have been detected.

(3) Leaf-treatment.

The induction of mutations at the S-locus by means of leaf-treatment with X-rays, EMS, UV and fast-neutrons and subsequent plantlet-production by means of the adventitious bud technique did not yield constructive or negative mutations in the progeny derived from the treated leaves.

- The analysis of the factors and mechanisms involved in the generation of new S-alleles. (test-species: *Lycopersicon peruvianum* Mill).

To test the theory of Edström that constructive mutations are due to reactivation of allelic copies previously stored during outbreeding, several S_1S_2-plants (selected from the crosses $S_1S_6 \times S_2S_6$; $S_1S_6 \times S_2S_7$; $S_1S_7 \times S_2S_6$; $S_1S_7 \times S_2S_7$) have been inbred via hormone treatment and heatshocks. The first seeds have been obtained and it is hoped that a new specificity will be found, which should be the same as the specificity which was stored during previous outbreeding.

The detection and origin of spontaneous mutations at the S-locus of inbred plants of *Lycopersicon peruvianum* Mill. - D. DE NETTANCOURT, B. DONINI.

In order to elucidate the mechanism operating when inbred plants of *L. peruvianum* spontaneously generate new S-alleles (de Nettancourt *et al*, Theor. Appl. Genet. 1971), the same S_1 and S_2 alleles which had been studied previously in one inbred genetic environment were placed, after a complete diallel analysis, test-crosses and identity tests, in several different genetic backgrounds for ascertaining the influence of such backgrounds on the frequency and specificity of spontaneous constructive mutations at the S locus. As the S-genotype of the staminate parent which was used for introducing modifications in the genetic background is known (S_4S_5), the same material will permit the detection of an eventual relation between mutation specificity and the identity of the S-alleles which have been associated, one generation earlier, to S_1 and S_2.

In 1972, the work had involved the conduction of a diallel test (20 plants) in the progeny of $S_1S_2 \times S_4S_5$ crosses, the determination of each of the 4 genotype classes present in the progeny and the production, by means of crosses between S_1S_4 or S_1S_5 and S_2S_4 or S_2S_5 individuals, of S_1S_2 genotypes in new genetic backgrounds. Such S_1S_2 plants were submitted in 1973 to obligate inbreeding and 137 seeds were obtained from 1.451 hand-pollinations after heat shocks and hormone treatments. These seeds will be sown in 1974 and the resulting populations submitted to detection tests (reciprocal crosses to S_1S_2) and to ancestry tests (reciprocal crosses to S_1S_4, S_1S_5, S_2S_4 and S_2S_5) to find out if a new specificity is present and if this new specificity is identical to S_4 or S_5.

The induction of self-compatibility in allogamous species - D. DE NETTANCOURT, B. DONINI.

With the practical aim of detecting self-compatible mutants in varieties of *Prunus avium* L. which are adapted to the climatic conditions of Italy, extensive screening tests were carried out on the morphological mutants previously induced by means of radiation treatment. Up to now, not a single self-compatible individual has been found. In 1974, special attempts will be made to induce self-compatible mutations in the two "compact mutants" which have been recently selected and which appear so promising for cultivation in Italy.

Using *Lycopersicon peruvianum* Mill and the self-incompatible hybrid *esculentum* x *peruvianum* in which electron microscopy has shown (de Nettancourt *et al*, 1973 and in press) that the formation of a concentric endoplasmic reticulum (CER) in the pollen tubes was one of the first symptoms of the self-incompatibility reaction, attempts were made to use abscissic and giberellic acids for breaking down self-incompatibility. The motivation for such experiments stemmed from the recent finding that these chemicals could lead to an elimination of the CER and to an initiation of metabolic activity in dormant potato buds. Several hundreds peruvianum flowers were sprayed 48 hours before incompatible pollinations and relatively high fruit sets (ranging from 10 to 100%) and seed-sets (ranging from 1 to 50 seeds per fruit) were recorded in the G.A. series (as compared to practically none after application with abscissic acid or distilled water). No seeds could be obtained, however, in the cases where giberellic acid was applied to the interspecific hybrid. These results, if they are confirmed, suggest that:

- giberellic acid, via a possible action on the circular endoplasmic recticulum, is an effective chemical for inducing temporary self-compatibility,
- sterility barriers are superimposed to self-incompatibility in the interspecific hybrid which prevent applications of G.A. to result in seed formation upon self-pollination.

Establishment of linkage relationships with the S-locus of self-incompatible plants and identification of the S-bearing chromosomes - A.J.G. VAN GASTEL, D. DE NETTANCOURT, F. CARLUCCIO.

- Establishment of linkage relationships in *Nicotiana alata* Link and Otto.

  The aim is to induce marker mutations on a large scale. The induced mutations will be tested for linkage with the S-locus. This approach

may permit early identification of S-genotypes. Up to now detached leaves have been treated with X-rays, E.M.S., U.V. and fast neutrons. Only three morphological mutants (after X-rays), which did not transmit their phenotype to the next generation have been recorded. These negative results might be due to very low mutation frequencies, the small amount of plants and the homozygosity of the material. In case the material is homozygous dominant the induced mutations can only be detected in the bud-selfed progenies. Therefore, plants derived from treated leaves have been bud-pollinated. The resulting seeds were sown and selection for marker mutations was carried out in the seedling stage.
Almost all progenies obtained from the fast neutron experiment have been analysed and not a single mutation seemed to be segregating. Therefore, no visible mutations (AA → Aa, Aa → aa) have been induced. The absence of mutations still might be due to the small number of plants analysed or to homozygous recessive material.

- Identification of the S-bearing chromosome.

According to the competition theory plants, which are trisomic for the S-bearing chromosome, are self-compatible. Via cytological analyses of trisomic plants we try to identify the S-bearing chromosome. Trisomic plants can be expected in the progeny of triploid *N. alata* x diploid *N. alata*. Thirty plants (out of 200) have been tested for self-compatibility. Two plants seemed to be slightly self-compatible and may, therefore, have an additional chromosome on which the S-locus is located.

- Analysis of the mechanism leading to the formation of self-compatibility mutations with a centric fragment in *Nicotiana alata* Link and Otto.

In 1973, the cytogenetical analysis of *N. alata* individuals which are unstable at the S-locus was extended on a very large scale. There is absolutely no doubt that the original clone and part of its progenies are heterologous for chromosome 3 and that one of the chromosomes in the pair carries a duplication of the satellited region. Very clear evidence, from analysis of more than one hundred meristematic cells in root tips, has been obtained which shows that the duplicated region can detach itself from the short arm of chromosome 3 and become a free fragment very much similar to the centric fragment characterizing certain types of self-compatibility mutants. Hence, it does not only appear that the mechanism by which accessory chromosomes are formed has been elucidated but it also seems that the origin of self-compatibility fragment is now known.
The results of a segregation test for the modified chromosome 3 and the S-locus (S_4 as labelled by a stylar part mutation) indicated, however, that the S-locus, as far as stylar specificity in the style is concerned, is not located on chromosome 3. If one accepts the view that chromosome 3 is the origin of self-compatibility promoting centric fragment, this finding implies that either the specificity segment governing the incompatibility reaction in the pollen is not located on the same chromosome as the specificity segment active in the style or that the centric fragment does not carry an S-locus (as assumed by the competition theory) and does not even originate from the S-bearing chromosome (as assumed by the restitution theory).

- The identification of the S-bearing chromosome of *Lycopersicon peruvianum* Mill by means of the trisomic method

  Triploids have been produced after crosses between self-compatible tetraploids and self-incompatible diploids. Although these triploids appear to be essentially self-incompatible (which casts doubts on the validity of the competition theory and on the possibility to use the trisomic method for identifying the S-bearing chromosome), backcrosses have been made to the diploid parent and a limited number of seeds were obtained which will be sown early in 1974.

- The ultrastructure and the genetics of self- and cross-incompatibility in an interspecific hybrid in the genus *Lycopersicon* - D. DE NETTANCOURT, M. DEVREUX, U. LANERI.

  In 1973, thanks to the very valuable cooperation of the laboratory of electron microscopy at the University of Siena (Professor G. Sarfatti, Drs. Cresti and Pacini), a detailed analysis was made of the genetical and ultrastructural features of incompatibility in an interspecific tomato hybrid produced at the Casaccia.

  It was found, in the course of this study, that fluorescence techniques and electron microscopy allowed, after self-pollination of the hybrids or after reciprocal crossing between the hybrid and the parental species, a distinction between pollen tubes inhibited by a unilateral incompatibility reaction and pollen tubes inhibited by a self-incompatibility reaction. The observed differences, assumed to be real and reliable, were such that it was possible to demonstrate that unilateral incompatibility in *esculentum* pollen tubes is governed by a single gametophytic factor which is either linked or allelic to the S-locus. The finding was discussed in relation to the reports recently made (see Hogenboom 1973) that unilateral incompatibility is controlled, in *peruvianum* styles, by a number of different dominant genes and the conclusion was reached that these dominant genes, the S-locus of self-incompatibility and the gametophytic factor regulating the unilateral incompatibility reaction in *esculentum* pollen belong to the same linkage group.

  The strong sterility barriers which prevent practically all backcrosses between the hybrid and the parental species were shown to be independent of the factors regulating stylar incompatibility.
  L. peruvianum is heterozygous for the sterility genes which prevent fertilization or embryo formation when the interspecific hybrid is crossed, as pistillate parent, to different accessions of *L. peruvianum*. One *peruvianum* stock was found which, as a pollinator, was highly cross-fertile with the hybrids.
  The presence of a concentric endoplasmic reticulum in inhibited pollen tubes was observed to be a constant feature of both the self- and the unilateral incompatibility reactions and was interpreted as an indication that incompatibility leads to a general cessation of protein synthesis.
  Although incompatible tubes resemble very much, in this respect, the pollen tubes cultured *in vitro*, the conclusion was reached that the inhibition of pollen tubes in incompatible styles does not result from an absence of growth promoting substance but from the presence of a metabolic inhibitor.

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

Changes in peroxidase isoenzyme patterns of unpollinated, self-pollinated and cross-pollinated styles of *Nicotiana alata* Link and Otto were investigated in order to obtain information which could be used to prove or to reject Pandey's hypothesis that peroxidase is involved in the formation of the incompatibility barrier. Compatible growing pollen tubes need almost four days (progamic phase) to reach the ovary. The differences in peroxidase iso-enzyme composition of the whole style after self- and cross-pollination were rather small during the progamic phase (see figure 22) and did not support the idea of PANDEY (1967) that a new peroxidase should be formed in self-pollinated styles which is supposed to be active in the incompatibility reaction. We did not find a new peroxidase which was specific for self-pollinated styles. The new peroxidase (No. 13) in self-pollinated styles also occurred in cross-pollinated styles. On the contrary the new peroxidase No. 8 only appeared in cross-pollinated styles. On the one hand, it is possible that the activity of peroxidase 8 is necessary for continuation of pollen tube growth. On the other hand, however, the differences in peroxidase isoenzyme activities after self- and cross-pollination might be a consequence rather than a prerequisite of differences between compatible and incompatible pollen tube growth. Up to now this question remained unanswered.
When the compatible pollen tubes have reached the ovary after about 4 days the activities of most peroxidase isoenzymes show a striking increase (see t = 117 hrs) in contrast with self-pollinated styles in which the peroxidase activity remains more or less the same. In the latter case, the pollen tubes do not reach the ovary. When isolated styles were used instead of detached flowers several peroxidase isoenzymes do not increase in activity after cross-pollination. Apparently the increase of these isoenzymes is caused by activation of the growth centre in the ovary which would regulate the enzyme activities of the flower.

- The population genetics of the S alleles of a sporophytic incompatibility system - I. BOS, G. HEEMSTRA.

The analysis of the relations between S-alleles in a heterozygous S-genotype has been finished for 9 different S-heterozygotes. This analysis was based on reciprocal backcrossing of Sweet Alyssum *Lobularia maritima* (L.) Desv.) plants, obtained through forced selfing, and the appropriate parent (HARUTA's method). Because of the considerable variation in (in)compatibility (measured as fruitset) between parent and selfed progeny, it was not possible to guarantee, bythis method alone, the correctness of the determination of the relation (dominance or codominance in the pollen producing tissue and in the stigma) and therefore the few I_1 plants with the most apparent (in)compatibility expression were subjected to a diallel cross. This is a slight modification of FABIG & NOWAK's method of analysis: the number of I_1 plants to be crossed in the diallel set of crosses is diminished through selection based on the results of the HARUTA analysis. The results of both types of determination of the relation of S-alleles should be the same and confirm each other.

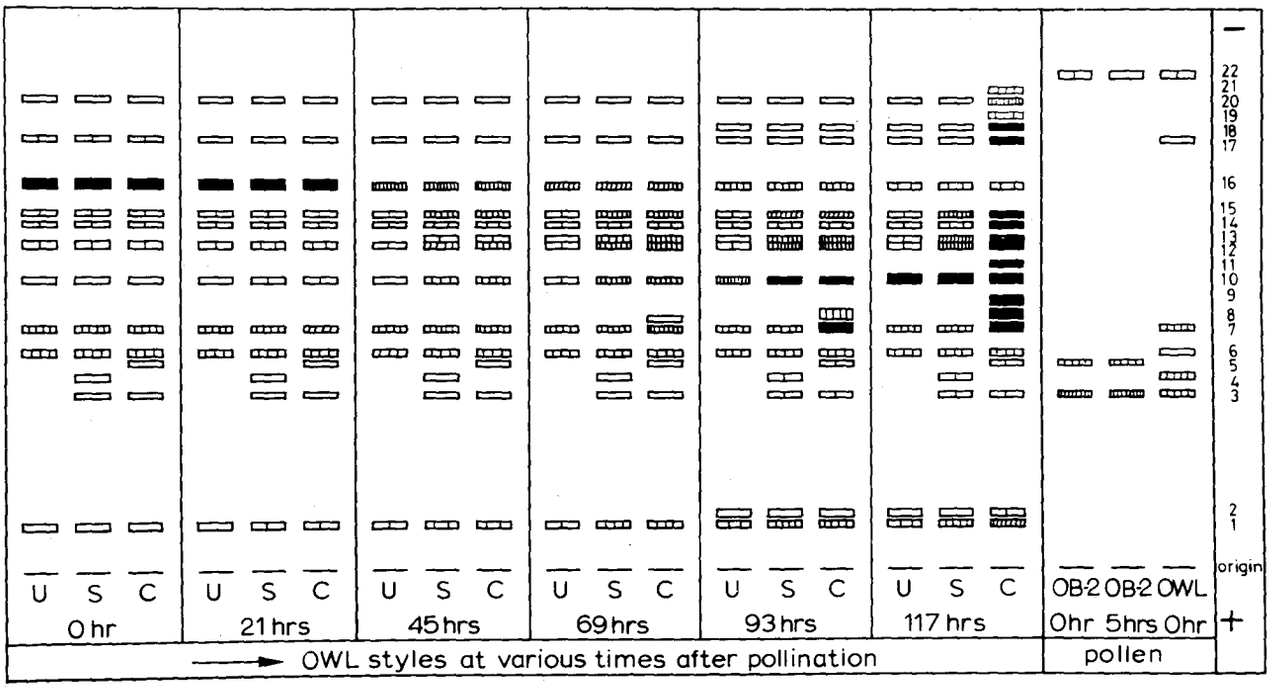


Figure 22 - Diagram of the electrophoretic patterns of peroxidases from un- (u), self- (s) and cross- (c) pollinated *Nicotiana alata* styles at various times after pollination and from germinated OB-2 (5 hrs) and ungerminated OB-2 and OWL pollen (0 hrs). The degree of hatching approximates the staining intensity. No hatching means that the bands on the starch gels were just visible.

- Effect of X-ray irradiation of pollen on its growth - L.N.J.W. GILISSEN.

  The aim of the research is to find out in what way X-rays can be used in solving problems related to the incompatibility reaction in *Petunia hybrida* L.
  During irradiation, radicals are formed. There is evidence which suggests that the amount of radicals formed after X-ray irradiation, the decay of radicals and the biological damage are proportional. Radical concentrations are measured with an electron spin resonance spectrometer (ESR). The dose-effect curve of X-rays on pollentube growth in compatible and incompatible pollinations showed that the incompatibility reaction was not broken down. The only effect was a retardation of the growth rate during the first phase of pollentube growth. Experiments in which irradiated pollen was compared to pollen that was washed after irradiation revealed that washed pollen, which have lost their radicals, germinate as well as unwashed pollen irradiated with the same dose.

- Effect of X-ray irradiation on the growth of pollen of *Pseudotsuga* sp.- J.A.W.M. VAN DER DONK, G.K. LIVINGSTON.

  Although irradiation (64 krad) stimulated the growth of this pollen, no difference was found, both in content and synthesis of RNA. Experiments were carried out to study protein synthetic capacity *in vitro* of extracted polynbosomes. The first results indicated that the stimulation of amino acid incorporation into proteins was higher by polynbosomes extracted from irradiated than by polynbosomes from unirradiated pollen. Lack of viable pollen, however, forced us to stop these experiments.

- Activation of the ovary upon pollination - H.F. LINSKENS, J.J.M. DEURENBERG.

  Activation of the ovary was studied at three levels: changes of the fresh and dry weight, the protein, amino acids and RNA content, the changes of the polynbosome profiles and the protein synthesis *in vitro* with the polysomal fraction of different developmental stages after both self- and crosspollination. The activation of the ovary after crosspollination occurred before fertilization took place and started with the carpels and the placenta. Results after selfpollination showed no significant differences with those obtained after crosspollination up to 24 h after pollination.

- Qualitative analysis of RNA synthesized 24 hours after pollination - J.A.W.M. VAN DER DONK.

  These analyses were carried out by SDS-polyacrylamide tandemgel electrophoresis. RNA labelled with ^{3}H-orotic acids showed characteristic profiles for self- and crosspollinated styles. In crossed styles messenger-like RNA was found with a main peak of about 200,000 MW. This peak was present in all compatibly pollinated styles tested and not in selfed styles.

- Synthesis of RNA and proteins as a function of the time of pollentube-style interaction - J.A.W.M. VAN DER DONK.

  Different patterns of synthetic activity were found upon self- and crosspollination. In selfed styles, the rate of RNA synthesis (measured

as incorporation into RNA of ^{3}H-orotic acid after a pulse of 3h) was maximal at 3 and 13h after pollination, whereas the rate of protein synthesis (measured as the amount of 14C-algal protein hydrolysate incorporated *in vitro* into proteins stimulated by polynbosomes extracted from styles) was maximal at 6 and 14h after pollination. In crossed styles only one maximum was found (6h for RNA, and 10h for protein synthesis). In both selfed and crossed styles the rate of synthesis of RNA and proteins was increasing from 18 to 24, and from 21 to 24 h after pollination respectively.
Analysis of the messenger activity of the newly synthesized RNA is started using egg-cells of *Xenopus levis* as an *in vitro* system.

- Electrophysiology of the style in relation with the incompatibility reaction - H.F. LINSKENS.

  At this level, a rapid reaction of the style to pollination was found: at first, a positivation was observed followed by negativation of the potential. The reaction was different related to compatible and incompatible pollination. The same patterns were found in different clones.

- Translocation and accumulation before and during anthesis - H.F. LINSKENS

  The behaviour of labelled phosphorus, amino acids and carbohydrates suggests the existence of an internal shifting system within the flower. In mature flowers, the amino acid pool in the anthers diminishes quickly and the protein pool in the ovary increases.

RELATED STUDIES ON PLANTMATERIAL

Respiratory and physiological properties of bi- and trinucleate pollen - F. HOEKSTRA.

In humid air of 97% relative humidity, binucleate pollen have a low respiration rate over a long period, and a high percentage of viability can be maintained. Trinucleate Compositae- and Gramineae-pollen on the contrary, have a high rate of respiration during a short period, and a quick loss in vitality.
In order to gain insight into the origin of these differences, pollen of several plant species have been examined. Among these are *Nicotiana alata* Link et Otto, *Typha latifolia* L. and *Narcissus poeticus* L., *Cosmos bipinnatus* Cav., *Tanacetum vulgare* L. and *Zea mays* L. for the trinucleate type. For the experiments, over 120 portions of pollen have been collected under controlled conditions. After 20 hours of drying over sodium hydroxide pellets at 5°C, they were kept under deepfreeze circumstances in closed vessels. In October, the samples have been tested for their germination ability and respiratory properties at 97% R.H. and 30°C.
Within the samples of one species with binucleate grains, the variation of the rate of respiration was very limited. In general, the respiration rate of the binucleate grains ranges from 0,060 to 0,120 µmol CO_2/mg pollen/hour.
Within the samples of one species with trinucleate grains, however, considerable differences in respiration rate could be established. This phenomenon could also be simulated by short time experiments: *Cosmos bipinnatus* pollen can respire for 7 hours at a rate of 0,45 µmol CO_2/mg/hour. After incubation for 1½ hours, followed by quick desiccation over NaOH-pellets at 5°C, reincubated pollen gave a reduced respiration rate of 0,20 µmol CO_2/mg/hour, during 5 hours. Preliminary incubation for 3 hours, followed by quick desiccation in the cold, totally blocked the respiration ability of the re-incubated material. It was observed, that retarded shedding of the ripe trinucleate pollen reduced the germination ability and respiration rate. For this reason, attention has to be paid to the homogenity of the trinucleate pollen sample, when required for biochemical purposes.
The respiration process of both types of pollen apparently was almost not affected by X- and γ-irradiation. Low doses did not stimulate, nor reduce the respiration rate. A decrease of the rate and duration of respiration was seen with the trinucleate *Cosmos bipinnatus* pollen at doses, in the range from 350 krad up to 900 krad ^{60}Co γ-rays. The binucleate *Narcissus poeticus* pollen showed a continuous increase in the respiration rate, starting at a dosis of 450 krad, and reaching twice its initial value at 650 krad.
The increase is presumably due to membrane damage: the duration of the respiration in fact decreased, starting from the 450 krad dose level. Such high doses deteriorate the DNA and RNA of cells of higher plants irreparable. De novo synthesis of proteins will be disturbed entirely. Even at these high doses, the binucleate type did not show a decrease in the respiration rate. The normally low respiratory activity of the binucleate types therefore, has probably no relation with a slow de novo synthesis of proteins. Both types of pollen have their respiratory system present at the moment of shedding.

Another factor in relation to the respiration rate of binucleate pollen, that might be limiting, is the rate of uptake of water vapour. The uptake of tritiated water vapour gave practically no differences in the accumulation of radioactivity between bi- and trinucleate types of pollen. The estimated amounts of water, that have been taken up, appeared to be in accordance with the measured increase in weight of the pollen mass. Initially the uptake of water vapour is very rapid. Eventually, both types reached nearly a doubling of their initial weight. This clearly demonstrates, that the observed differences in the respiration rate can not be the result of supposed differences in the uptake of water vapour. More detailed studies will be required, concerning the nature of the available substrates and the mitochondrial activity in the course of the respiration process.

S O I L - P L A N T S T U D I E S

U P T A K E O F S P E C I F I C E L E M E N T S B Y P L A N T S

Kinetics of ion uptake by intact riceplants (*Oryza sativa* L. cv. Arborio) - G. VERFAILLIE.

In 1972, the qualitative interference of various ionic components of a Hoagland nutrient solution on the proton uptake by rice plants from a proton-phosphate binary system (H-P) had been put in evidence.
In 1973, more quantitative results have been obtained about the ionic balance prevailing during the ion uptake by the plants from binary and more complex systems.
The most interesting results obtained in this study are:

- Proton- and phosphate uptake from an H-P binary system

  In a pure 1.5 N H_3PO_4 solution phosphate uptake was followed by radioactive tracing with ^{32}P.
  Total acid uptake (bound protons and H^+-ions) has been calculated as a function of the phosphate uptake and of the pH variations according to the theory, presented in the annual report 1972.

  - The pH increases regularly from pH 3.4 and tends towards an equilibrium value vicinating 4.2 (figure 23 curve A).
  - The uptakes of respectively phosphate and of the protons, expressed in micro-equivalents per gram fresh root and plotted against time, give two curves exactly overlapping each other (figure 23 curve B).
  - After the experiment, no trace of any other ion could be detected in the nutrient solution indicating that no ionic exchange occurred during uptake.
  - The ratio $\Delta H/\Delta P$ remained constant and equal to 3; this means that the uptake of each mole phosphate has been balanced by:
    2 equivalents of bound protons ($H_2PO_4^-$)
    1 equivalent of H^+-ions.

- Uptakes from the H-P-K ternary system

  In a nutrient solution of 1 mM KH_2PO_4 acidified with H_3PO_4, potassium uptake has been calculated by difference.

  - The presence of the K^+-ions increases the phosphate uptake rate and, consequently, the proton uptake rate (figure 23 curves C and D).
  - The pH does not vary as long as the concentration of K^+ has not been reduced to a value below 0.2 mM (figure 23 curve F).
  - The charge balance is realized by the uptake of K^+-ions. (figure 23 curve E).

  The ratio $\Delta H/\Delta P$ for K^+ concentrations smaller than 0.2 mM increases from 2 to 2.2 when the K^+ concentration decreases. This means that each mole of phosphate taken up is ionically balanced by:
  2 equivalents bound protons ($H_2PO_4^-$)
  1 to 0.8 equivalent K^+-ions.
  0 to 0.2 equivalent H^+-ions.

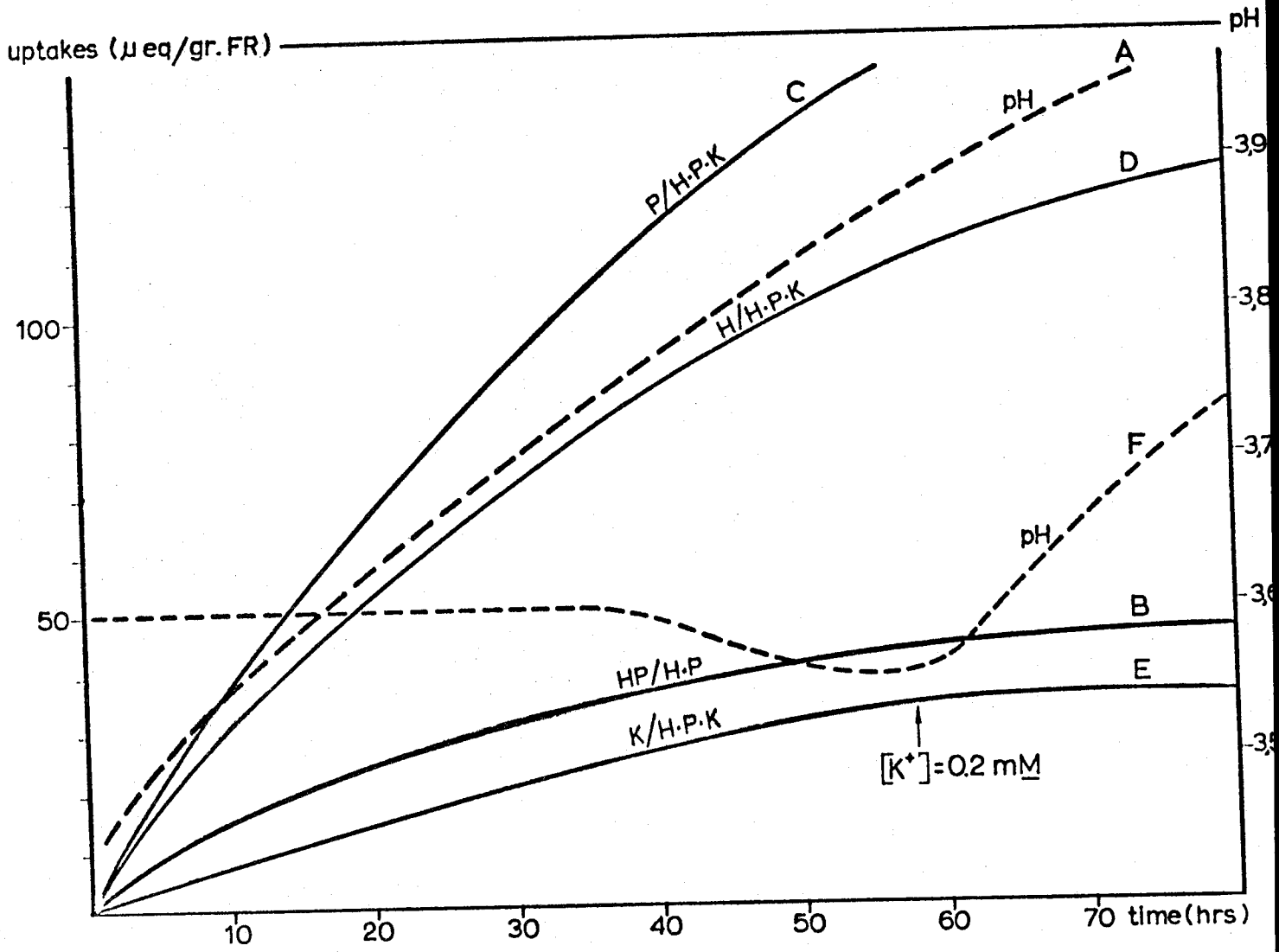


Figure 23 - Ionic balances during the uptakes from H-P and H-P-K systems.
Curve A: pH variations for binary H-P system.
Curve B: proton- and phosphate uptakes for H-P binary system.
Curve C: phosphate uptake for H-P-K ternary system.
Curve D: proton uptake for H-P-K ternary system.
Curve E: potassium uptake for H-P-K ternary system.
Curve F: pH variations for H-P-K ternary system.

- Interference of the nitrate uptake with the H-P-K ternary system (figure 24).

  In a nutrient solution of 1 mM KH_2PO_4, without acidification but with a later injection of KNO_3 bringing the nitrate concentration up to that of a Hoagland solution (15 mM), the following was observed:
  - Before the nitrate injection, the pH decreases from 4.7 and tends towards the equilibrium value. Immediately after the nitrate injection, the pH as well as the rate of acid uptake increase rapidly but the phosphate uptake slows down.
  - The ratio $\Delta H/\Delta P$ passes suddenly from 2.05 to 8.8 indicating that the nitrate uptake is accompanied by a consumption of H^+ with a high stoichiometry.

- Interference of the sulfate uptake with the H-P-K system (figure 25).

  In a nutrient solution of 1 mM KH_2PO_4 at equilibrium pH (phase I), getting a first injection of $MgSO_4$ at the level of 2 mM (phase II), and a second of KNO_3 at the level of 5 mM (phase III):
  - The pH decreases immediately after the injection of the sulfate. This is predicted by the theory if SO_4^{--} is the only sulfate species taken up by the plants (shift in the equilibrium of the ionic dissociation of the diprotic sulfuric acid).
  - The proton uptake rate shows a small increase bu the increase of the phosphate uptake rate is high.
  - The ratio $\Delta H/\Delta P$ passes from the value 2.24 (phase I) to the value 1.53 (phase II). This means that for each mole of phosphate taken up, 1.47 equivalent H^+ and bound protons are involved in the process of sulfate uptake.
  - The later injection of nitrate produced the rapid reincrease of the pH (phase III) for the reasons explained in the preceding paragraph.

- Interference of the chloride uptake with the H-P-K system (figure 26).

  In a nutrient solution containing acidified 1 mM KH_2PO_4 at equilibrium pH (phase I), and receiving an injection of KCl^- at the level of 5 mM (phase II) the results suggest that:
  - the pH increases linearly after the injection of the chloride.
  - the ratio $\Delta H/\Delta P$ passes from the value 2.3 (phase I) to the value 3 (phase II). Each mole phosphate taken up is exactly balanced by 2 equivalents bound protons and 1 equivalent H^+-ions as in the binary system H-P (see figure 23A). To put this better in evidence, the coordinates have been shifted in order to bring the origin at the KCl injection.
  - everything looks as if the Cl^- could suppress the degree of freedom produced by the presence of K^+ or as if the Cl^- uptake would be linked to that of $H_2PO_4^-$ with an extra stoichiometric consumption of 1 H^+-ion.

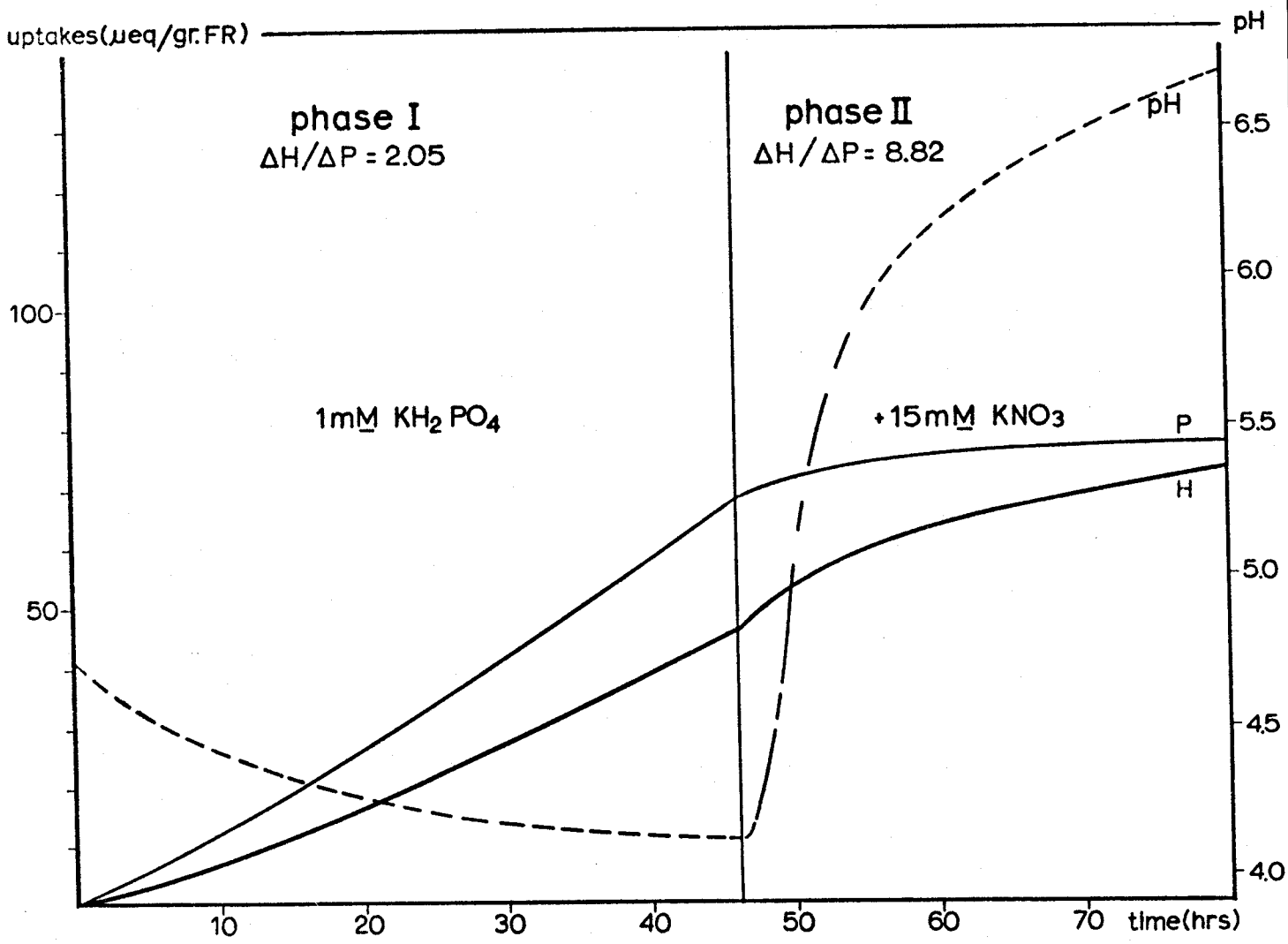


Figure 24 - Influence of the nitrate uptake on a H-P-K system.
Phase I : H-P-K reaching dynamic equilibrium.
Phase II: effect of the nitrate uptake after injection of KNO_3.

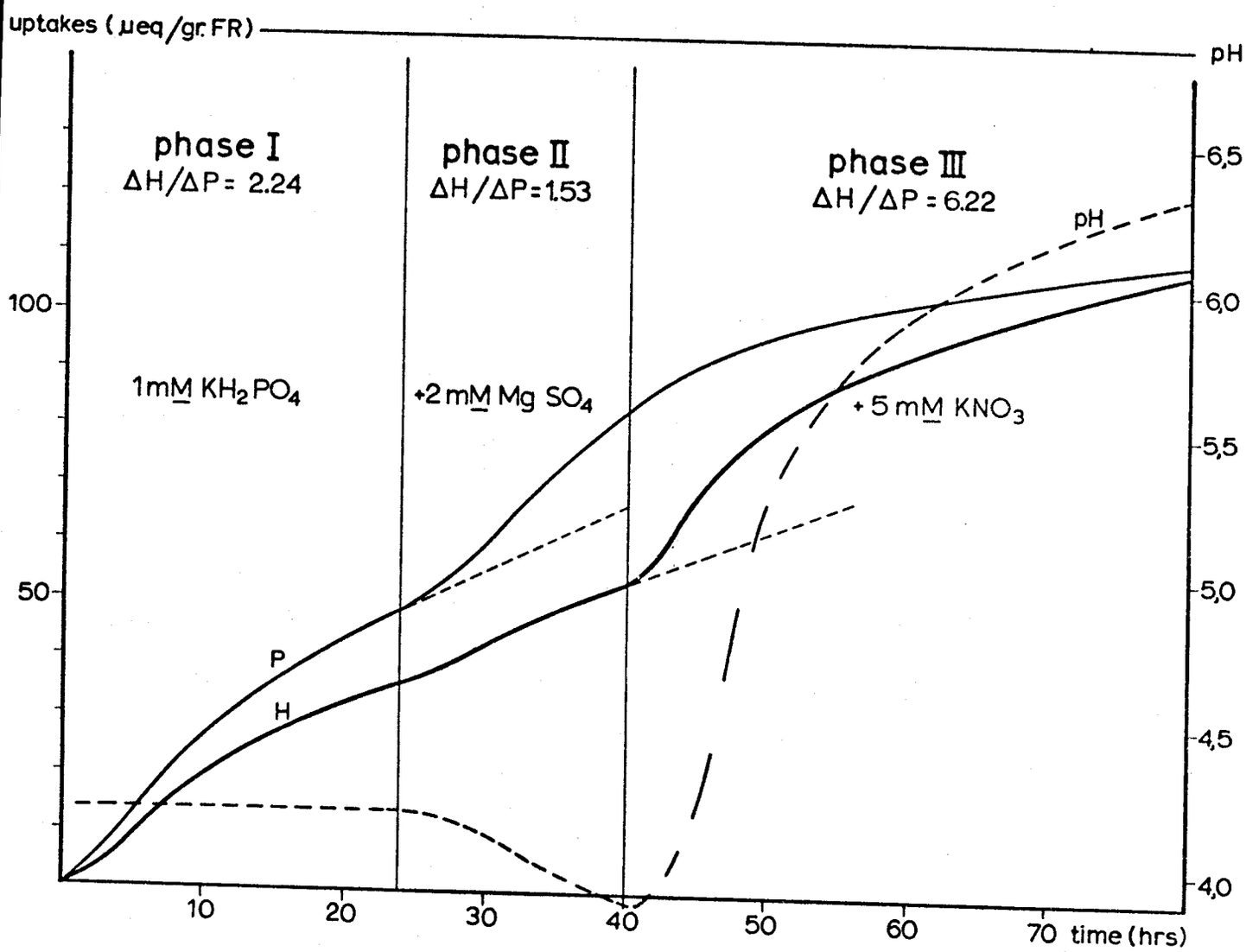


Figure 25 - Influence of the sulfate uptake on a H-P-K system.
Phase I : H-P-K in dynamic equilibrium.
Phase II : effect of the sulfate uptake after injection of $MgSO_4$.
Phase III: cumulated effect after injection of KNO_3.

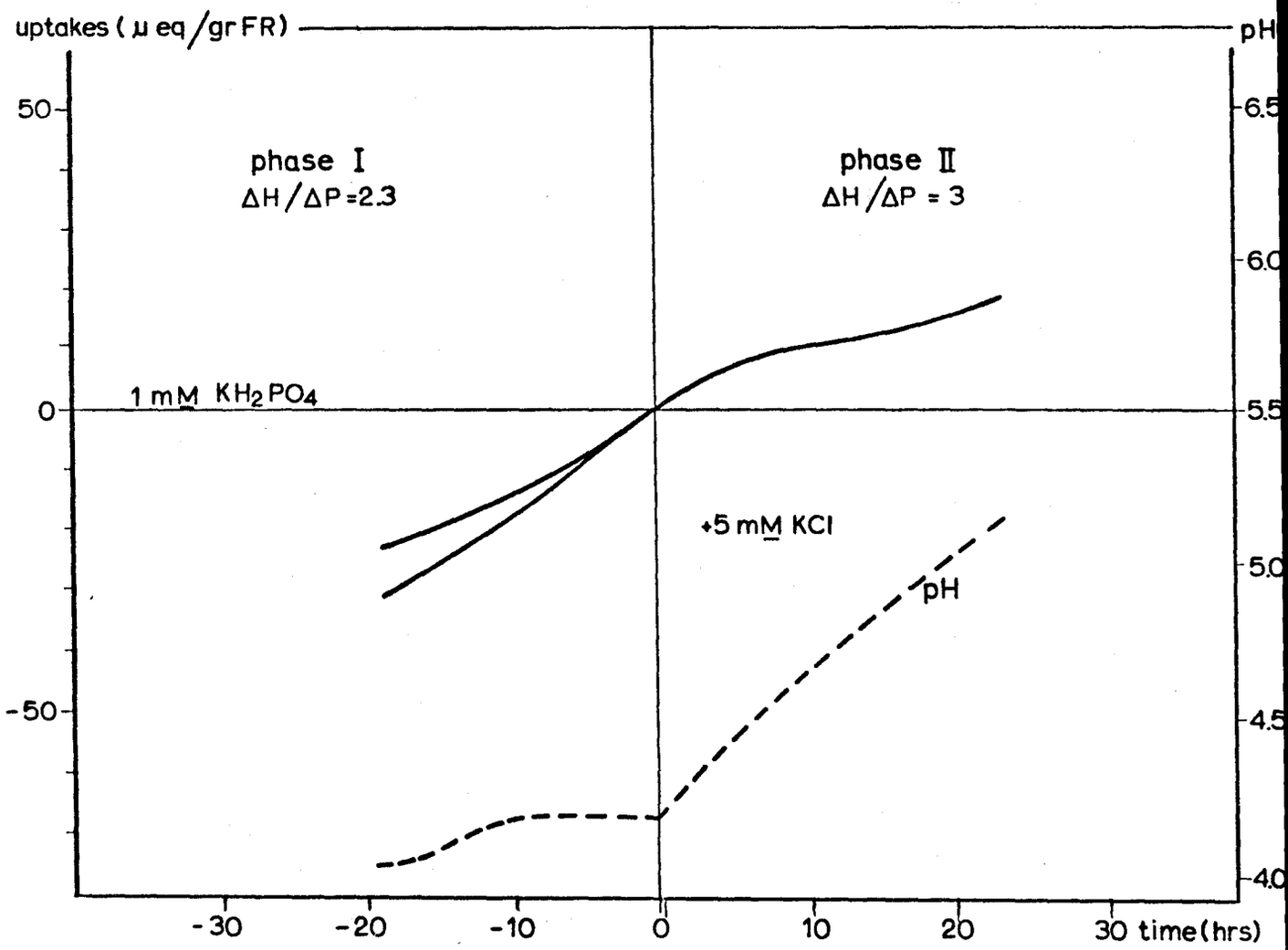


Figure 26 - Influence of the chloride uptake on a H-P-K system.
Phase I : H-P-K in dynamic equilibrium.
Phase II: the chloride uptake reduces the behaviour of the H-P-K system to that of a H-P system.

Uptake of mineral solutes by plants from a dilute environment - H. LAUDELOUT, TANG VAN HAI.

The work carried out during 1973 has been concerned with the uptake of P, K, Mg and K by the rice plant (*Oryza sativa* L. cv. I.R. 8) from dilute solution with various concentrations of a given element keeping all the others constant. With regard to P the range of deficiency symptoms is 0.16 - 3.2 µM, for K the range is 51-102 µM, for Ca: 6.2 - 12.0 µM.
No symptoms of Mg deficiency were recorded at the lowest concentration used (8 µM). Maximum growth was observed at 200-250 µM Ca, 10-25 µM, P, 125-250 µM K and 30-32 µM Mg.
The uptake of N by the same plant was then investigated using optimum concentrations of P, K, Ca and Mg. The range of N concentrations was very broad (70 to 7140 µM as ammonium sulfate). At low N concentrations (below 285 µM) deficiency symptoms were observed. A threefold increase in dry matter production was observed up to about 3 mM nitrogen. Root growth was found to have an inverse relationship with nitrogen concentration, maximum root length being observed at the lowest nitrogen concentration.
A kinetic analysis of the rate of nitrogen uptake vs. nitrogen concentration showed that a Michaelis type of relationship was obtained.
The K_m value did not change with age and remained at .2 to .25 mM while the V_m decreased with age from 5.8 $mg.g^{-1}h^{-1}$ for 25 days old plants to .9 $mg.g^{-1}h^{-1}$ for 100 days old plants expressed on the basis of the dry root weight.
The work has also been extended to the uptake pattern of N, P, K, Ca and Mg at varying levels of N by the soybean plant *Glycine max.* L.Merr.). Saturation of the NH_4^+ absorption mechanism was observed at .7 mM. It was observed that inoculation of the soybean plant depressed the V_m value of the Michaelis relationship without affecting K_m.

Ion uptake by intact plants from the soil solution or from the equilibrium solution of a synthetic resin. Experimental and simulation model approach - J. SINNAEVE, F. VAN DORP, M.J. FRISSEL, P. POELSTRA.

The CSMP-computer simulation model, written last year, described the ion uptake of one cylindrical root penetrating in a soil cylinder. Further data concerning root geometrics, mineral nutrition balances with regard to the major elements and parameters of growth media (e.g. water content, hydraulic conductivity, ion concentrations in the equilibrium solution, diffusion constants of ion, redoxpotentials), however, are needed.
A first step in the evaluation of the suitability of a synthetic medium was the determination of the immobilization rates of the major elements (Ca^{++}, Mg^{++}, K^+, $H_2PO_4^-$ and NO_3^-) as well as the transpiration rates for several plant species (barley (*Hordeum vulgare* L., cv. Aramir), maize (*Zea mays* L., cv. Ona), spinach (*Spinacea oleracea* L. cv. Winterreus) and pea (*Pisum sativum* L. cv. Allround)), in gravel culture using a Hoagland-Arnon I nutrient solution. Two seventy days experiments with the four plants species in two different plant densities have been carried out. Growth vessels with a constant solution level within the gravel and equiped with a window for photographic registration of root development and penetration were used. The results, from which one series (plants at a distance of 7 cm) is presented in figure 27, will be used to check the nutrient availability from the selected synthetic medium.

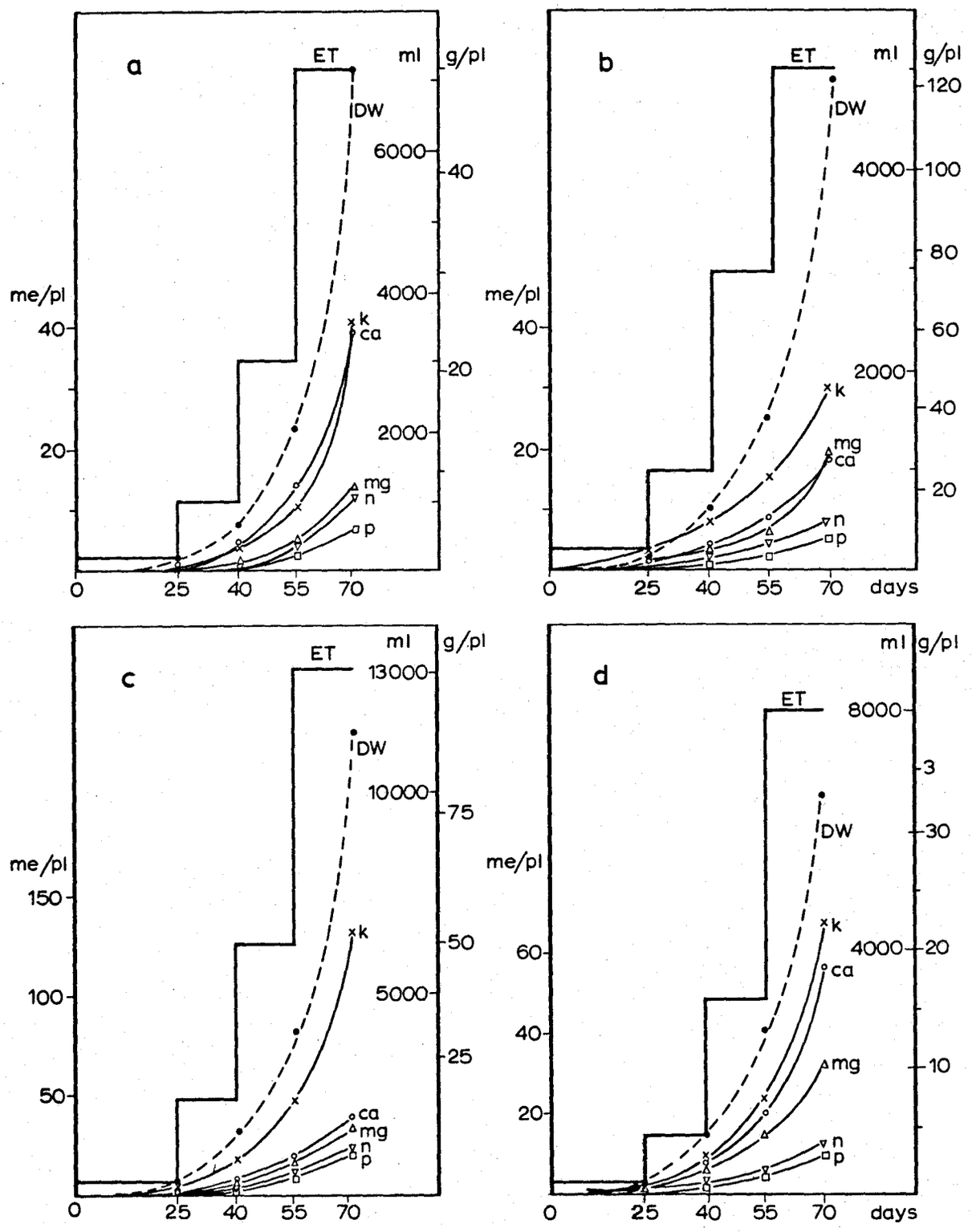


Figure 27 - Growth experiments with pea (A), maize (B), barley (C), and spinach (D) plants at a distance of 10 cm from eachother. The concentration of K^+, Ca^{++}, Mg^{++}, NO_3^- and $H_2PO_4^-$ is given in milliequivalents per plant (me/pl). The dry weight (DW) is given in grams per palnt (g/pl) and the evapotranspiration (ET) in millilitres (total amount over the period considered (3 times half an hour aeration per day).

With respect to the resins used in the synthetic media, the physical properties of the adsorption complex of an anionic and cationic exchanger, respectively Dowex 21K with an exchange capacity of 1.2 me/ml wet resin and Dowex 50W with an exchange capacity of 1.9 me/ml wet resin were studied. To saturate the exchange complex, five ml of Dowex 50W were brought in a perspex column and percolated with 2 litres Hoagland-Arnon I nutrient solution (20 mc/l). Figure 28A gives the concentration of Ca, Mg and K in the effluent and figure 28B gives the composition of the adsorption complex in function of the time. Equilibrium conditions were reached after percolation of 1.5 litres; the adsorption complex was charged with 84% calcium, 12% magnesium and 4% potassium. According to literature data, this reflects an acceptable ionic balance. The preparation of sufficient quantities of resin for plant growth would, however, require enormous volumes of nutrient solution. Therefore, a 10 times-concentrated nutrient solution was used. The results are given in figures 29A and 29B. The equilibrium composition of the resin contains less calcium (76%) and more potassium (12%). This procedure, however, would require important volumes of solution and a more concentrated solution can not be prepared as $CaSO_4$ precipitates. This imposed a procedure in which fractions of the resins were saturated separately with different ions. The composition of the media used in a growth experiment with barley (*Hordeum vulgare* L. cv. Aramir) is given in table 15. None of the mixtures gave satisfactory results (strong growth retardation and nutrient deficiency) which probably was due to the insufficient aeration of the medium, the unadapted water management (unsufficient capillary rise with a water table at a depth of 30 cm) and the strong cationic selectivity of the cation exchanger.

The physical properties of the medium were improved by replacement of the sand fraction. A non-sieved coarse agraperlite (up to 4 mm particle diameter) and a sieved medium coarse agraperlite (0.6 to 2.5 mm) were used. Agraperlite is a porous material made of vulcanic stone and heated at 1200 - 1500°C. It has no significant exchange capacity and has a specific weight of 2.4 g/ml; the pore volume is 96%. Figure 30 gives the volumetric distribution of air and water in agraperlite in function of the water tension.

The four plant species specified above were used and the plants were grown from the seedling stage on, in small columns (5 cm inner diameter) with the water level at a depth of respectively 30, 35, 40 and 45 cm. The medium used in the columns consisted of 61.6% agraperlite, 11.5% NO_3^- resin, 2.5% SO_4^- resin 2.5% $H_2PO_4^-$ resin, 15.3% Ca^{++} resin, 3.3% Mg^{++} resin and 3.3% K^+ resin. The aeration of the medium was considerably improved and the growth of the plants was less retarded although nutrient deficiency appeared later on.

In a similar way a study with the strong base exchanger Dowex 1 and the weak acid Amberlite exchanger IRC50 was started. The resins have an exchange capacity of respectively 3.7 and 10.3 me/g dry resin and a specific weight of 0.7 and 0.6 g/ml with a pore volume of 55% in the wet material for both types. The Amberlite type is more suitable for plant growth than the strong acid Dowex 50W as amberlite in the potassium form, when percolated with water, releases measurable amounts of potassium (figure 31). The irregularities in figure 31 may

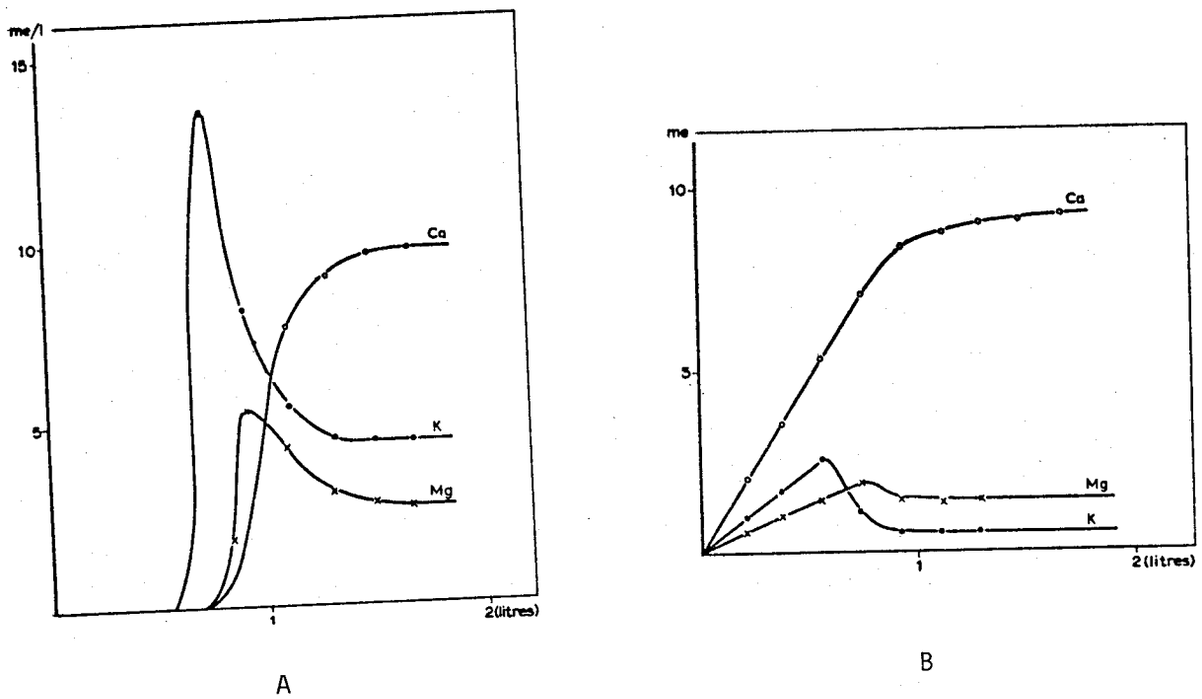
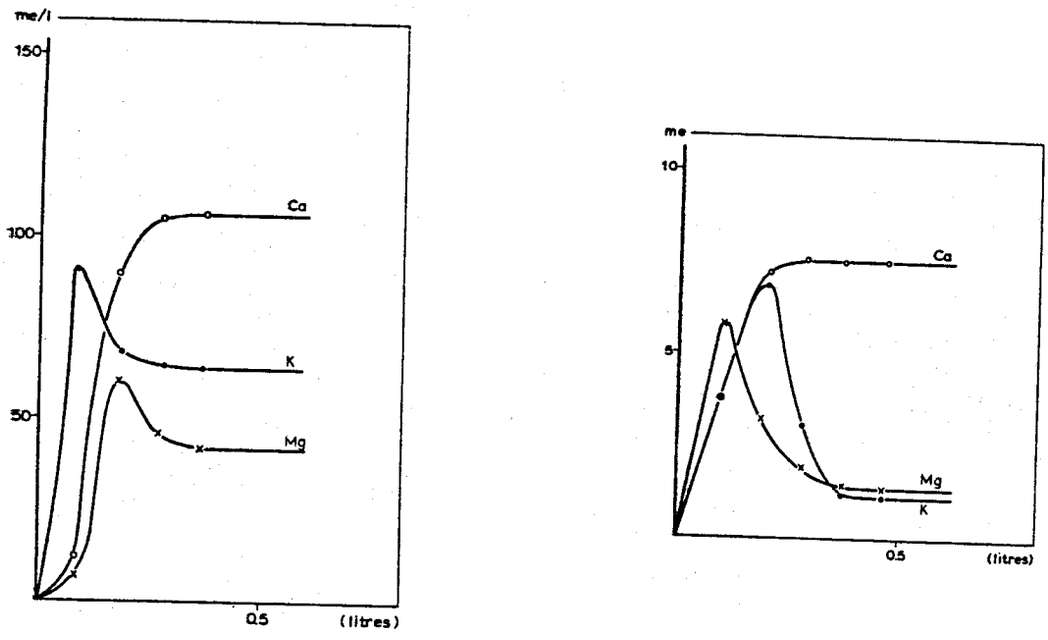


Figure 28 - Percolation of Dowex 50W with a Hoagland-Arnon I nutrient solution (5 ml resin in a column).
A: me/l in the effluent in function of the amount (liters) of solution percolated.
B: nutrients adsorbed (me/5 ml resin) in function of the amount (liters) of solution percolated.



A

B

Figure 29 - Percolation of Dowex 50W with a 10 times concentrated Hoagland Arnon I nutrient solution (5 ml resin in a column)
A: me/l in the effluent in function of the amount (liters) of solution percolated.
B: nutrients adsorbed (me/5 ml resin) in function of the amount (liters) of solution percolated.

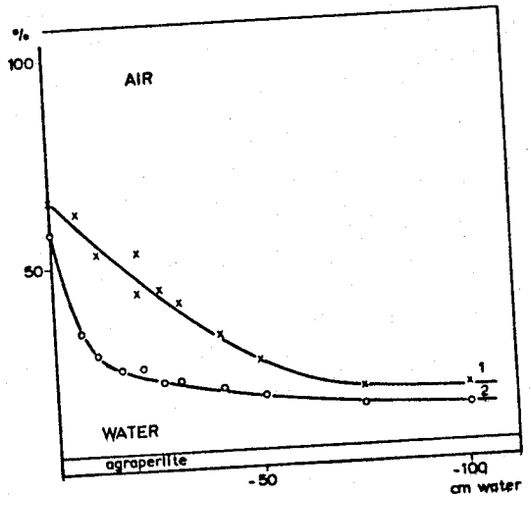


Figure 30 - Distribution of air, water in agraperlite medium coarse (line 1) or agraperlite coards (line 2) in function of the water tension.

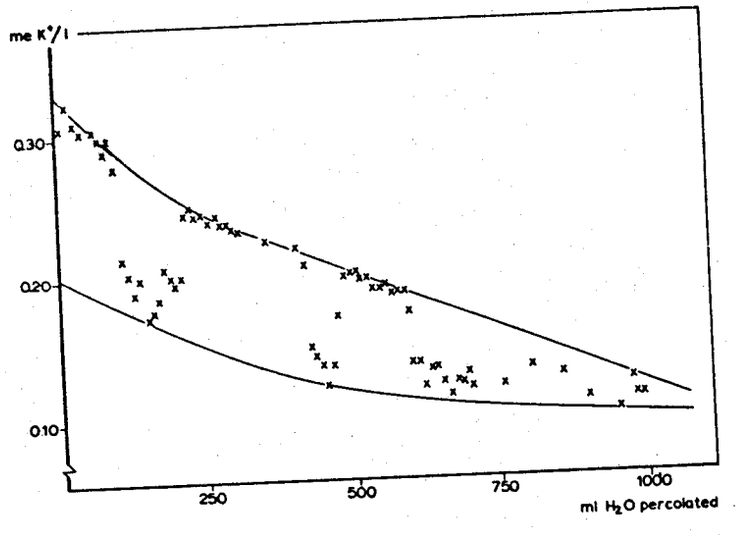


Figure 31 - Potassium concentrations (me/l) in the effluent of 0,19 dry amberlite IRC-50 (saturated with K^+) percolated with water (47 ml/hour).

be due to changes in the velocity of percolation. The high preference of Amberlite IRC50 for H^+ gave some difficulties saturating the resin with calcium and magnesium but by mixing the resin with suspensions of calcium hydroxide or magnesium hydroxide, a complete saturation of the adsorption complex apparently was possible.
Instrumentation to study the concentration of nutrients in the equilibrium solution is worked out. Small amounts of the equilibrium solution are continuously extracted with a micro-pump (1 ml per 24 hours) and ionic activities are measured with microelectrodes. An apparatus for measuring diffusion constants of ions in the media at different water tensions has been designed and experiments will be conducted in 1974.
A detailed description of two types of growth chambers with different illumination geometrics and an adapted air conditioning unit controlling windspeed, temperature, relative humidity and CO_2 concentration was worked out.
This type of set-up will enable the evaluation of all growth factors to be taken into account in the simulation models and, therefore, the root development in relation to solid substrates. The environmental parameters for root and shoot development are indeed studied in relation to the overall control of all plant-activities (e.g. photosynthetic rate).

Table 15 - Composition of different synthetic resin media used in a preliminary growth experiment with barley. Two plants were growing in 4.2 litres of substrate. All quantities are expressed in litres.

No	anionic resin	cationic resin	pure sand	NO_3^- resin	SO_4^{--} resin	$H_2PO_4^-$ resin	Ca^{++} resin	Mg^{++} resin	K^+ resin
1	.84	.84	2.52	.40	.40	.040	.70	.10	.03
2	.42	1.26	2.52	.20	.20	.020	1.00	.14	.05
3	.28	1.40	2.52	.15	.15	.015	1.09	.16	.05
4	1.26	1.26	1.68	.60	.60	.060	1.00	.14	.05
5	.63	1.89	1.68	.30	.30	.030	1.50	.22	.07
6	.42	2.10	1.68	.20	.20	.020	1.68	.24	.08
7	1.68	1.68	.84	.80	.80	.080	1.34	.19	.06
8	.84	2.52	.84	.40	.40	.040	2.02	.29	.10
9	2.10	2.10	0.00	1.00	1.00	.100	1.68	.24	.08

Double labelling in ion-uptake experiments - J. SINNAEVE, S. JAIT, S.C. VAN DE GEIJN, M.J. FRISSEL, G. VERFAILLIE, A. RINGOET.

Double labelling experiments were carried out to investigate their reliability. (ESNA report of the joint sessions of the working groups 5 and 7 (Nuclear techniques in the study of soil-plant relationships and Environmental pollution) of the annual meeting in Budapest 1972).
Tomato plants (*Lycopersicon esculentum* Mill. cv. Marette VF) were grown in controlled conditions using a diluted (1/10) Hoagland-Arnon I nutrient solution (3.02 me/l). Two elements were considered: calcium as an element already present in the plant material and cesium as an element originally

not present. Three different concentrations of the stable elements combined with different ratios of the two isotopes of the element considered were used. The stable calcium and cesium concentrations were varied between respectively 0.2 and 20 mg/l and 0.3 and 30 mg/l. For each concentration of the stable element, the two isotopes (respectively ^{45}Ca and ^{47}Ca and ^{134}Cs and ^{137}Cs) were given at different ratios ranging from 0.02 to 50 and a specific activity (μCi/mg) ranging from 0.0017 to 17 for the cesium absorption experiments and from 0.025 to 62.5 for the calcium absorption experiments. The ratios of the concentrations of the two isotopes in roots, stems and shoots were compared to the corresponding ratio in the nutrient solution.
Another experiment only considering cesium and its both isotopes, has been done to study the accumulation of the ions in different plant organs. Roots, stems and different leaf samples (cotyledonous leaves, first composed leaves, etc., and tops) were constituted. Only one stable cesium concentration (0.2 me/l) combined with four labelling ratios (ranging from 0.1 up to 10), was considered; the specific activities were 10 to 50 times higher than those in the former experiment.
The calcium samples were counted making use of the Cherenkov effect for the ^{47}Ca isotope (β-radiation of 1.98 MeV and 0.67 MeV) and normal scintillation counting for the ^{45}Ca isotope. The treshold value for Cherenkov counting is admitted to be about 0.7 MeV but unfortunately it appeared that even softer emitters (e.g. the 0.6 MeV β-radiation of the ^{47}Sc daughter of ^{47}Ca) were counted in bottles with good light scattering properties (plastic counting vessels). The interpretation of the results is hazardous because, not expecting the influence of the softer β-radiations of the scandium daughter, we did not allow sufficient time for the establishment of equilibrium between ^{47}Ca and ^{47}Sc.
The cesium samples are actually counted with a germanium-lithium crystal connected to a multichannel analyser. Peak areas are computed by polynomial fitting and background correction.
A detailed statistical analysis of the counting procedure has been done. Based on these observations, two counting times, in function of the isotopic ratio, were chosen (500 minutes for samples with a ratio equal to 0.02 and 50 (1.4% standard deviation) and 167 minutes for samples with a ratio equal to 0.1 or 10 (2.2% standard deviation). Due to the long counting times and to the number of samples (more than 300), statistical interpretation of the results is not yet finished.

Calcium absorption by isolated chloroplasts - G. DESMET, A. DE RUYTER, E. CARAFOLI, F. NOVETTI, M.F. DALGAL.

The chloroplasts are isolated as described in previous reports from spinach plants (*Spinacea oleracea* L. cv. Amsterdams Reuzeblad). However, a more complex isolation medium is used, consisting of 5×10^{-2} M TES pH 7.6, 0,35 M saccharose, 0,1 M KCl, and a variable concentration of Mg^{++}.
The influence of the Mg^{++}, present in this medium, on the Ca^{++} absorption by dark chloroplasts is investigated. The result is presented in figure 32. From this experiment, it becomes clear that Mg^{++} shows a concurrency for the Ca^{++} absorption sites of the chloroplasts.

In research at the University of Modena, efforts have been concentrated on the fractionation of chloroplasts, and on the isolation from their membrane system of protein fractions capable of binding calcium and

strontium with high affinity. Mild hypotonic shocks were applied to chloroplasts. The soluble extract obtained was concentrated on diaflo-membranes, and fractionated on preparative polyacrylamide gel electrophoresis columns. Under these conditions, it is possible to isolate from animal mitochondria an acidic glycoprotein with high affinity for calcium; no acidic protein capable of binding calcium was, however, present in the chloroplasts extracts which have been analysed so far. The absence of easily-dissociable glycoproteins capable of binding divalent cations from the chloroplasts membrane is "per se" of interest. Research is now in progress on chloroplast extracts obtained with exposure to chaotropic agents, which have been shown to extract firmly-bound proteins from other membrane systems. Among them, lithium di-jodo salycilate, which is apparently specific for extracting glycoproteins, appears to be the most interesting.

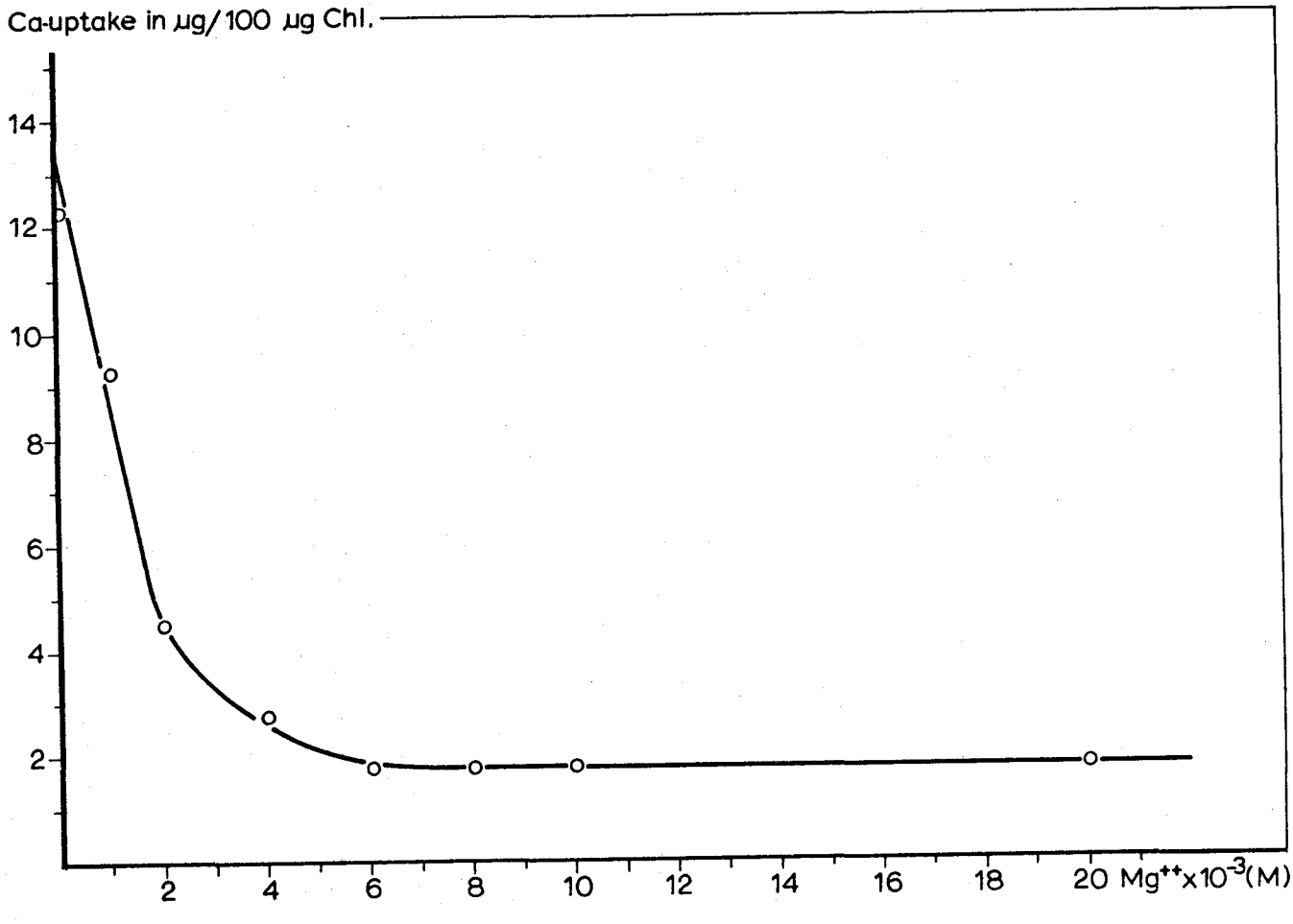


Figure 32 - Influence of Mg^{++} on the Ca^{++} absorption by chloroplasts.

BEHAVIOUR OF SPECIFIC ELEMENTS IN THE SOIL AND WATER ENVIRONMENT

Ion movement and exchange in soils - H. LAUDELOUT, R. VAN BLADEL, N.K. FAGERIA.

Most of the work in 1973 has been carried out on the modelling of ion movement through soils and on the experimental determinations of the relevant parameters to be used as input in the models. Work is being done simultaneously on several sub-models to be later incorporated in a single large model which should allow a fairly wide range of forecasting actual situations. One of the submodels which is now operating satisfactorily describes the movement of cations through the soil under saturated conditions taking account of exchange and solubility reactions. The novel feature of this submodel consists in that the full ion exchange isotherms are taken into account rather than empirical exchange relationships as done previously. This has enabled us to make full use of the fundamental data collected in this laboratory on ion exchange thermodynamics. A second submodel is now in the process of development, its purpose is the description of mineral nitrogen transformation as it moves down the soil profile. Again the relevant parameters are determined experimentally or are taken from previous work done in this laboratory on the kinetics of nitrifying bacteria. A very satisfying agreement between the predictions of the model and the observed rate of transformations of mineral nitrogen has been found in mixed cultures of nitrifiers.

Verification of predictions (mainly derived from computer simulation models) concerning ^{90}Sr and ^{137}Cs behaviour in soils of Western Europe - M.J. FRISSEL, P. POELSTRA.

Sampling has been carried out on some sites, but, due to illness and other activities of the soil group, their analysis for ^{90}Sr-content has been postponed to 1974.

Quantitative description of the behaviour of nitrogen in soils - M.J. FRISSEL, F. VAN DORP, J.A. VAN VEEN.

The project was started with an extensive investigation of the literature concerning the microbial aspects of nitrogen behaviour in soils. In consult with fellow-workers of the Institute of Soil Fertility, Haren (Gr.), it was further decided first to make an evaluation of the existing models described by Paul and Domsch (Braunschweig), Knowles et al. (Stevenage), McLaren (Berkeley), Cho (Winnipeg) and Beek & Frissel.

Influence of phosphate on nitrogen fixing algae - J.H. BECKING.

The experiments on the effect of nitrogen-fixing algae on the eutrophication of natural water were continued. The uptake and the utilization of different sources of phosphate (ortho-, meta-, and poly-phosphate) were tested with *Nostoc* sp. and *Anabaena* sp. strains in pure culture growing at the expense of molecular nitrogen from the air. It was confirmed that these blue-green algae can grow on meta- and poly-phosphate as sole source of phosphorus; the growth on poly-phosphate was better than on meta-phosphate. This indicates that blue-

green algae under natural conditions with molecular nitrogen can produce growth and proliferation on poly-phosphates released by the introduction of detergents and synthetic soaps containing phosphates as effluents in surface water. In the analyses the difficulty was encountered that no chemical method was found to distinghuish between ortho-, meta-, and poly-phosphate. Therefore, no insight could be obtained in the source of phosphate contamination occurring naturally in surface water. It is quite feasible that the amounts of phosphates released to surface water are partly ortho-phosphates from agricultural practice of phosphate (superphosphate) dressing and not due to sewage contamination. Moreover, it is possible that the blue-green algae split first the phosphate polymer in a monomer before utilization. Such a reaction can not be followed, because of the lack of a chemical method discriminating between both phosphate forms. Blue-green algae responded to extreme low phosphate levels (0.1 - 0.3 mg P per litre) in the medium. Therefore it is possible to use these algae as an accurate biological assay method for the determination of available phosphate in natural waters. In figure 33 the relation between the growth of the blue-green algae (mg N per 100 ml medium) and phosphate levels in the medium (0.1 - 1.0 mg P per litre) is given. The hyperbolic shape of the curve indicates a saturation effect at the higher phosphate levels like in an adsorption curve. Plotting the reciprocal values, i.e. 1/growth against 1/phosphate concentration, a linear relation is obtained for the lower phosphate concentrations. Also a chemical method was tried to determine inorganic phosphate in solution. The very sensitive test with sodium molybdate producing phosphate molybdate, which was subsequently complexed with a methyl green dye was used (Van Belle, Analytical Biochemistry 33, 1970: 132). This method gave less satisfactory results than the proposed bio-assay and moreover its best lower limit was about 3 mg PO_4-P per litre being about 10 times higher than the bio-assay method.

Algae occurring in the surface water of lakes (Veluwe Meer) were classified in order to get an insight in the eutrophication process of the water. In normal lake water green algae belonging to *Scenedesmus* species (Protococcales) proved to be common. In the eutrophication process these algae are replaced by blue-green algae such as *Aphanizomenon flos aquae* (L.) Rafls, *Anabaena flos aquae* (Lyngb.) Brb., other *Anabaena* species and *Nostoc* species. Very polluted water (such as near the harbour and the beach of Harderwijk) contained as dominant organism the blue-green alga *Oscillatoria agardhii* Gomont. In laboratory experiments *Oscillatoria agardhii* proved to be rather difficult to cultivate and it is likely that this species is non-nitrogen fixing. Presumably it grows saprophytically on complex organic compounds produced by the lysis of preceding algal associations.

So far not a systematic survey was made of the algal populations with regard to season, time of the day, temperature, light intensity and chemical quality of the water. Such determinations are rather time consuming and laborous. Since it was found that the Rijksdienst of the IJsselmeerpolders does routinely such determinations at a large number of sites in the IJsselmeerpolders and moreover to avoid duplication, I asked the Direction of the IJsselmeerpolders for a cooperation in research. It was suggested that the IJsselmeerpolder service would do the more ecological research as they have done so far and would provide us with

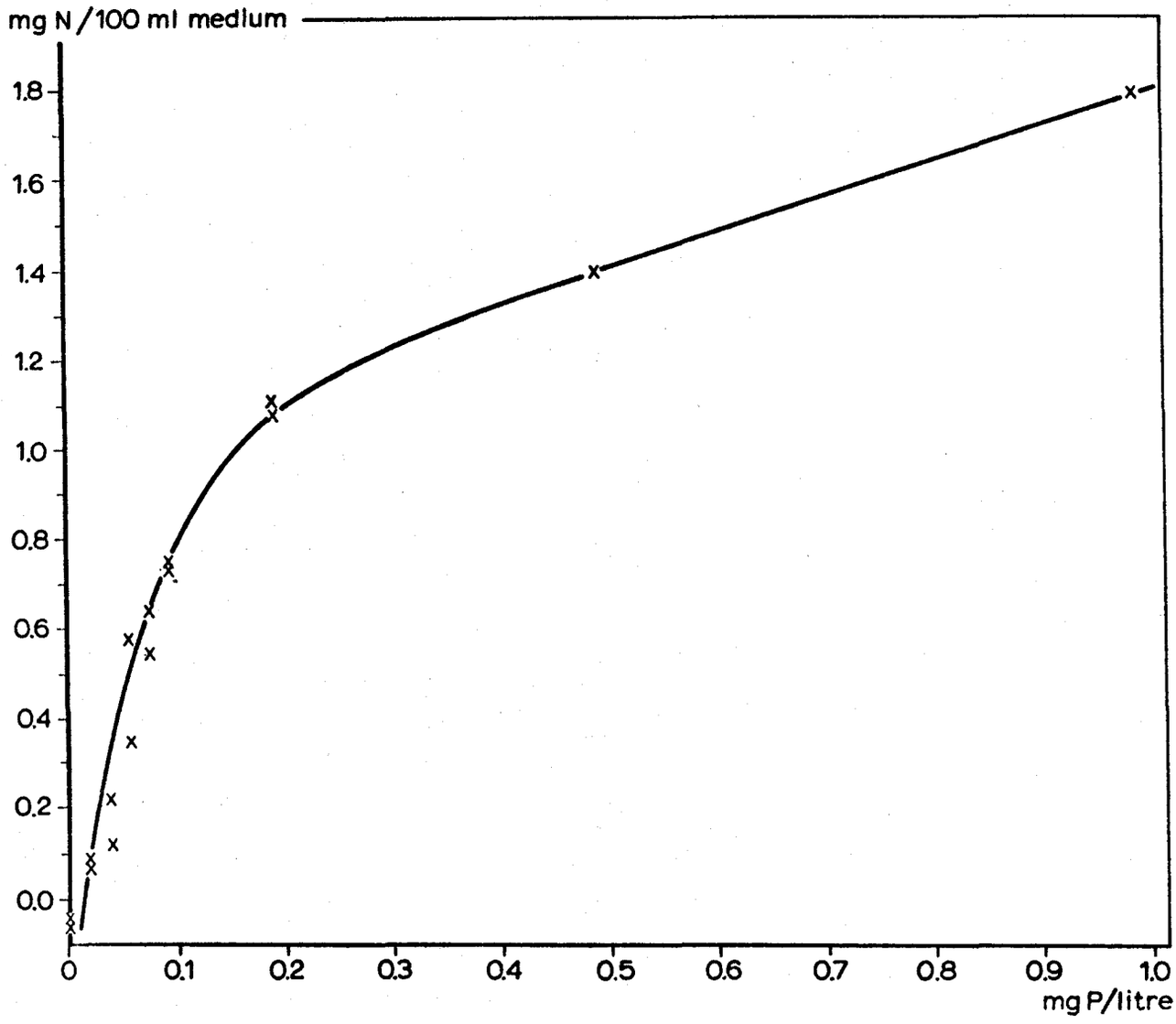


Figure 33 - Nitrogen fixation by *Nostoc* sp. (mg N per 100 ml medium) in relation to phosphate levels (mg P per litre) of the medium.
Incubation at 29°C on a rotary shaker, light intensity 10,000 lux.

the parameters (chemical quality of the water, pH, pO_2, etc.), related to a bloom of *Oscillatoria agardhii*. Our contribution would then be to do laboratory experiments with chemostats (continuous cultures) testing the parameters obtained in the field and simulating such an eutrophication process *in vitro*.

An exchange of results was suggested with Prof.Dr. D.W. Tempest and Dr. L.F. Mur (Laboratory of Microbiology, University of Amsterdam), who recently have started a project on the effect of blue-green algae on the eutrophication of natural waters. In relation to this, it was advisable to join the working group "Aquatic Ecology" of the BION, coordinating all aquatic ecological research in The Netherlands. Moreover, a cooperation was suggested with Ir. G. Berger of the Rijksdienst of IJsselmeerpolders at Lelystad with regard to the planned chemostat experiments simulating the eutrophication process of water by *Oscillatoria agardhii* in model experiments. With regard to the latter, a positive answer was recently received.

Transport of insecticides and herbicides in soils - J.P. ROLLAND, M.J. FRISSEL, P. POELSTRA.

The main processes which affect pesticide action in soil are considered to be the extent at which the pesticide is absorbed by soil, the rate at which it is lost from the soil by decomposition, the evaporation and/or leaching. The effectiveness of a biocide in controlling many undesirable effects is primarily due to its persistence, which represents the final result of interactions of all biological, physical and chemical processes occurring in soil, and its mobility in the environmental system.
Two compounds, one insecticide and one herbicide were further tested on their adsorption properties and their movement in soil columns. Trichloronate ($C_{10}\ H_{12}\ O_2\ Cl_3\ P\ S$), an insecticide, and Bam (2,6-dichloro-benzamide, $C_7\ H_{11}\ O\ Cl_2\ N$), a microbial decomposition of Dichlobenil ($C_7\ H_9\ Cl_2\ N$), an herbicide controlling perennial crops, were sprayed at a dose of respectively 6 and 4 mg on two homogeneous sand columns (diameter 12 cm; length 90 cm). A continuous artificial rain (daily 2.55 ml/cm^2) containing 0.005 N $CaCl_2$, KCl, NaCl and regulated by an automatic rain simulator, was applied at the top of the column. Every day an effluent sample was taken and analysed by Gas Liquid Chromatography, Electron Capture detector in order to detect the leaching of Trichloronate or Bam from the columns.
Every week a complete scanning of the columns was made using a two-gamma-sources (^{241}Am and ^{137}Cs)-detector. After calibration and careful determination of the mass attenuation coefficient characteristics for the two sand columns, simultaneous measurements of dry bulk density and volumetric moisture content were performed and compared with satisfying agreement to final analysis occurring at the end of each experiment.
The columns profiles were cut in layers of 1 or 2 cm at the end of the experiment and the final distribution of Trichloronate and Bam residues was determined using a common extraction method with ethylacetate. Furthermore, a mixture of soil and solution (150 g dry soil with 400 ml pesticide solution at various concentrations) was shaken during 6 hours in 700 ml flasks. At varying time-intervals, 5 ml aliquots were pipetted

and, after centrifugation (10 min at 3000 rpm) and extraction with ethylacetate, the supernatant was withdrawn for GLC analysis. Estimates for the adsorption rate constant for Bam were obtained using experimental data and an algorithm programme in order to evaluate the first order kinetic equation constants given by Fave and Eyring (J. Phys. Chem. 60, 890 (1956)); this equation also takes into account the desorption reaction.

The mobility of Trichloronate is very small in comparison with Bam. After 61 days of artificial rainfall (170 cm H_2O) 55% of the initial amount of Trichloronate (52 µg/cm^2) remained in the upper 11 cm (figure 34) of the column while no traces of the pure compound were found in the effluents (amount decomposed: 45% corresponding to a half live of 60 days).

In contrast, 68% of the sprayed Bam (35,4 µg per cm^2) was leached through the profile (see breakthrough curve figure 35) and the residues were uniformely adsorbed along the column (12.10^{-2} µg per cm^3 bulk soil); no decomposition occurred.

These experiments were conducted at approx. 40% of the maximum water saturation (figures 36 and 37) and, according to the moisture content determined during the experiment by the two-gamma-sources-detector and the final measurement, a constant water flow was attained; unfortunately, the accuracy of the measurements by this non-destructive method is, for the time being, unacceptable for heterogeneous profiles.

Several computation models were worked out, which considered successively convection, diffusion and hydrodynamic effects.

Analytical solutions (plate model of Glueckauf, chromatographic equation of Lapidus and Amundson and other solutions of the Focken Planck equation with appropriate boundary conditions) are compared with corresponding numerical solutions computed by CSMP programmes. In the numerical solutions the spreading of the concentration over the soil profile is overestimated. This phenomenon, called the artificial spreading, is caused by the use of finite intervals in space and time instead of infinitesimal intervals. A few procedures are tested to suppress this artificial spreading and are applied in more elaborate models.

A first mathematical model taking into account an instantaneous equilibrium between solid and liquid phase (linear adsorption isotherm) was compared with a model considering first order kinetics for adsorption and desorption. The adsorption isotherm was again considered as linear, but the desorption reaction is only partly reversible. In both cases convection and diffusion processes (in liquid and gas phase) were involved, also diffusion from the soil surface was taken into account. Attention was paid to the apparent diffusion coefficient and suppression of artificial spreading effects. Furthermore decomposition of the pesticide both in solid and liquid phase was included. The instantaneous equilibrium model was applied to the leaching of Trichloronate in a sandy soil column. Although some important parameters, as the rate factor for the decomposition (2.10^{-3} fractions per day) and the distribution ratio between solid and liquid phase (20 µg per g soil/ µg pest. per cm^3 soil solution) were determined independently, the agreement between experimental and calculated values is moderate (see figure 34). The application of the non instantaneous model will be carried out as soon as the required parameters (adsorption and desorption rates) are measured.

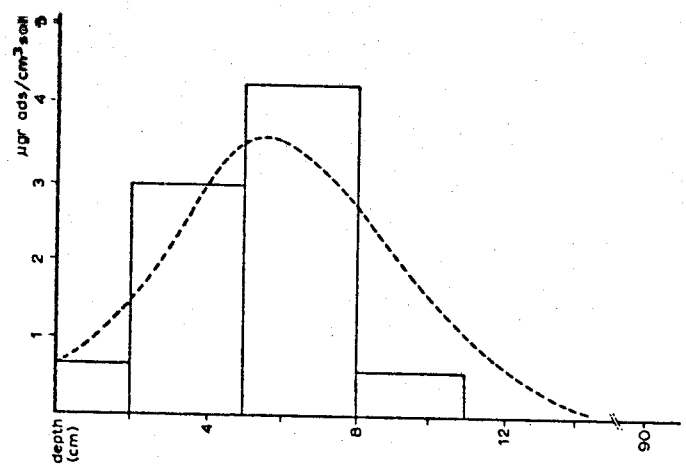


Figure 34 - Trichloronate adsorbed in relation to depth after 61 days of artificial rain and application of 50 µg.cm^{-2} (column 90 cm length).

———— experimental result

-------- theoretical curve (instantaneous equilibrium and decomposition coefficient of 2.10^{-3}).

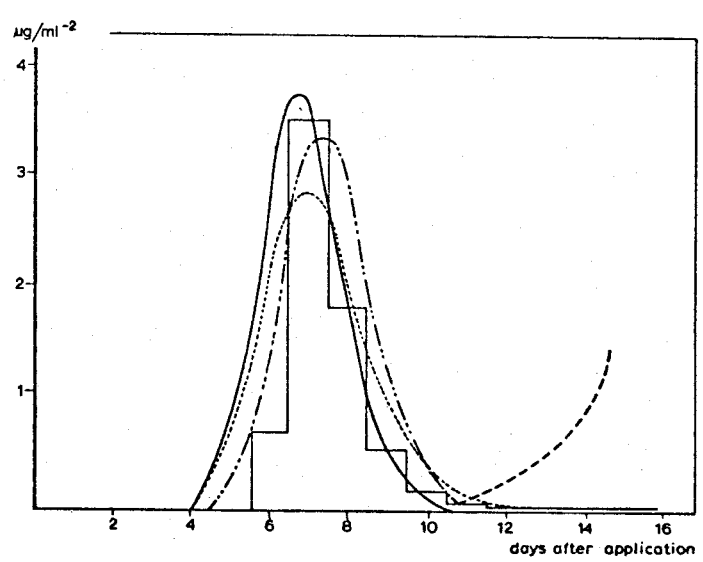


Figure 35 - Breakthrough curve of Bam in function of time after spray (35.4 µg.cm^{-2}) application.

———— experimental result (disturbed sand column)

..... theoretical curve (non-instantaneous equilibrium simulation model). KSW (distribution ratio between solid and liquid phase) = 0.03, ADSRT (adsorption rate) = 5.6, RELRT (desorption rate) = 1.

-.-.-.-. theoretical curve (instantaneous equilibrium s.m.) KSW = 0.23.

-------- theoretical curve (inst. equil. s.m.) KSW = 0.02

-..-..-..- theoretical curve (inst. equil. s.m.) KSW = 0.03

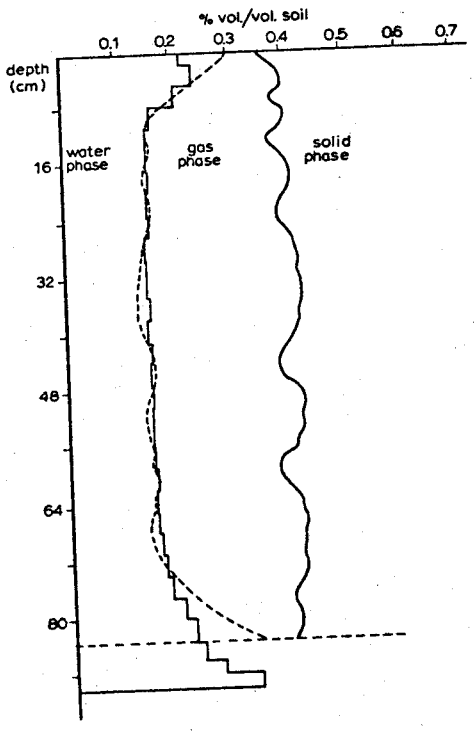


Figure 36

Physical description of the sand profile used for Bam leaching experiment.

——— moisture content: analysis determination

-------- moisture content: two-gamma-sources detector.

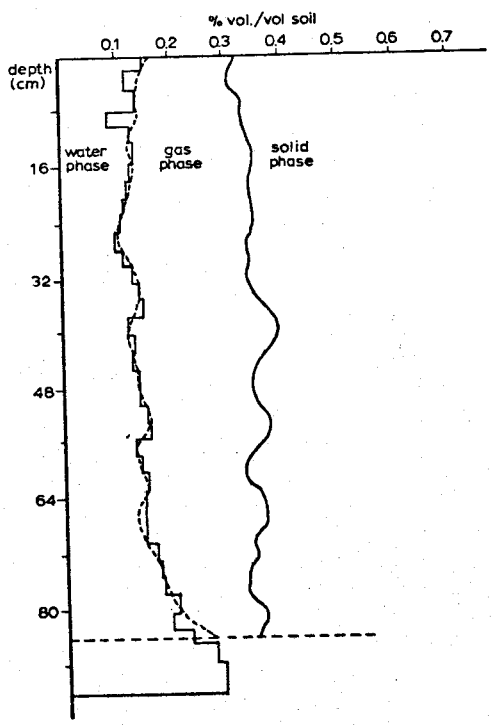


Figure 37

Physical description of the sand profile used for Trichloronate leaching experiment

——— moisture content: analysis determination

-------- moisture content: two-gamma-sources detector.

The just mentioned parameters were already determined for Bam: adsorption rate constant (5,6 fractions per day), desorption rate constant (1 fraction per day), distribution ratio between solid and liquid phase (0.23 µg pest. per g soil/µg pest. per cm^3 soil solution), decomposition rate (negligeable for the duration of the experiment). From the leaching experiment it appeared, however, that irreversible adsorption plays a significant role (approx. 0,12 mg per cm^3 bulk soil remained absorbed under the experimental conditions). This irreversibility invalidates the determined distribution ratio of 0.23 $cm^3\ g^{-1}$. This is clearly demonstrated in figure 35. Application of a distribution ratio of 0.23 $cm^3\ g^{-1}$ in the equilibrium model results in a breakthrough curve which is delayed with respect to the experimental one. The curves for the, at this moment arbitratily chosen, values 0.02 and 0.03 $cm^3\ g^{-1}$ show better agreement. (Multiplication of the distribution ratio (0.025 µg pest. per g soil/µg pest. per cm^3 soil solution) with the bulk density (1.7 g soil per cm^3 bulk soil) and dividing by the water content (0.2 cm^3 soil solution per cm^3 bulk soil) gives an R_F of 1/(1+0.025 x 1.7 : 0.2) = 0.82). The equilibrium model does not consider the irreversible adsorption, the amount remaining in the column is neglected, which makes the agreement less good than the curves suggest. The result of the application of the non-instantaneous equilibrium model is therefore much better, it takes logically into account the irreversibly adsorbed amount and shows the tailing effect which is so typical for many pesticide leaching experiments. The value for the distribution ratio (0.03 $cm^3\ g^{-1}$) is the only one adapted to fit the experiments. An independent determination of the distribution ratio is not possible for such systems.

HEAVY METALS IN PLANTS AND SOILS

As part of a European collaboration programme e.g. with the Biology Division at Ispra, the information on the behaviour of heavy metals in plants and soils has been grouped in this separate chapter.

Behaviour of mercury and mercury compounds in soils - M.J. FRISSEL, P. POELSTRA.

The mercury monitoring programme, in 1972 restricted to the Netherlands, has been extended to soils situated in Germany, France and Italy. For location of the sites see figure 38; for description of the soils as well as for their mercury content see table 16; mercury profiles are given in figure 39.
The soils sampled may be divided into 3 groups:

- In the first group (1 to 10 are reference soils) no mercury has been introduced into these soils. No profile disturbances occurred during the last 20 years.
  The mercury content in the top 20 cm layer of these soils ranged from 0.02 ppm up to 0.10 ppm, and averaged at 0.07 ppm. The distribution over the profiles reveals that the mercury content diminishes with depth rather quickly and at the below 20 cm level it is never more than 0.04 ppm. The "distribution over Europe" suggests that the mercury content is higher in regions with high industrial activity e.g. sampling sites nos. 1, 2 and 3 in the Netherlands and no. 10 in Italy. So the figures suggest the existence of mercury fallout originating from man's activities.
- The second group consists of 2 soils from the bulb-growing area in Holland (11 and 12). The application of mercury as a fungicide in these areas has been common practice for 50 years. The soils had a mercury content of approx. 0.15 ppm in the top 20 cm layer and a distribution pattern over the profile different from the one in the reference soils (figure 39). It is obvious that a limited mercury accumulation does occur in the treated soils.
- The third group are foreland soils from the heavy polluted Rhine river. Such soils are frequently flooded, usually in winter. They showed mercury contents of over 10 ppm, which obviously stem from the Rhine and are strongly adsorbed by clay.

In cooperation with the waterworks company of the city of Amsterdam, soil sampling has been done up to a depth of 150 cm, from infiltration canals in dunes where Rhine water is purified to drinking water. The top soil of the infiltration canals showed a mercury content of 0.16 ppm and 0.33 ppm, respectively, in the 0 and 20 cm layer, whereas in non irrigated dune a content of 0.03 ppm was found. The sludge from these sites show a mercury content of up to 1 ppm.

Migration experiments have been carried out, under controlled conditions, with γ-emitting mercury compounds in columns with undisturbed soil profiles. These columns, 100 cm long with an inner diameter of 12 cm, are provided with rain heads and automatic control units for maintaining a proper supply of the influent in small droplets with fluxes to be varied from 0.6 mm to 180 mm per day.

The top 5-cm layer of the soil is homogeneously labelled with ^{203}Hg either within mercury chloride or monomethyl mercury chloride or metallic mercury.
A γ-scanner records the distribution pattern of the compound. Leaching was performed with a flux of 24 mm per 24 hours with an artificial soil solution (0.003 N CaI_2, 0.001 N KCl, and 0.001 N NaCl). Air was sucked continuously over the top of each column to remove and to trap volatile compounds which might escape from the soil. Four columns were used for each mercury compound, two of them were leached continuously, the other two periodically for 16 hours a week, keeping the net waterflux equal in all columns. The temperature was kept at 20°C. From the monthly scanning results it can be concluded that migration of metallic mercury and $HgCl_2$ does not occur; CH_3HgCl is initially somewhat leached downwards; thereafter migration was negligible (figure 40).
Evaporation was slow for all the compounds, whether they were leached continuously or periodically. Within the frame of a joint project with the Forschungsanstalt für Landwirtschaft at Braunschweig, Germany, mercury migration studies have also been carried out on soil columns, taken from the sewage fields at Braunschweig. The leaching solution containing 0.0244 M (Ca, Na, K) Cl (ratio of 3:1:1), was applied with a flux of 24 mm per 24 hours for a period of 3 months. ^{203}Hg labelled $HgCl_2$ is mixed with the top layer of the soil in the column before starting the experiment. When the whole system is kept under anaerobic conditions no movement of mercury is observed.
The column experiments and the field measurements indicate a strong adsorption of mercury and its compounds in the top layer of the soil. Leaching is hardly involved. Evaporation does occur to some extent, but its rate is very low.

Applying a modified computer simulation programme for the behaviour of mercury in the ecosystem (from Anderson and Anderson (1972)), it could be calculated that the amount of mercury present in the reference soils could not be explained by global mercury fallout only.

A regional multiplication factor had to be introduced. For Bari this factor amounted to approx. 1, for Ahrweiler and Amiens approx. 5 and for the soils near Alkmaar, Hilversum and Amersfoort as well as for the soils near Hannover and Ispra approx. 10. This indicates that the contribution of man-made mercury is higher in regions with high population density and high industrial activity.
So the high mercury levels in soils can probably be explained by accepting local fallout due to man's activities (See publications symposium Helsinki, 1972).

The methylation of mercury compounds or their conversion into volatile products has been considered via a thoroughly executed literature search. This literature search, recorded in a report (W. Tap, Methylation of mercury) together with results from experiments made by M. van de Steene, has shown that methylation of mercury compounds or their conversion into volatile compounds is of little significance for soil systems, that the reverse reaction-demethylation-further diminishes its effects and that conversion rates are not yet quantitatively known.

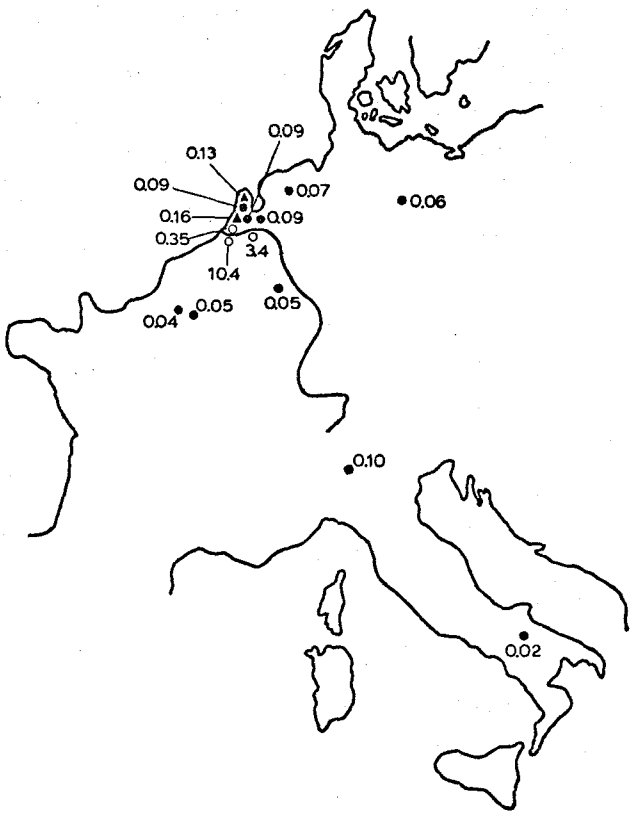


Figure 38

Location of sampling site. Data denote mercury content in 0-20 cm layer of soils in ppm (● pastures; ▲ soils, bulb-growing area; o foreland soils, River Rhine).

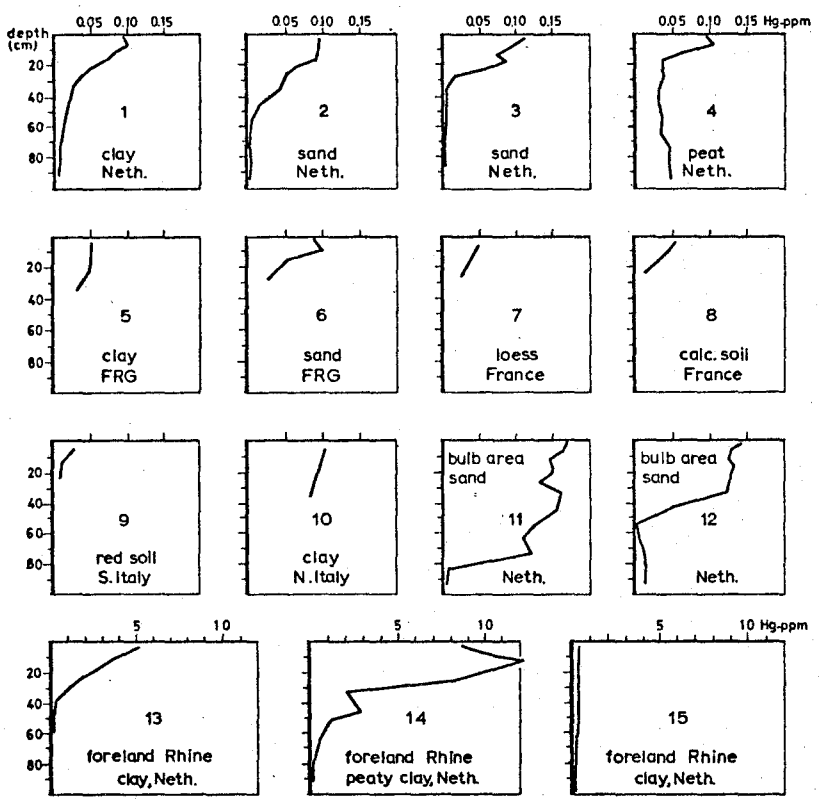


Figure 39 - Mercury distribution as function of depth (soil numbers correspond to table 16).

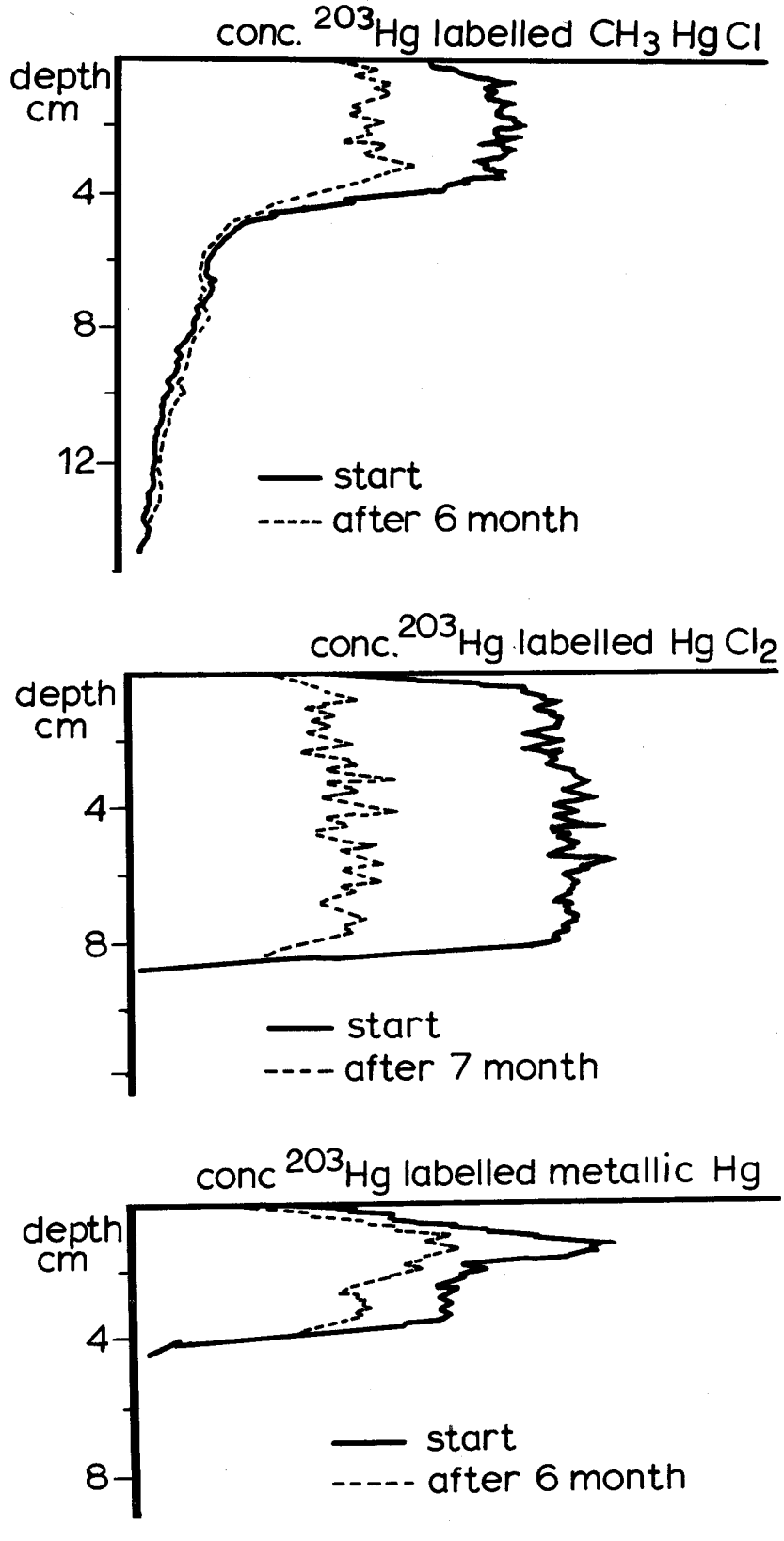


Figure 40 - Result of leaching experiments in a column with ^{203}Hg-labelled CH_3HgCl, $HgCl_2$ and metallic Hg respectively.

Table 16 - Description of soils and mercury contents.

Nr.	Soil			Mean Hg content ppm	
	location	identification		0-20 cm	20-100 cm
1	Alkmaar, Neth.	alluvial gley	(clay)	0.09	0.02
2	Hilversum, Neth.	podsol	(sand)	0.09	0.01
3	Amersfoort, Neth.	podsol	(sand)	0.09	0.01
4	Schoonebeek, Neth.	fen	(peat)	0.07	0.04
5	Ahrweiler, FRG.	pseudo gley	(clay)	0.05	-
6	Hannover, FRG.	podsol	(sand)	0.06	-
7	Amiens, France	rendzina	(calc.soil)	0.04	-
8	Amiens, France	Para brown earth	(loess)	0.05	-
9	Bari, Italy	terra fusca	(red soil)	0.02	-
10	Ispra, Italy	pseudo gley	(clay)	0.10	-
11	Hillegom, Neth.	dune	(sand)	0.16	0.10
12	Den Helder, Neth.	reclaimed sea soil	(sand)	0.13	0.05
13	Valburg, Neth.	alluvial	(clay)	3.4	0.45
14	Biesbosch, Neth.	alluvial	(peaty clay)	10.4	2.6
15	Dordrecht, Neth.	alluvial	(clay)	0.35	0.26

Transport and accumulation of chromium in soils - M.J. FRISSEL, P. POELSTRA.

In cooperation with the FAL, also chromium migration studies were set up in undisturbed soil columns taken from the sewage field. These migration studies are carried out, respectively under aerobic and anaerobic conditions, with a waterflux of 24 mm per 24 hours, using a solution of 0.0244 M (Ca, Na, K)Cl in a ratio of 3:1:1 with 1 ppm Cr of the Cr-compound studied. The radioactive Cr-compound (^{51}Cr) is mixed to the top layer of the soil at the start of the experiment. Cr-compounds investigated are $CrO4^{2-}$ at pH 8 under aerobic conditions and Cr^{3+} at pH 5 under anaerobic conditions. The redox potential is measured in the centre of the column at a depth of 15 cm. From figure 41 can be concluded that movement of the chromium compounds do not occur under aerobic or under anaerobic conditions. The redox potential gradually changes, in the aerobic system, from -40 mV to approx. -160 mV, and remained rather constant afterwards.

A chromium monitoring programme comparable to the mercury-programme has been set up on the same soils. In figure 42 the chromium distribution over the profile is shown as a function of depth in two foreland soils from the river Rhine (Valburg and Biesbos) and they are compared to an Alkmaar soil (permanent pasture for over 20 years) as a reference. In the reference, containing 50 ppm Cr in the top soil, some chromium may be introduced by fertilizer dressing (phosphates, which may contain Cr up to 500 ppm), which is gradually distributed over the top soil by biological mixing. The foreland soils, however, show a much higher Cr content: Valburg, 150 ppm in the top soil and the Biesbos up to 450 ppm. The difference in chromium content in the 3 sub soils is probably caused by differences in chromium content of the parent material. The distribution pattern, over the profiles of the sampling sites, suggests that migration of chromium in soils does not occur.

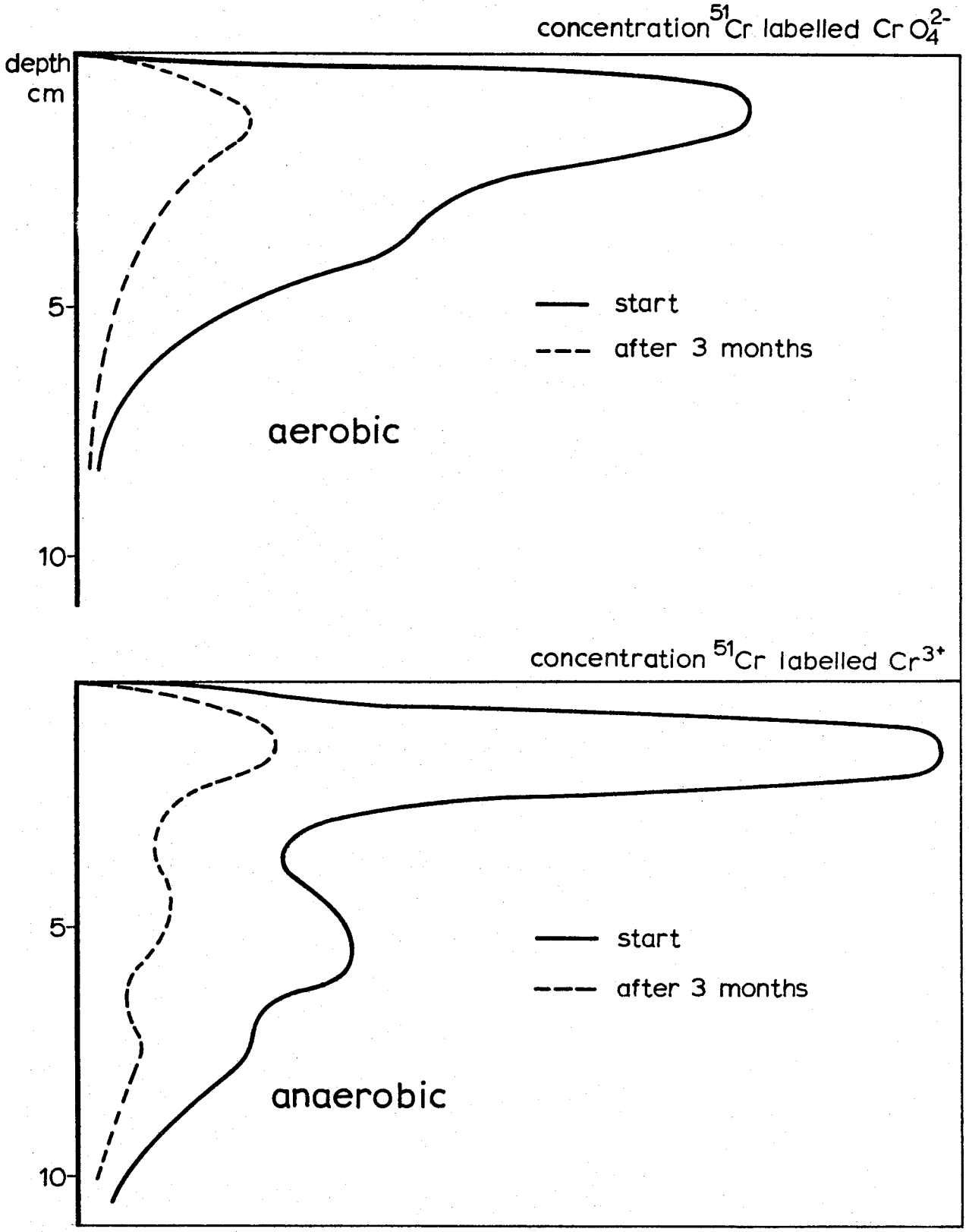


Figure 41 - Results of leaching experiments in a column with ^{51}Cr labelled K_2CrO_4 and $CrCl_3$ resp. Reduction in concentration after the experimental period is due to the fast decay of ^{51}Cr ($t_{\frac{1}{2}}$ = 27.8 days)

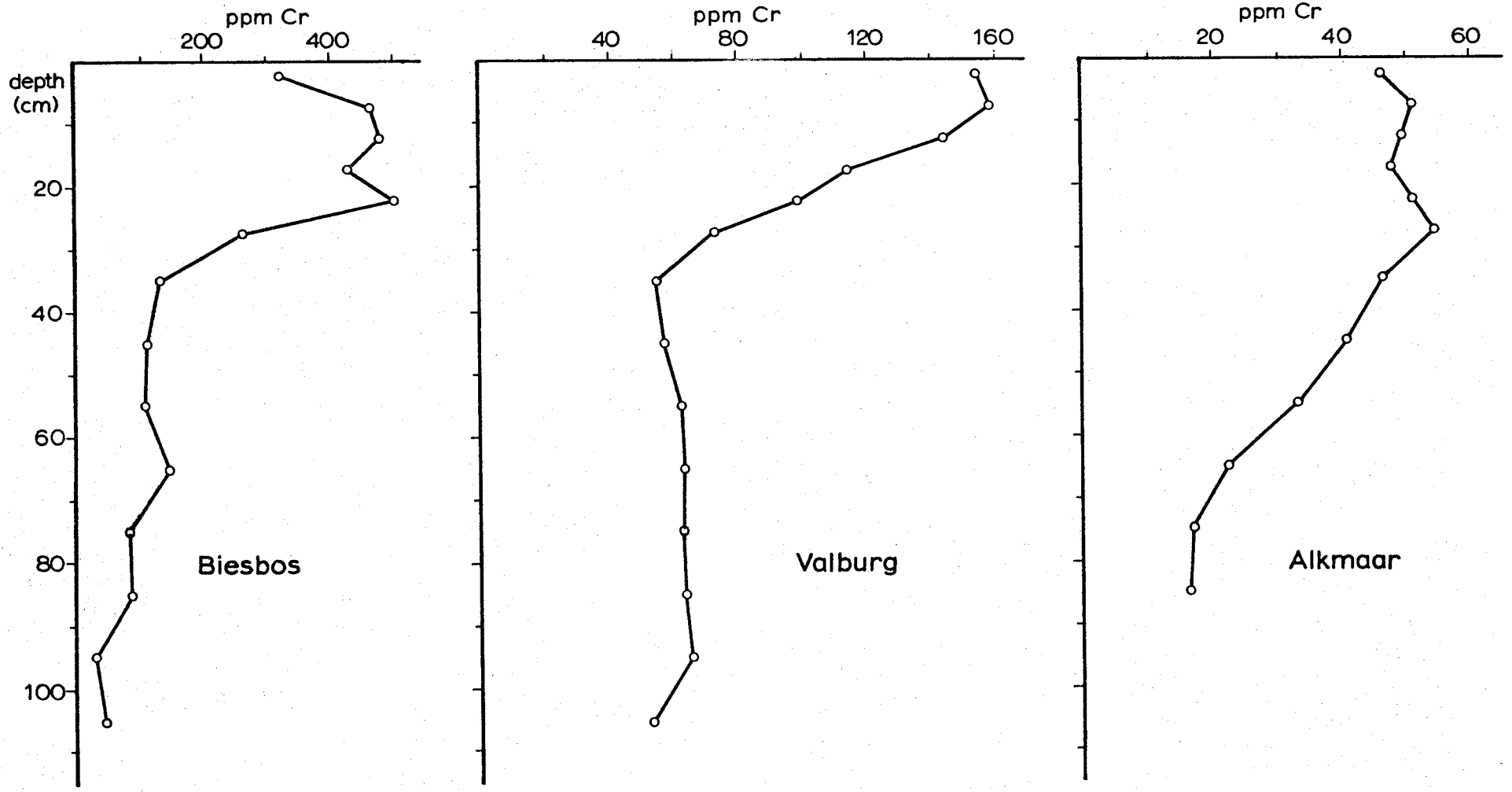


Figure 42 - Chromium distribution as a function of depth.

Transport and accumulation of zinc in soils - M.J. FRISSEL, P. POELSTRA.

Within the frame of a collaboration with the Forschungsanstalt für Landwirtschaft (FAL) at Braunschweig, zinc migration was studied in undisturbed soil columns from the Braunschweig sewage fields. These migration studies were carried out under aerobic conditions with 1 ppm Zn in the leaching solution. Undisturbed soil columns were also taken from an untreated field close to the treated one. ^{65}Zn labelled $ZnCl_2$ was added on the top of each column; the migration was followed by the soil column scanner. Zinc moves approx. 1.4 times faster in the sewage treated field than in the untreated field (figure 43).
A preliminary simulation model for the behaviour of zinc under the operating conditions was developed by two students of the Agricultural University at Wageningen (J.M.C. Dirven and K. van Staveren, Simulation of zinc migration in a sandy soil). Experimental data, like adsorption isotherms at different depths of the columns, the content of zinc as a function of depth etc., were used in the model.
A comparison between the results of the experiments and the calculations showed that the movement of zinc in the treated soil was about twice as fast as predicted by the simulation model. More research is needed to explain this difference.

Kinetics of chromium uptake by intact rice-plants (*Oryza sativa* L. cv. Arborio) - G. VERFAILLIE.

Started in 1972 with the kinetics of chromium uptake by intact rice plants (*Oryza sativa* L. cv. Arborio) from nutrient solutions containing respectively K_2CrO_4 and Cr^{III}-EDTA, the study has been continued in 1973 with the kinetics of chromium uptake from solutions contaminated with Cr^{+++} ions. Using the same methods as before, the range of Cr^{+++} concentrations has been extended from 10^{-7}M Cr^{+++} up to 10^{-4}M Cr^{+++}.

The results obtained in this study are:

- The uptake of chromium from a chromic solution occurs in 3 successive steps as it can be seen in figure 44 and figure 45.
  - A short phase of physical absorption at high velocity lasts for about 6 minutes.
  - A phase of chemical absorption on preexisting fixation sites or of chemical reaction with a pool of metabolites has a velocity decreasing parabolically during 8 to 10 hours (2d order interaction).
  - The last phase is continuous and corresponds to a real metabolic uptake proceeding at a low and slowly decreasing rate (1st order).

    This phase requires the continuous creation of new fixation sites and the continuous delivery of suitable metabolites by the leaves. Indeed, this 3d phase disappears when the aerial parts are removed, whereas the other phases do not, and even might be simulated by the exchange of new $^{51}Cr^{+++}$ injected without notable carrier amount (figure 46).
- The metabolic phase shows 2 isotherms both corresponding to saturation kinetics. Expressing the results with hyperbolic functions

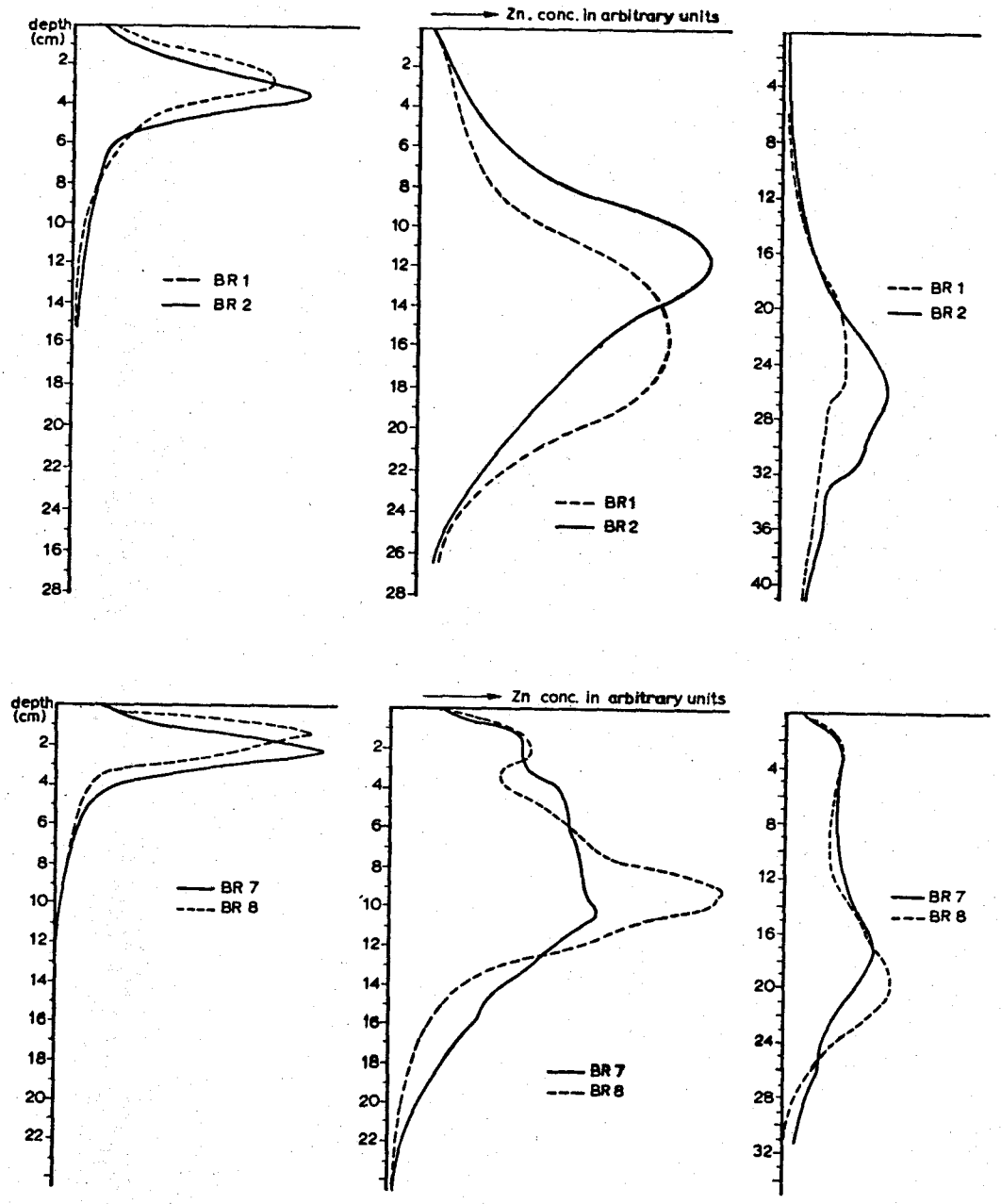


Figure 43 - Results of leaching experiments in columns with ^{65}Zn labelled $ZnCl_2$ in a sewage treated field (columns Br 1 and Br 2) and in an untreated field (columns Br 7 and Br 8). The results are recorded 10, 77 and 151 days resp. after the beginning of the experiment.

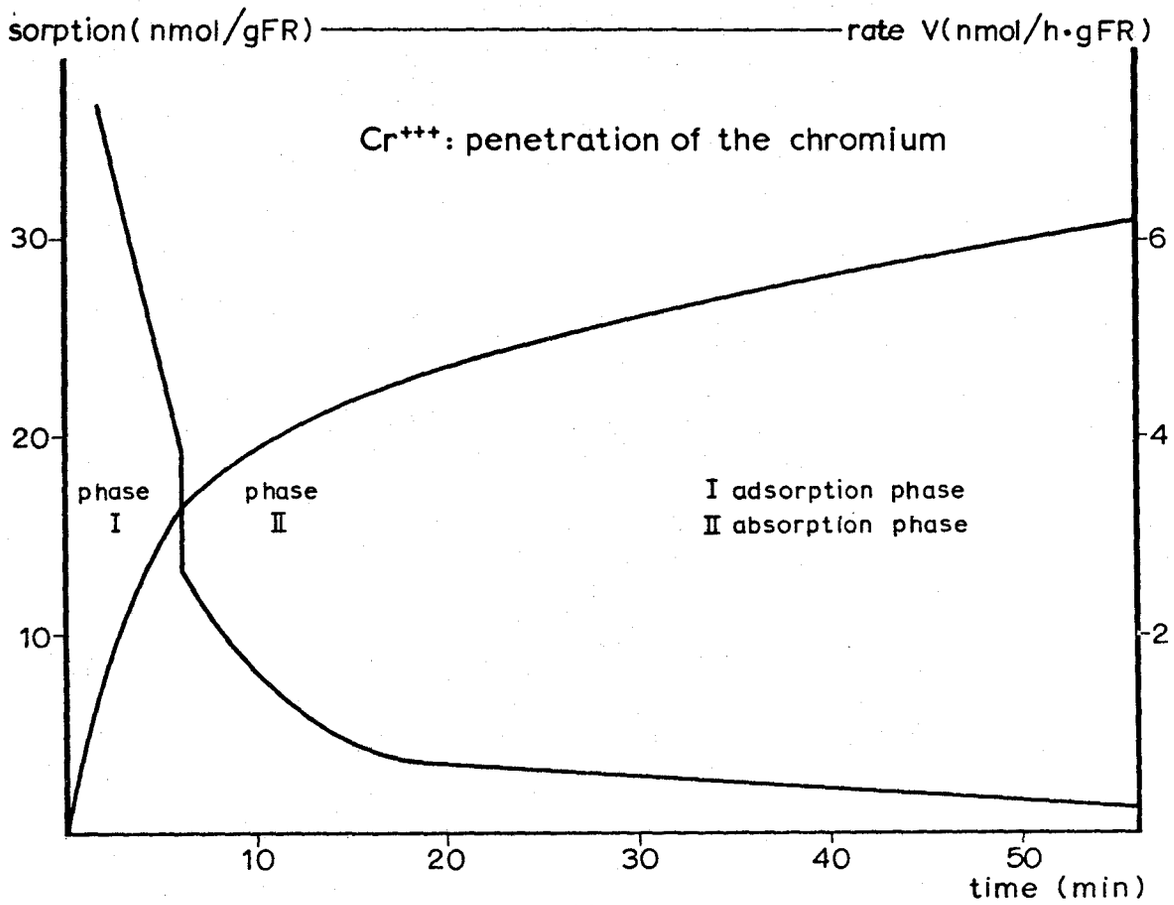


Figure 44 - The sorption of the Cr^{+++} ions : phase 1 and phase 2.

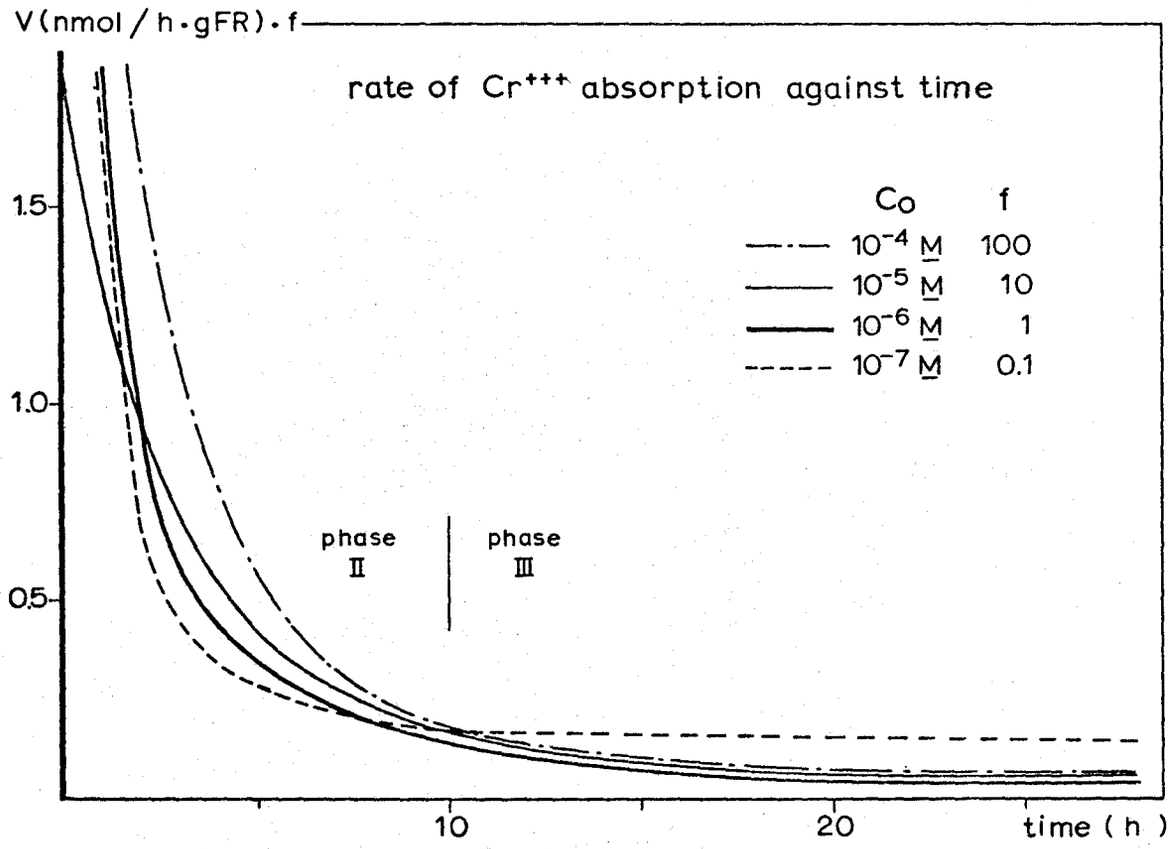


Figure 45 - The sorption of the Cr^{+++} ions : phase 2 and phase 3.

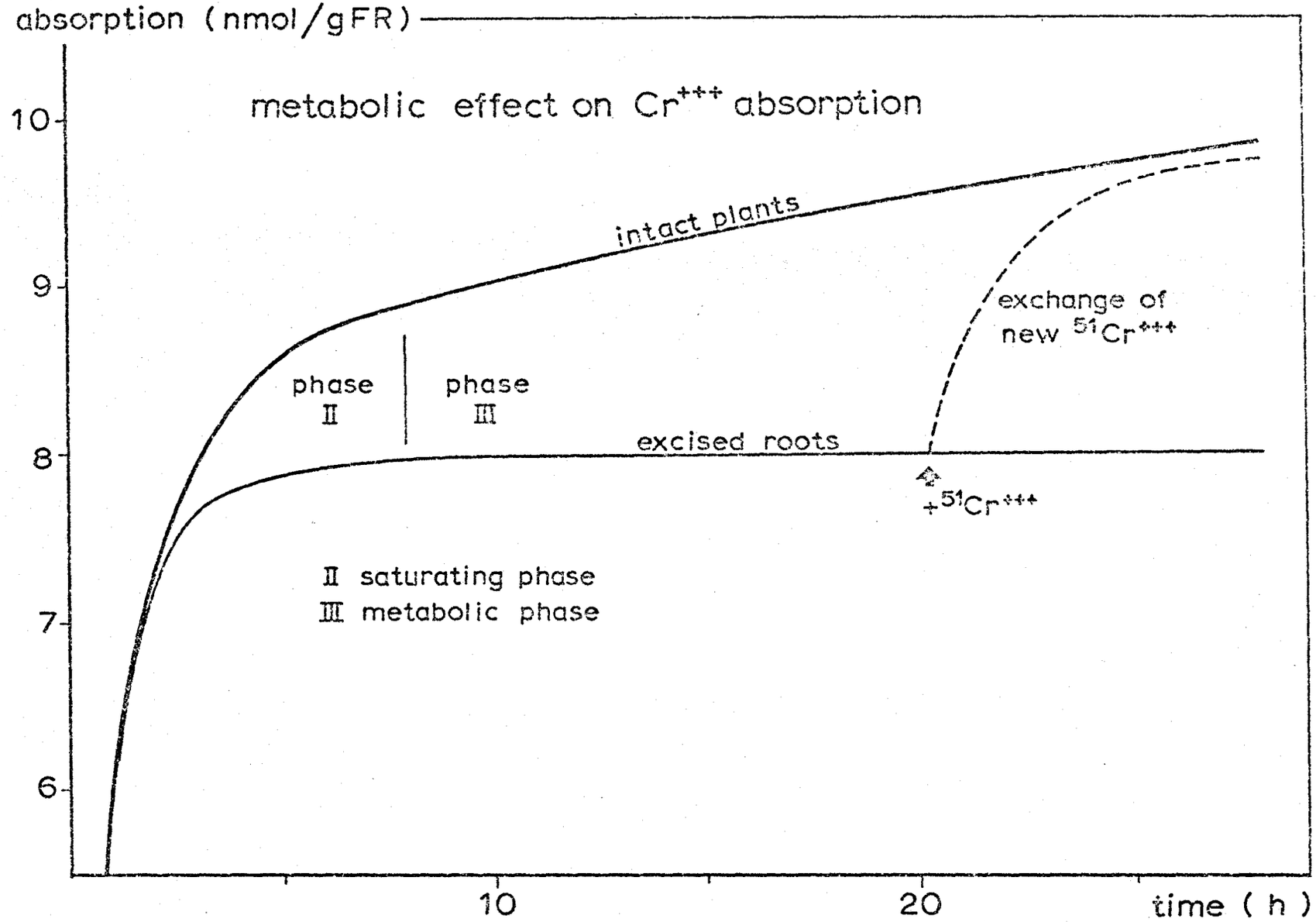


Figure 46 - The metabolic effect on the Cr^{+++} absorption.

similar to those used by Michaelis (figure 47) and Hofstee (figure 48), the following kinetic parameters have been calculated, the threshold concentration being T = 1.72×10^{-5} M Cr^{+++}.

Parameters	Below threshold	Above threshold
V_m	0.52 nMol/h.gFR*	3.4 nMol/h.gFR
K_m	5.14×10^{-6} M	2.65×10^{-5} M
$F_m = (V/C)_m$	0.1 ml/h.gFR	0.14 ml/h.gFR

- The distribution of the chromium after absorption is identical to that found for the chromate form, the largest part remaining fixed on and in the roots (95 to 98 percent).
- No physiological effect on the plants could be observed. It must, however, be emphasized that the threshold concentrations found by us for both chemical forms correspond exactly to the toxicity levels found by our colleague C. Myttenaere at Ispra, Italy for long term contamination by chromium compounds.
- From a synthesis of all results in 1972 and 1973, it can be concluded that, as far as the aerial parts of the plants are concerned in the food chain, the plant mineral nutrition cannot be considered as a vector of the pollution by chromium, whatever its chemical form, either because of the extremely low rate of absorption by the roots (Cr^{III}-EDTA) or because of the very small translocation of the element to the aerial parts (Cr^{+++} and $HCrO_4^-$). Compared kinetic functions are also given in table 17.

Full details of the techniques, of the methods and of the results will be found in the proceedings of the symposium held in Otaniemi, Finland, (see publication list).

Table 17 - Kinetic functions compared for chromium and phosphate. (W = transpiration rate)

Elements	Cr (maximum values)			P (10^{-3} M)
functions/units	Cr^{III}-EDTA	K_2CrO_4	$Cr(NO_3)_3$	KH_2PO_4
V nMol/h.gFR	0.35	34.2	4.0	440
F = V/C ml/h.gFR	0.018	4.8	0.1	0.44
D = F/W unities	0.082	8.2	0.5	1.2

Uptake and transport of cadmium in tomato-plants - C. PETIT, G. VERFAILLIE

The results which are available for the moment were obtained for cadmium in the tomato plant *Lycopersicon esculentum* (Mill), cv. Marette. Attention was mainly paid to the accumulation in plants grown in a polluted substratum and to the movement of cadmium inside the stem.

Growth in a cadmium-polluted substratum

Ten days old seedlings are put on Hoagland solution which contains, expressed in mM: 6K, 5 Ca, 2 Mg, 15 No_3, 2.01 SO_4, 1 H_2PO_4, 0.0091 Mn,

*gFR = gram fresh root.

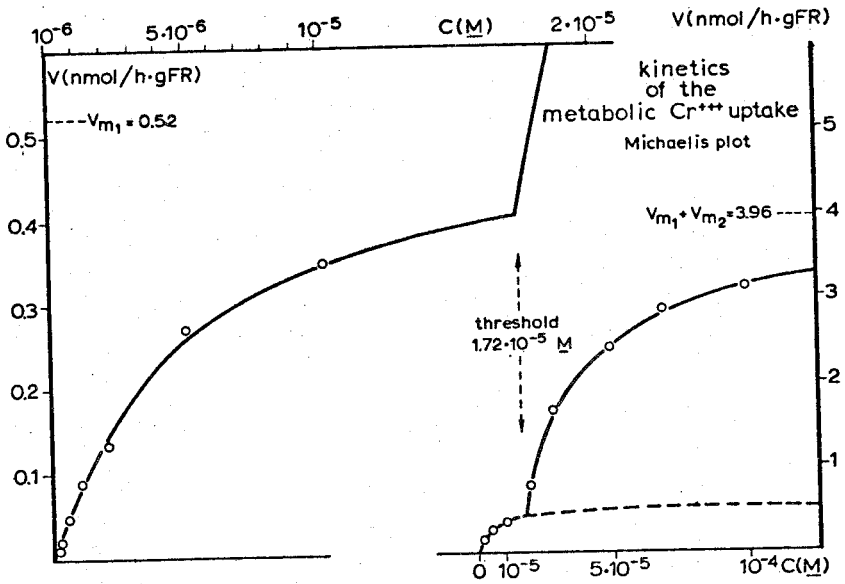


Figure 47 - Kinetics of the metabolic Cr^{+++} uptake: Michaelis plot.

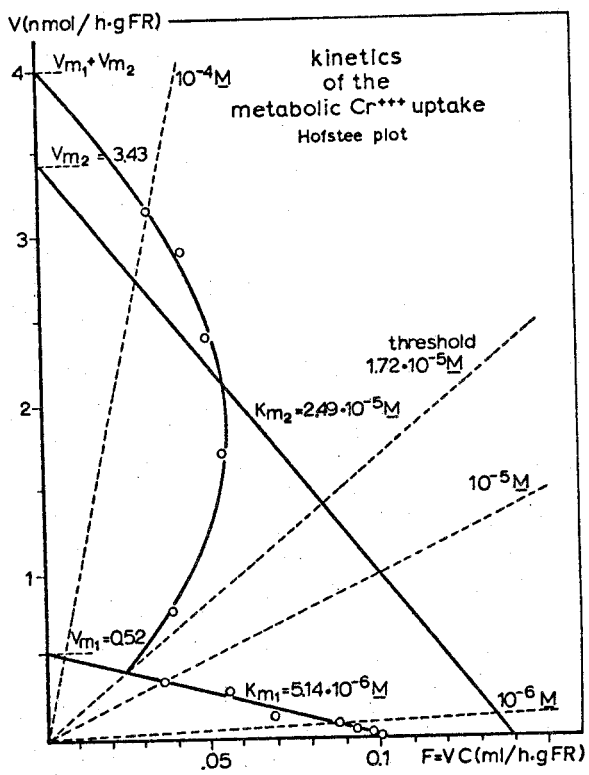


Figure 48 - Kinetics of the metabolic Cr^{+++} uptake: Hofstee plot.

0.00076 Zn, 0.00031 Cu, 0.082 Fe(EDTA), 0.082 Na. To this solution, weekly replaced, Cd^{++} was added at different concentrations, in the following range: 0., 0.00324, 0.01621, 0.03241, 0.08104 mM. The plants are harvested after 6 weeks, dried, weighed and the cadmium content is determined.

Both decreasing yield and symptoms of toxicity begin to appear at 16,21 µM: yellowing of the leaves, longer internodes, delayed appearance or absence of flowers and lateral buds.
At the highest concentration, growth is almost nihil.
Cadmium mainly accumulates in the roots while the transport index-

$$\frac{\text{Cd aerial part}}{\text{Cd total}}$$

decreases with increasing pollution (figure 49).

The pH variations in the various solutions were measured during the last week of the experiment. The plants alcalinise their substrate at a smaller rate at higher cadmium concentrations.

The EDTA, used in the nutrient solutions in order to maintain a sufficient level of iron, also results in the chelation of all minor-elements. Being interested in the divalent ionic form, Cd^{++}, we have three possibilities to avoid at the same time, deficiency in iron and cadmium complexation.

- Iron is supplied by foliar aspersion. In this respect, a test has been conducted, but without success.
- Iron can be maintained in the nutrient solution by using EDDHA-ethylene diamine di(o-hydroxyphenil acetic acid) - which chelates iron more specifically than EDTA. The knowledge of the formation constants of the various metallic EDDHA chelates (H, Fe, Ca, Mg, Cu, Zn, Cd) allows the determination of the percentage of chelated cadmium in equilibrium with a solid phase of ferric-hydroxide. From a theoretical study it results that the complexation of cadmium is still relatively too sensitive to the variation of EDDHA concentration.
  This variation depends upon the initial Zn and Cu level which is itself modified in the process of uptake by plants. However, by continuously changing the solution all variation can be eliminated.
- Iron is still present in the nutrient solution when no chelating agent is used, because it is in equilibrium with the solid phase of $Fe(OH)_3$. By maintaining a low pH (so as to obtain a maximum concentration of Fe^{+++}) and by constant replacing of the solution, one may hope to avoid the deficiency of iron and to keep the concentration of all elements at a constant level.
  The pKs of $Fe(OH)_3$ being 37, the concentration of Fe^{+++} is equal to 10^{-10}M at pH 5. As appears from figure 50 A and B, no significant coprecipitation with $Fe(OH)_3$ occurs in a Hoagland solution containing $5.\ 10^{-7}$M $Cd(NO_3)_2$ as well as 10.10^{-6}M $Fe(NO_3)_3$ at pH 5.

Movement of cadmium in the stem - C. PETIT, S.C. VAN DE GEIJN.

Using a semiconductor detector assembly (see previous report), it has been possible to observe the accumulation and the redistribution of cadmium in the stem, when it is traced with ^{115m}Cd (E_{max} = 1,63 MeV). Cd can be detected in the cotyledons of a three weeks old plant, 30 min

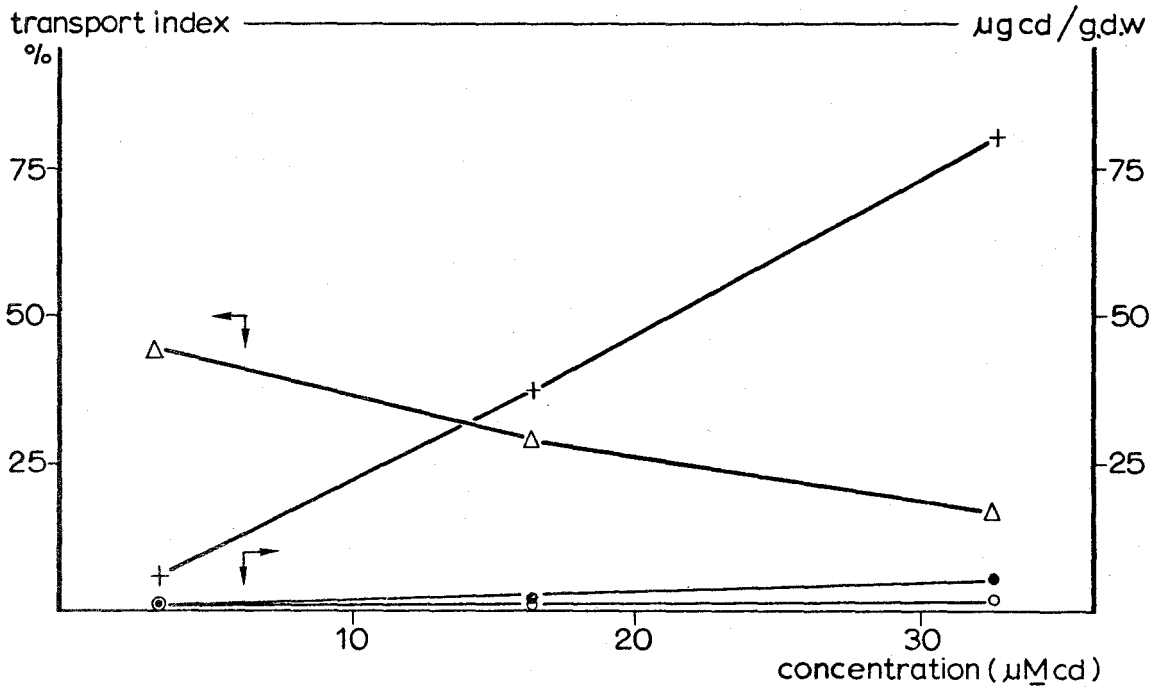


Figure 49 - Transport index of cadmium (Δ) and cadmium content of the roots (+), of the leaves (●), of the stem (o), in relation to various cadmium concentrations in the nutrient solution.

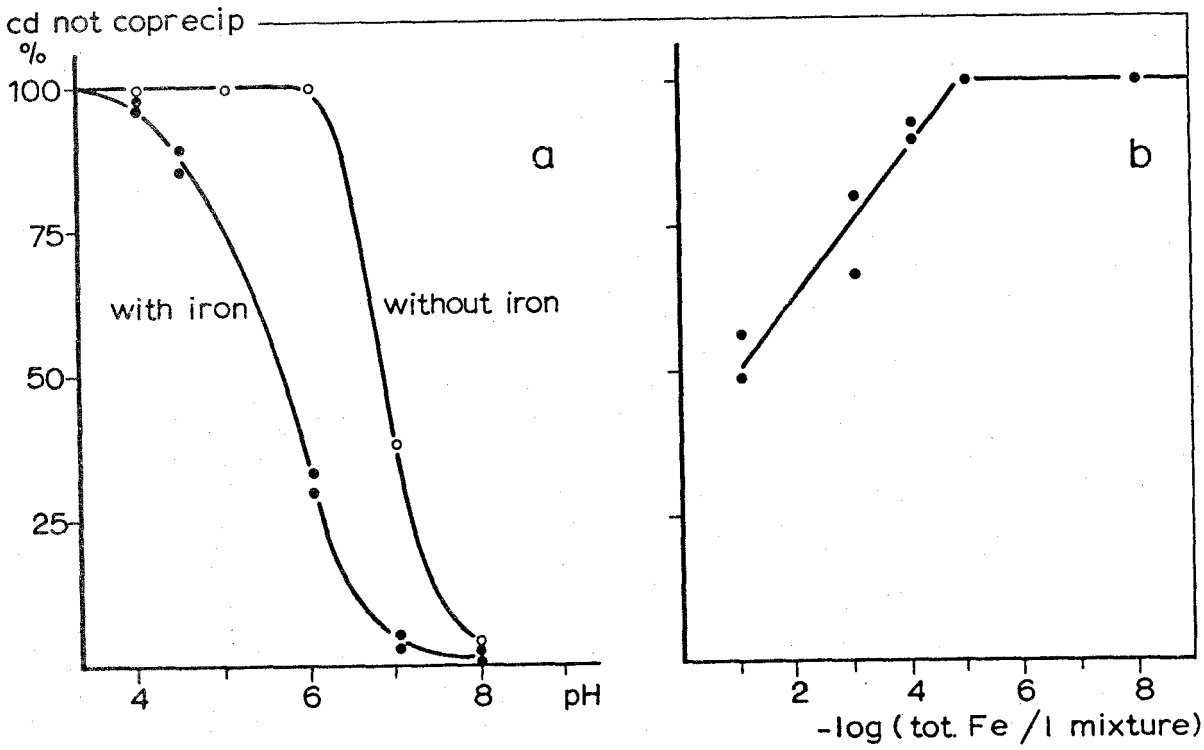


Figure 50A
Effect of the pH on the coprecipitation of cadmium. Chelate-free Hoagland solution without iron or after adding 10^{-3} Mol $Fe(NO_3)_3$ $9H_2O$ per liter, Cd concentration: 5.10^{-7} M.

Figure 50B
Effect of various addition of $Fe(NO_3)_3$ $9H_2O$ on the coprecipitation of cadmium. Chelate-free Hoagland solution at pH 5. Cd concentration: 5.10^{-7} M.

after the roots have been dipped in a Hoagland solution containing 6.10^{-6}M $Cd(NO_3)_2$ and 0.1 mCi ^{115m}Cd per liter. When the radioactive solution is replaced either by demineralised water or by an aqueous solution of 10^{-5}M $Cd(NO_3)_2$ the rate of local accumulation of ^{115m}Cd decreases gradually towards zero. However, when the washing solution is a cadmium-free Hoagland solution or a pure 13.10^{-3}M $Ca(NO_3)_2$ solution, the accumulation rate vanishes, approximately after two hours and becomes negative.

Cadmium may be exchangeably bound to some sites in the vessels of the stem from which it can be removed by other ions, such as calcium.
Computing of the energy spectra of ^{115m}Cd, recorded during these experiments, is in progress.
It will give more information concerning the lateral movement of cadmium inside the stem

On chromium uptake by isolated chloroplasts - G. DESMET, A. DE RUYTER.

The uptake and metabolism of chromate by chloroplasts isolated from spinach leaves (*Spinacea oleracea* L, cv. Amsterdams Reuzeblad) had been studied at pH 7.6. Work with chloroplasts at pH below 7 is without physiological sense.
Cr^{3+} has not been investigated as it precipitates at high pH.
The uptake of CrO_4^{2-} is measured by a combination of centrifugation and visible light spectrophotometry, using the extinction spectrum at approx. 372 nm. From the decrease of extinction of chromate in the supernatant an uptake saturation of about 40 ng CrO_4^{2-} per µg chlorophyll is observed.
The question whether chromate is transformed during its absorption by the chloroplasts was tackled by the comparison of the spectra of chromate, absorbed by the chloroplasts, with those of chromate in solution. A decrease of the extinction of the absorbed chromate has been found. Therefore partial transformation of chromium by the chloroplasts, eventually from Cr(VI) to Cr(III) is a possibility. This transformation is compared to the real uptake in figure 51. Because the molecular extinction coefficient of Cr(III) at its maximum is very small compared to the one of CrO_4^{2-}, no Cr(III) spectrum was measurable.
The standard redox potential E'_o of CrO_4^{2-}/Cr^{3+}, in alkaline medium, is about -0.13 V. Light activated chloroplasts are known to generate a redox power of at least -0.42 V. Using this approach, it seemed worthwhile to consider the possible reduction of CrO_4^{2-} by illuminated chloroplasts. Measurements of the influence of chromate on the variable fluorescence and on the O_2 evolution of activated chloroplasts have been performed. From these experiments it became evident that chromate really can act as an electron acceptor in a photosynthetic electron transport chain.
Its active concentration in this reaction, however, depends on the presence of a concurrent electron acceptor e.g. $NADP^+$ or methylviologen (figure 52). CrO_4^{2-} has been mentioned to be an antagonist of SO_4^{2-} reduction. Before reduction SO_4^{2-} has to be energized by ATP. This does not apply to CrO_4^{2-} because, even in the absence of ATP formation due to the presence of an uncoupler CH_3NH_3Cl, chromate reduction occurs (figure 52).

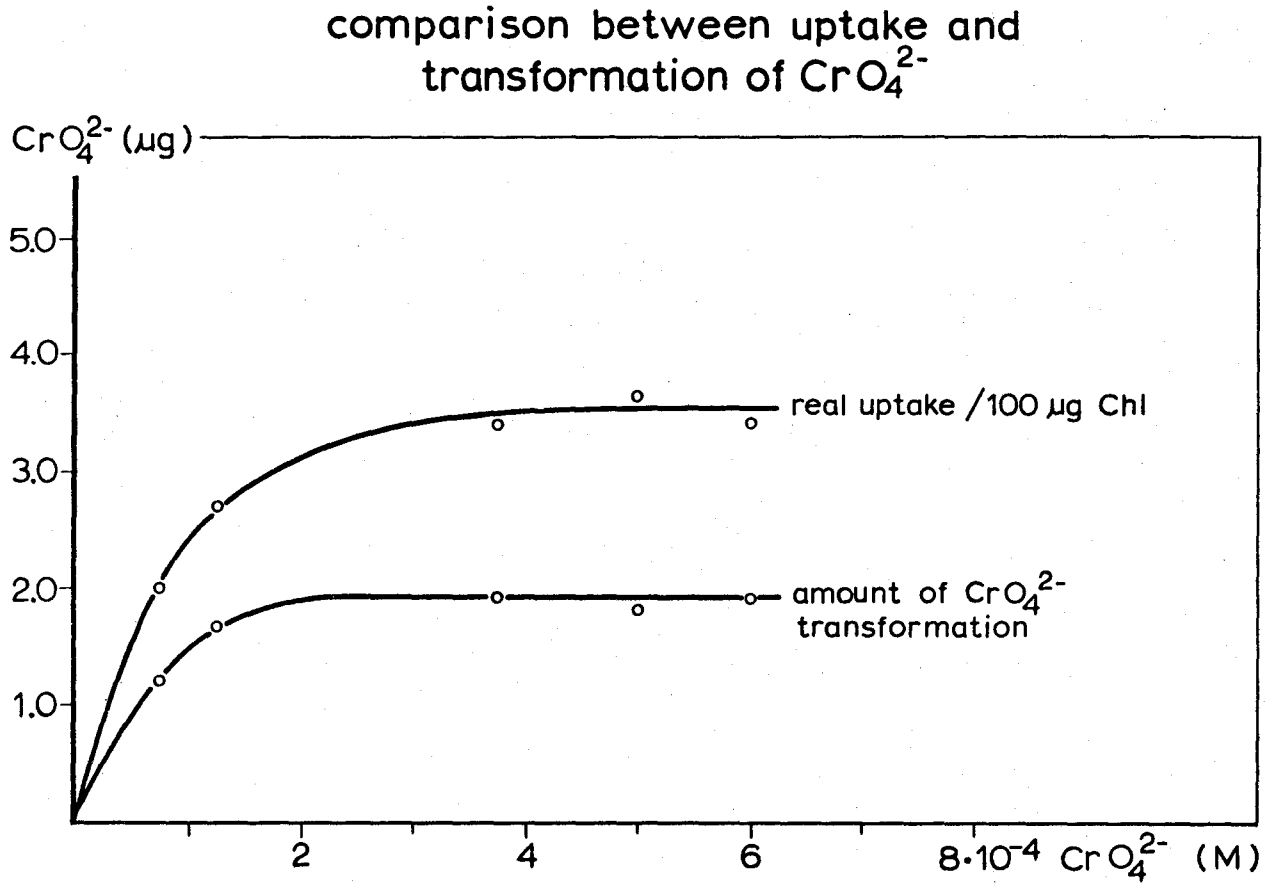


Figure 51 - Comparison between uptake and transformation of CrO_4^{2-} by isolated chloroplasts.

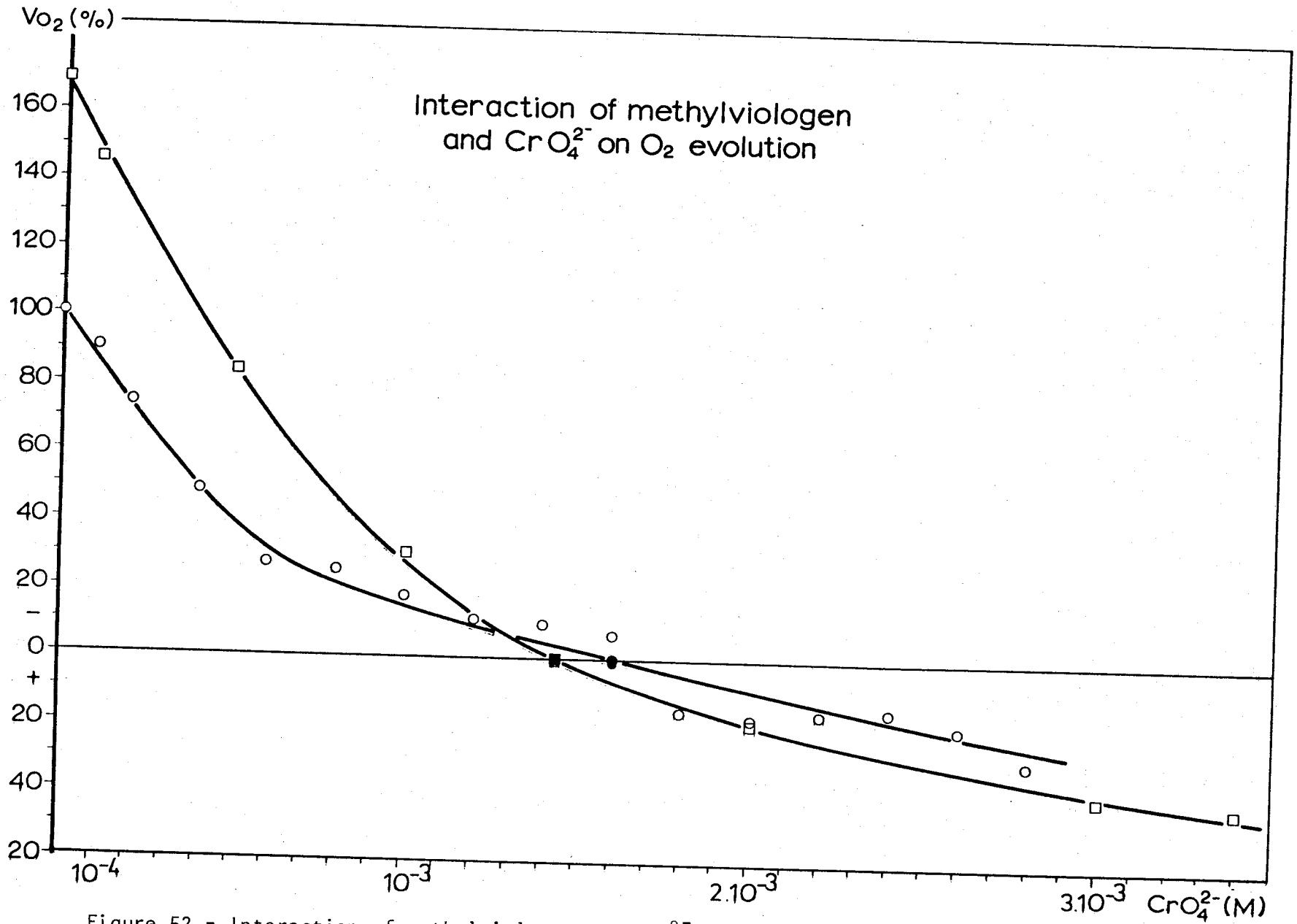


Figure 52 - Interaction of methylviologen and CrO_4^{2-} on O_2 evolution.
□——□ in the absence of an uncoupler
o——o in the presence of an uncoupler (CH_3NH_3Cl).

On cadmium uptake by isolated chloroplasts - M. MATTEOLI, G. DESMET.

In this research two important topics were considered: (1) the physicochemical behaviour of the element Cd with respect to different ligands; (2) the influence of the Cd^{2+} on the metabolism of active chloroplasts. The ligand properties of the different substances belonging to the buffered chloroplast medium have been investigated. This work has been done by means of a Cd^{2+} sensitive electrode system. The medium contains 0.25 M saccharose, 0.01 M $MgCL_2$, 0.035 M KCl, 0.05 M TES at pH 7.6. In this medium up to 70% of Cd^{2+} is complexed. A detailed investigation showed Cl^- and TES to be responsible for this complexation (figure 53).
From the study concerning the influence of Cd^{2+} on the O_2 evolution of chloroplasts it became clear that Cd^{2+} inhibits the electron transport (figure 54a). Besides it interacts with the energy metabolism of the chloroplasts. Indeed in the absence of a pretreatment with the uncoupler CH_3NH_3CL, an increase of the electron transport rate is observed at the lowest Cd^{2+} concentrations. At the higher concentrations the inhibitory action dominates (figure 54b). A detailed study of the precise interaction site of Cd^{2+} with the electron transport chain was also done. It was found that Cd^{2+}, although present at high active concentrations, is a rather specific inhibitor. It does not destroy the chloroplasts since most of the electron transport reactions remain intact. Cd^{2+} appears to interact with the H_2O splitting enzyme of photosynthesis and with the energy metabolism.

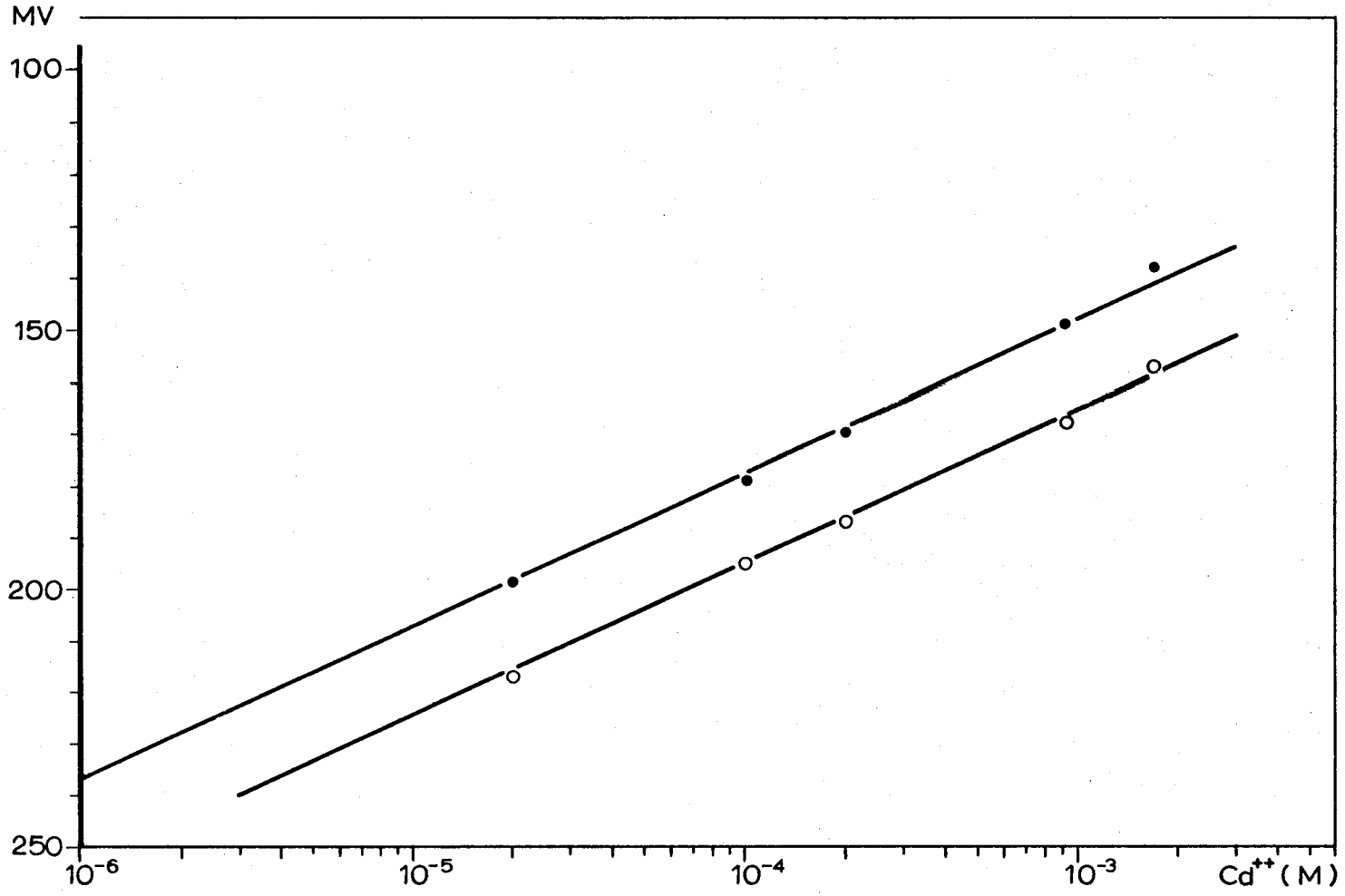


Figure 53 - Influence of the chloroplast medium on ligand complexation of Cd^{++}.

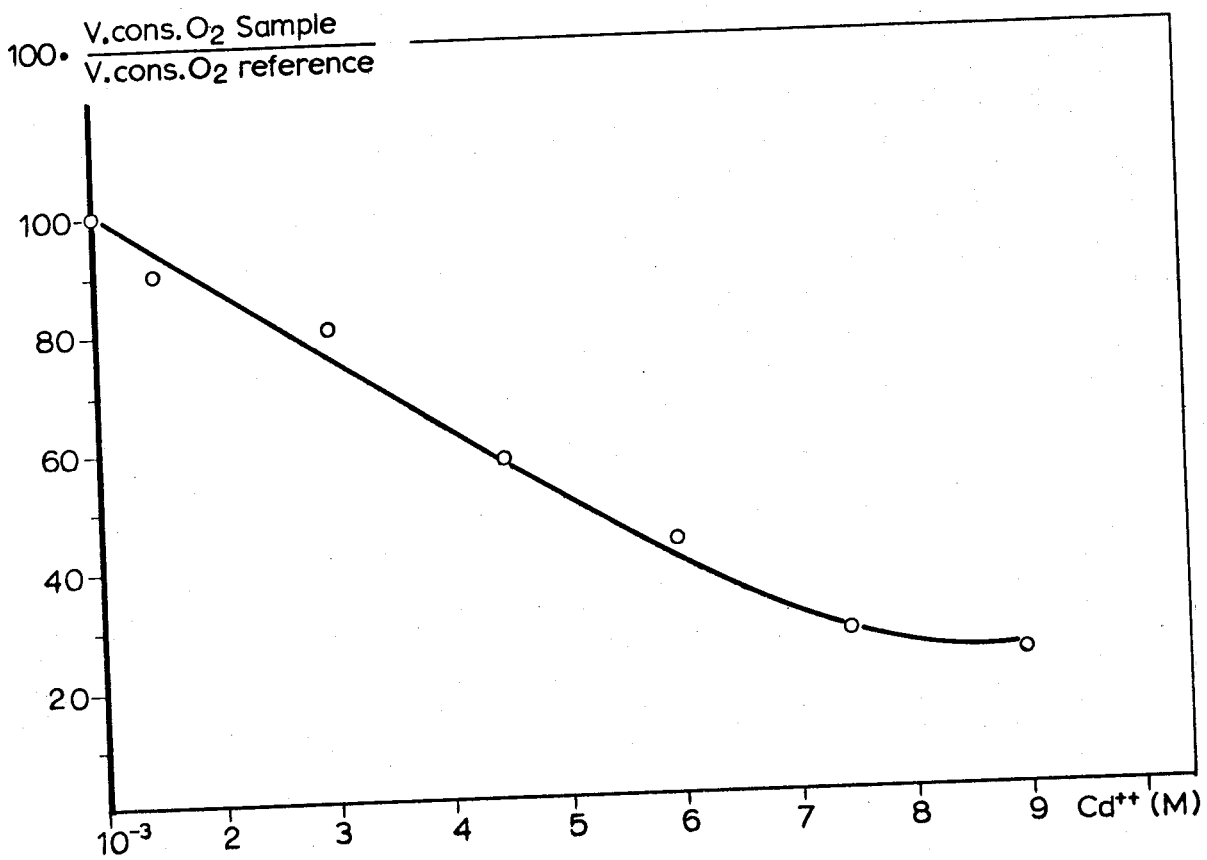


Figure 54a - Influence of cadmium on the photosynthesis rate of uncoupled chloroplasts.

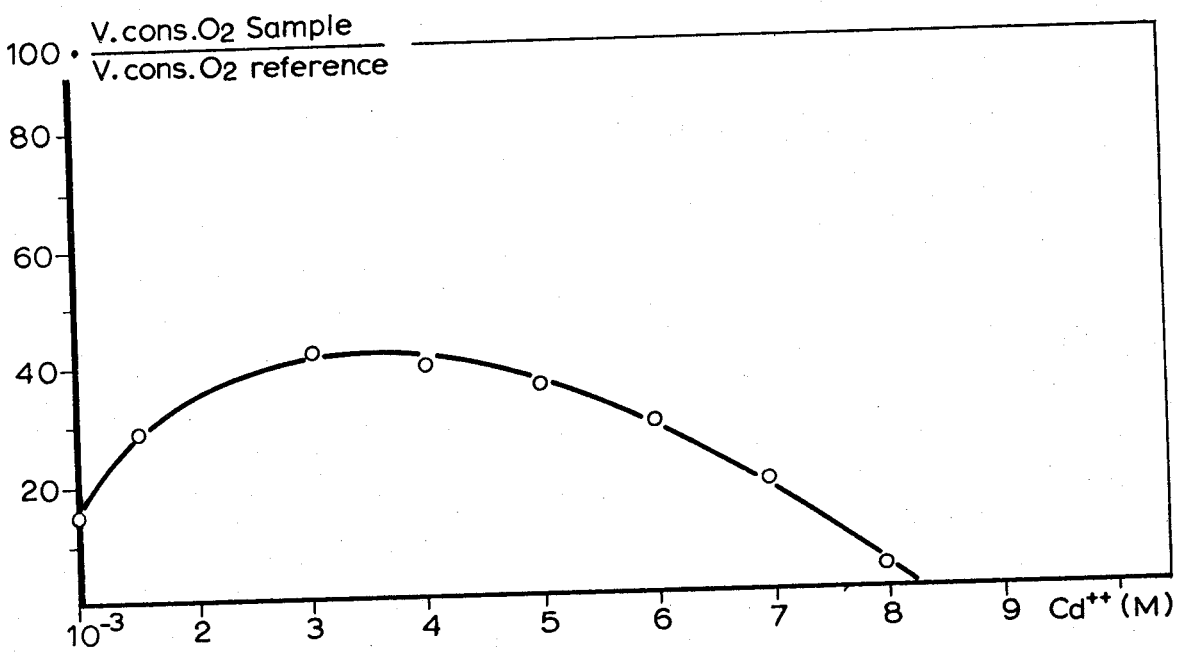


Figure 54b - Influence of cadmium on the photosynthesis rate of coupled chloroplasts.

METHODOLOGY

RELATED TO DOSIMETRIC, OTHER PHYSICAL AND INSTRUMENTAL STUDIES

Dosimetry - K.H. CHADWICK, K.J. PUITE.

Second EULEP intercomparison project on X-ray dose and dose distribution

A second intercomparison project on X-ray dose and dose distribution has been carried out between various institutes cooperating within EULEP. The aim of this second project was to check the improvements made after the first intercomparison studies, while also this project was more directed to the actual exposure conditions at the various institutes. The work has been carried out in close cooperation with the Radiobiological Institute at Rijswijk and the Laboratorio di Dosimetria e Standardizzazione at Rome and the standardization laboratory of the National Institute of Public Health at Bilthoven.
A test phantom with the size of a mouse and containing LiF and BeO TL dosimeters had to be exposed together with additional mouse phantoms in the case actually used for mice experiments at the participating institutes. From the TL reading of the dosimeters at the entrance, central and exit position in the phantom the absorbed dose acutally given and the dose distribution over the phantom could be calculated.
The total uncertainties for both the LiF and BeO absorbed dose data are estimated to be $\pm$ 2.5 to $\pm$ 3%. Although the experimentally found mean difference between the two independent systems is within the expected limit of $\pm$ 4 to $\pm$ 4.5%, there is an indication that some systematic discrepancy between the two systems does exist.
A schematic representation for comparing the LiF results of the 1971 and the 1973 intercomparisons is given in figures 55 and 56.
The Association Euratom-ITAL is listed in these figures with the number 14. It can be seen that improvement has been made, although 3 out of 11 institutes still obtain absorbed dose values which are not within 5% of the standard dose and 3 out of 13 institutes are not able to fulfil the requirements of performing class A irradiations (i.e. a ratio of less than 1.15 between maximum and minimum absorbed dose).

Treatment of liquid waste - A.F. GRONEMAN.

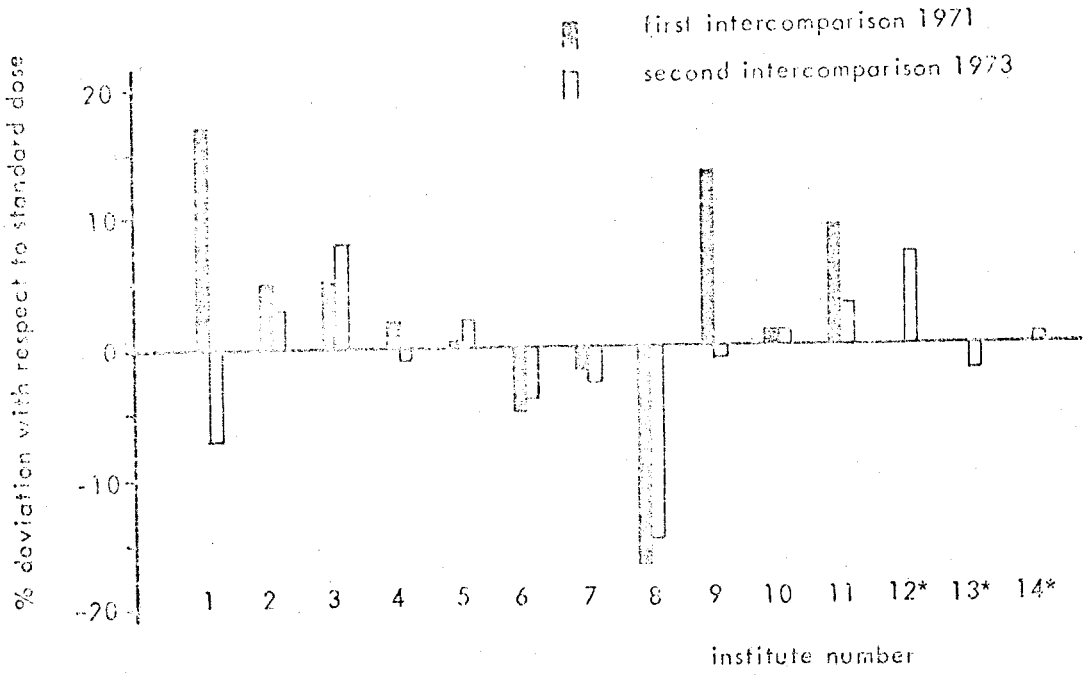
Research into the effects of radiation on sludge has started in November 1973.
A review of published research is in progress. Instruments and analytical methods are in the stage of development in order to measure the influence of irradiation on physical and chemical characteristics of sludge.

Electronic instrumentation - J.G. DE SWART.

Soilcolumn scanner for water movement studies

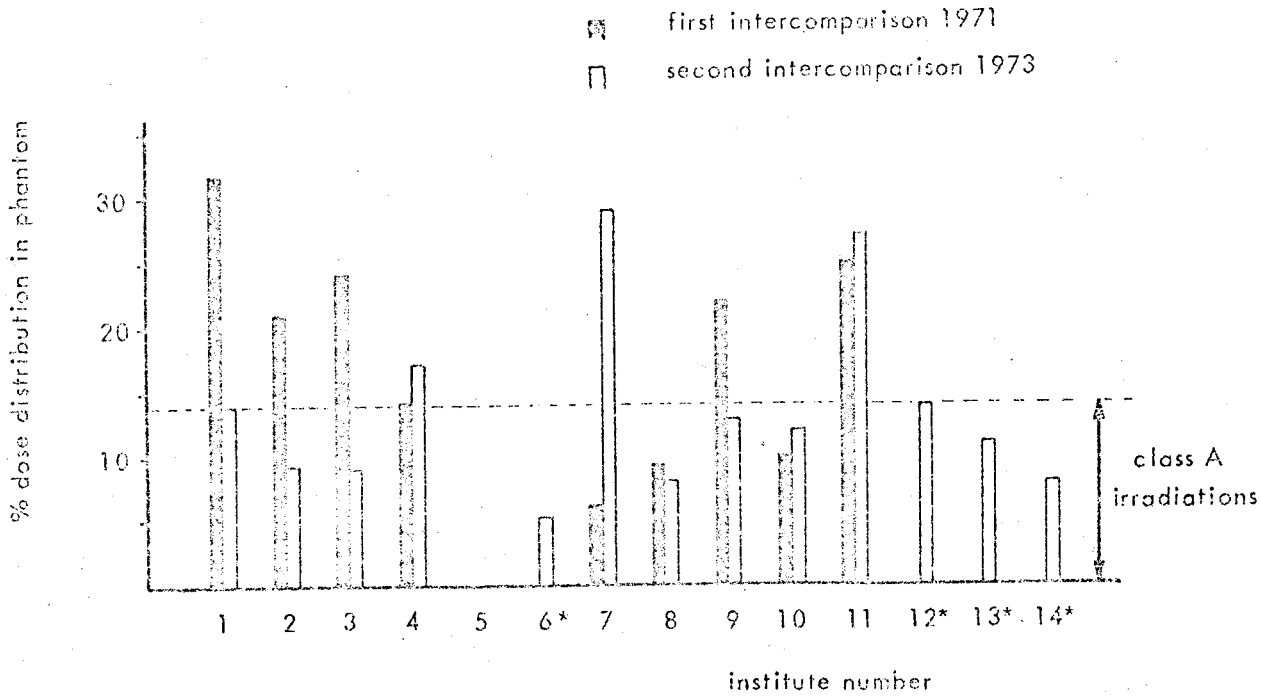
(Cooperation with ir. L. Stroosnijder, Agricultural University of Wageningen).

A publication about this subject has been accepted by Soil Science.



* these institutes participated only in the 1973 intercomparison

Figure 55 - Results of EULEP X-ray dose intercomparison.



* these institutes participated only in the 1973 intercomparison

Figure 56 - Results of EULEP X-ray dose distribution intercomparison.

Modifications of the Perkins-Elmer atomic absorption spectrophotometer

A fast peak detector with punch tape read out has been realized. The development of a sample changer for 50 samples has been postponed due to other priorities.

Protection system for maintenance workers during nights and week-ends at the Institute

An automatic alarm system was developed. The system operates via a radiotranceiver combined with a semaphone terminal in such a way, that the operator will be called automatically at fixed time intervals. If he does not react on these calls, the on-duty Health Physics functionary will get an alarm via his semaphone. The system became operational beginning 1973 and has proved to be very reliable.

Pneumatic samplechanger

The development of a samplechanger was started for the Ge(Li) semiconductor detector for the determination of low activity fall out products and activated corrosion products in fishery products and for the measurement of activated samples for the determinations of heavy metals in fishery products by means of neutron activation analysis. The detector will be shielded in this system with about 400 kg old lead (at least 170 years old).

Data collection system

The development of this system was started to facilitate the solution of actual measurement problems. It collects both digital and analog data and registration is done by punch tape, magnetic tape or parallel printer. Due to the introduction of the Racal cassette tape-recorder in Wageningen as a standard, the adaptation to our data-collection systems involved extra development work resulting in a delay in realization of our read-out facilities.

RELATED TO STUDIES ON BIOLOGICAL MATERIAL

Development of high-efficiency and high resolution ionographic methods. Applications to autoradiographic problems - R.V. RECHENMANN, E. WITTENDORP.

Methodology (collaboration of J. OORTWIJN)

- Study of the development process.

The usefulness of a systematical study of the photographic process has been shown by preliminary results previously obtained (Rechenmann, 1971; Rechenmann and Wittendorp, 1972), which have given rise to specific applications in the fiels of autoradiography (Ringoet et al, 1971; Sensenbrenner et al, 1971) and of microdosimetry (Rechenmann, Wittendorp, 1971; Rechenmann et al, 1973; Wittendorp et al, 1973). The increase in reproducibility, efficiency and resolution obtained by our methodology has anyhow to be considered only as a first step towards an optimal ionographic detector.
We continued therefore the studies concerning the reduction of silver bromide microcrystals, which is induced by a latent and/or a sub-latent image. Special attention has been paid to 1) the formulation of "compact grain" developers; 2) the study of the activation process; 3) the development temperature.

Compact grain developers.

It has been mentioned in former reports that we had started, in connection with our activation experiments, the development of new reducing solutions of high efficiency, producing silver grains of small diameter without a prohibitive increase of the chemical fog. It was found that a certain type of emulsion gives the best response with a given developer (Rechenmann and Wittendorp, 1972; Sensenbrenner et al, 1971).
A sub-group of these developers is formed by the so-called "compact grain" reducing solutions, which should reduce the AgBr microcrystals while preserving their form and dimensions to a certain extent; we added an even stronger requirement, i.e. these developers should be strong enough for developing microcrystals touched by low ionizing radiations, like electrons; a reducing solution of this type should also avoid the production of a too high chemical fog which may disturb the photographic recording or introduce unacceptable inaccuracies in eventual measurements.
Our first attempts to formulate reducing solutions of this type had been disappointing (Rechenmann and Wittendorp, 1972 and 1973): effectively, the developed silver grains varied not only in size, but also in shape from one experiment, performed in the same conditions, to another; in certain cases, an important general fog disturbed the image obtained.
For solving these problems, systematical experiments have been carried out by means of the same two basic agents which have already been used in our previous attempts, i.e. amidol and ferro-oxalate.
At a first stage, we tried to find an acceptable equilibrium between the "chemical" and the "physical" development mechanisms implied in the process producing photographic silver grains, by varying the relative amounts of the constituants of very solvent developers, and also by a careful adaptation of the chosen activation procedure. Unfortunately, a higher concentration of the "solvent" agents of a

developer (Na_2SO_3, KCNS, NaCNS, etc.) always results in a strong increase of the slope of the density-development time curve (figures 57 and 58). As a consequence, the reproducibility of the results may not yet be considered as satisfactory. In order to counterbalance the fast raise of the chemical fog due to these solvent developers, anti-fogging agents have been introduced in our developing formulas.
We are actually trying to overcome the disadvantages of these new types of developers, e.g. by a careful action on some of the factors governing the speed of the photographic development, like the temperature, or the negative ion layer protecting the AgBr microcrystals against charged developing agents.

Further studies on the activation process

Different formulas of gold activation solutions have been established for specific uses. A nearly complete eradication of the chemical fog could be obtained in the case of particularly favourable combinations emulsion-radiation-activation-developer.
Complexes of noble metals other than gold, like platinum, palladium, iridium, etc. have been used for predeveloping nuclear emulsions. It could be shown that if the gold is replaced by iridium in the activation solutions, the number of silver grains always remains inferior to the number of developed microcrystals due to a classical development. This "inactivation" of the latent image increases with the iridium concentration in the solution.
These results may lead to a photographic discrimination between γ-radiation and the influence of charged particles, even of different types. On the basis of such a procedure, the development of a new method of activation analysis by means of photographic detectors can eventually be foreseen.

Development temperature

In connection with the studies mentioned above, experiments devoted to the influence of development temperature on the rate of development as well as on the rate of chemical fog formation have been undertaken. A preliminary result is given in figure 59.

- Autoradiographic resolution

The programme on autoradiographic resolution had to be postponed; the responsible scientist left the laboratory.

Biological applications

Evidence of nucleolar DNA synthesis by activated trackautoradiography in adult rat liver cells

In a series of biochemical studies, M. Wintzerith, M.E. Ittel and P. Mandel (Centre de Neurochimie du CNRS - Strasbourg) had shown (Wintzerith et al, 1968) that nucleolar DNA of adult rat liver incorporates rapidly radioactive precursors of DNA as compared to chromosomal DNA of nuclei. These authors tried to confirm these results by applying the classical "stripping film" method. Their results showed that the sensitivity and the resolution of this classical autoradiographic technique are by far insufficient to get a confirmation of their hypothesis.

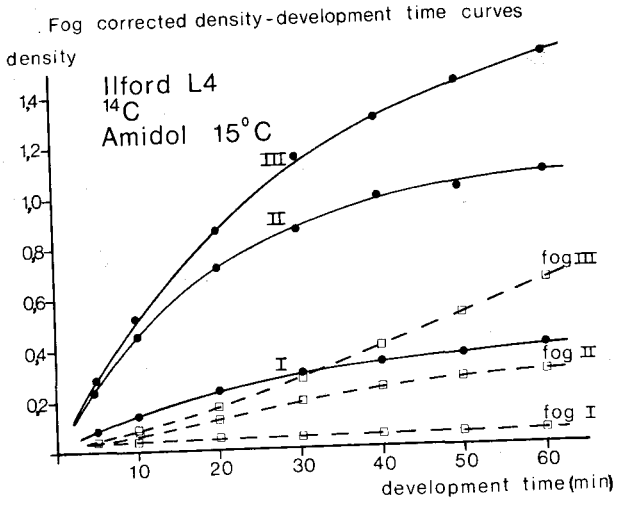


Figure 57

Optical density as a function of development time in amidol developers at 15°C, after activation.
Emulsion: Ilford L4 - Source: ^{14}C
I "Brussels Formula"
II "Compact grain" developer (0.5% KCNS)
III "Compast grain" developer (1% KCNS).

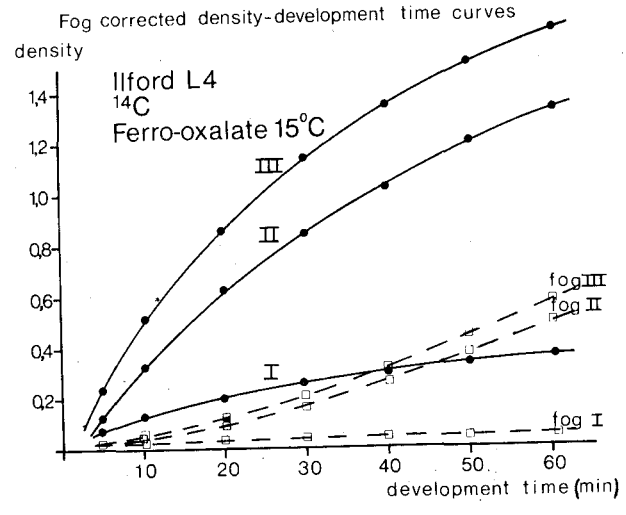


Figure 58

Optical density as a function of development time in ferro-oxalate developers at 15°C, after activation.
Emulsion: Ilford L4 - Source: ^{14}C
I Classical ferro-oxalate developer with added 1.8% Na_2SO_3.
II "Compact grain" developer (0.5% KCNS)
III "Compact grain" developer (1% KCNS).

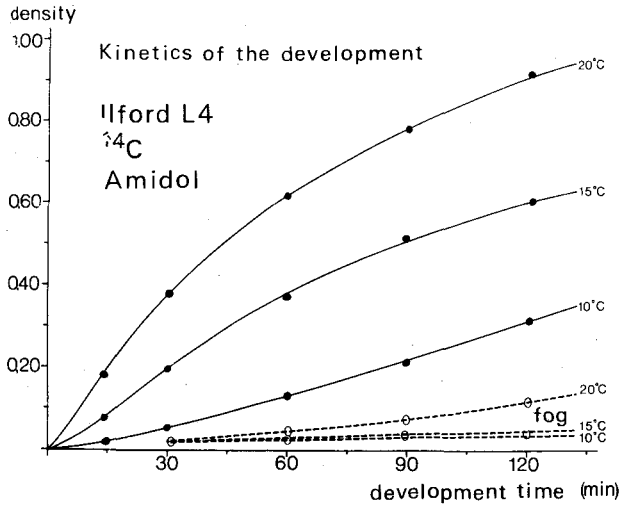


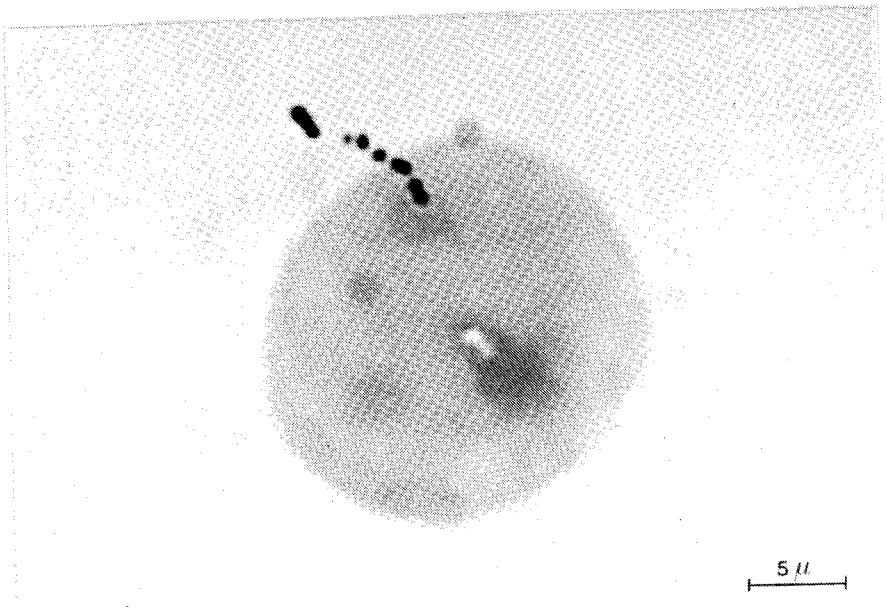
Figure 59

Optical density (fog-corrected) as a function of development time at different development temperatures (10, 15, 20 °C).
Emulsion: Ilford L4 - Source: ^{14}C
Developer: Amidol.

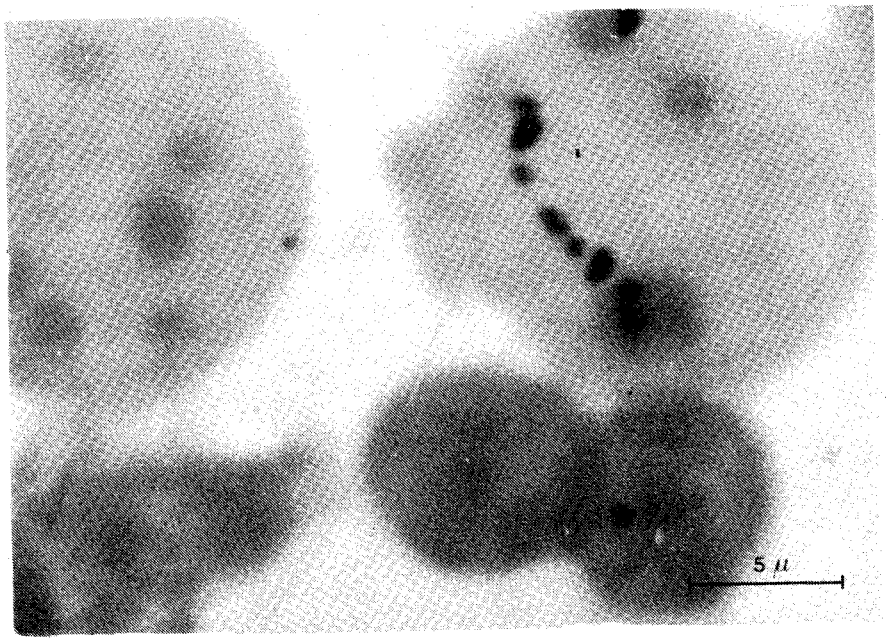
In collaboration with the authors mentioned, we have adapted the activated track-autoradiographic method specifically to this problem.
The nuclei and nucleoli have been prepared by the techniques of Chauveau et al (1956) and Muramatsu et al (1965) from adult rat livers labelled in vivo with ^{14}C-methyl-thymidine. Systematic tests have been carried out for determining the optimal staining technique, depending upon the combination tissue-emulsion-staining solution. The best results have been obtained with the Kodak NTB II emulsion, stained with Azur blue C after the development procedure, i.e. through the gelatin layer.
A thickness of 30 µm Kodak NTB II emulsion has been poured onto the slides. After 5 days exposure time at 5°C, the preparations have been submitted to gold activation before development for 50 minutes in a ferro-oxalate solution at a redox potential of -320 mV.
Light microscopical observations of the autoradiographic preparations showed that a significant number of electron tracks are emitted from nucleoli of the isolated nuclei. The incorporation of ^{14}C-thymidine within the nucleoli is clearly visualized on figure 60 a and b. Some heavily labelled nuclei have also been observed. These data are in good agreement with the mentioned biochemical results. A certain independence of nucleolar DNA and chromosomal DNA synthesis seems to be confirmed. An autoradiographic study on isolated nucleoli is foreseen.

Track-autoradiography of nerve cells in culture

Autoradiographic experiments on cultures of dissociated cells of chick embryonic brains had been undertaken (Rechenmann and Wittendorp, 1973) in collaboration with M. Sensenbrenner, G.G. Jaros and P. Mandel (Centre de Neurochimie du CNRS - Strasbourg). Let us recall that the purpose of this study is to correlate changes in the metabolism of nucleic acids with the appearance of morphological patterns of differentiation: the incorporation of ^{14}C-thymidine into neuroblastic cells should give a direct information on the multiplication of the undifferentiated cells and the control of their mitotic activity. The methodology, i.e. biological preparations and autoradiography, remained unchanged in its main features (Rechenmann and Wittendorp, 1973).
Nerve cells form brain cortex of 7-days old chick embryos were dissociated and cultivated in Rose chambers. The labelling of the cells took place immediately at the onset of the culture or after 1, 3 or 7 days by adding ^{14}C-thymidine (0.1 µCi/ml) to the culture medium for a duration of 24 hours. After periods varying from 1 to 21 days, the cultures were washed, fixed, impregnated with silver by the method of Holmes before applying the activated track-autoradiographic method.
Light microscopical observations of the autoradiographic preparations showed that nearly all the nerve cells incorporate radioactive thymidine when the tracer is added to the culture medium at the onset of the culture or after 1 day (figure 61 a and b). This result indicates a strong mitotic activity of the still undifferentiated cells, as well as their correct adaptation to the *in vitro* conditions. For protracted cultivation times (3, 4 and 7 days) before incorporation, the behaviour of the nerve cells, as described by their labelling, varied

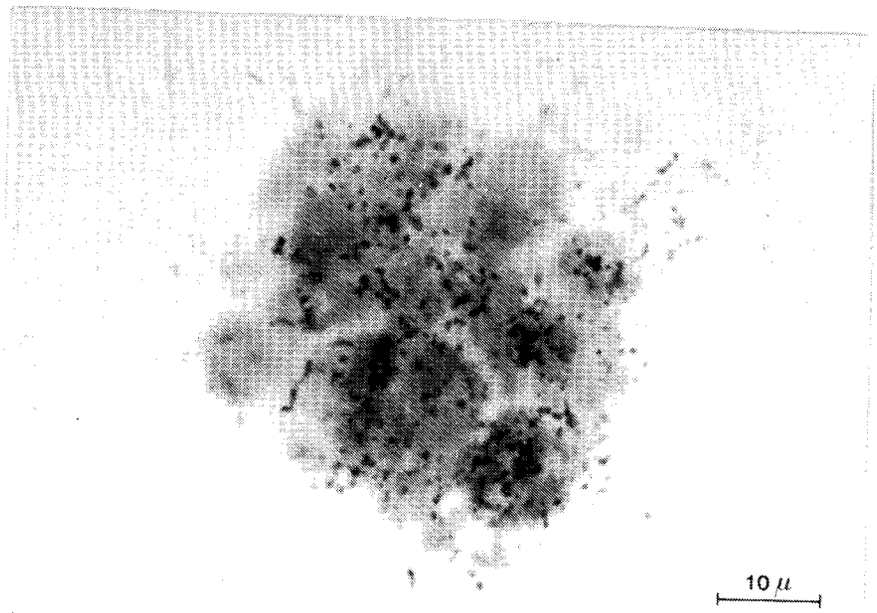


a

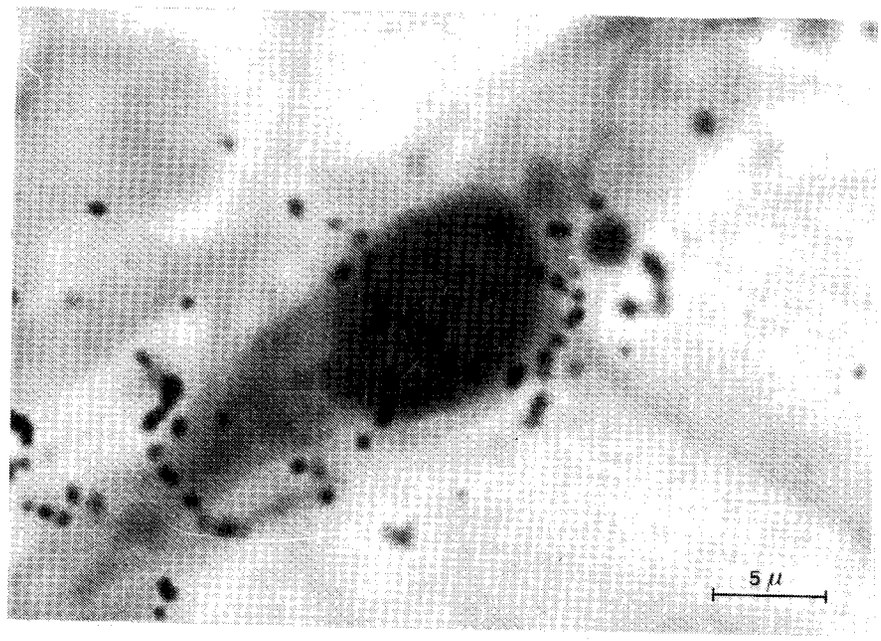


b

Figure 60 - Activated track-autoradiographs of isolated rat liver nuclei. The nucleoli are clearly visible. The ^{14}C-thymidine incorporated within the nucleoli can be easily located at the track origin of the electron emitted by the tracer element.
Emulsion: Kodak NTB II - Exposure time: 5 days
Development: Activated development for 50 min. in a ferro-oxalate solution at E_{redox} = -320 mV (15°C).
Coloration: Azur blue C after development, i.e. through the gelatin layer.
a) x 2700
b) x 4200



a



b

Figure 61 - Activated track-autoradiographs of dissociated nerve cells in culture (7-days old chick embryonic brains). Incorporation of ^{14}C-thymidine at the onset of the culture during 24 hours.
Emulsion: Ilford K5 - Exposure time: 17 hours.
Development: 50 min. in ferro-oxalate at E_{redox} = -320 mV (15°C), after gold activation.
Silver impregnation method of Holmes.
a) Incorporation of ^{14}C-thymidine in undifferentiated nerve cells after 48 hours of culture (x 1360).
b) Electron tracks originating from part of the neurons in the cultures, 2 weeks after exposure to the labelled thymidine (at the onset of the culture) (x 3000).

depending upon their nature: the neuroblasts differentiate rapidly into neurons, while the cells from the glial lines still remain at the undifferentiated stage, at least during the first week of culture. No marked divergencies between the observed behaviour of the nervous cells *in vitro* and their evolution *in vivo* could be observed.
These data justify the further use of cell cultures for the study of the morphological and metabolic patterns of the differentiation. Investigations by means of activated track-autoradiography concerning factors which are supposed to influence the differentiation of primitive nerve cells are foreseen in the next future.

Mass determination of plantations - H.P. SCHÄTZLER, W. KÜHN, H. KATANA.

Measurement of the aerial part

In 1973 a scanning device was completed to verify the results of preceding theoretical investigations. The most important part of the equipment is a strongly collimated beam of a 200 mCi ^{241}Am-source at a length of 2.5 m to get approximately parallel rays (length of collimator: 20 cm, area source: 40 x 10 mm^2, same collimator on the detector side). This beam scans the field in vertical and horizontal directions with help of synchronous motors. Measurement control and data compilation are performed by a multi-channel-analyser operating in the MCS mode. The spatial resolving power can be improved by varying the length of the measurement intervals. This measurement is indispensable, as could be shown with step-like absorption profiles, when the absorption varies largely. Investigations of the geometry of the measurement have proved that the cross section of the beam is almost constant along the path and matches the theoretically expected geometrical cross section of the absorbing material. The strongly collimated beam and the discrimination of the scattered radiation by spectroscopy guarantee the necessary exponential absorption for objects not smaller than the cross section of the beam. We have yet problems with boundary effects when measuring absorbing objects of still smaller size.
The penetrated mass of a certain unit area follows from the identity of the geometrical and the beam cross section. Summation over all area elements during scanning yields the total mass of the plantation. Preliminary measurements were carried out with a phantom of a corn field (with an effective atomic number corresponding to real crop). The culm density was varied while the lengths of the culms and spikes was kept constant. All experiments, performed so far, showed satisfactory agreement of the measured and the actual distributions of the weight per unit area.

Spatial distribution of the root system

Reasonable application of water and nutrients in semi-arid and arid cultures requires the knowledge of the spatial distribution of the root system in vertical and horizontal directions. Undirected irrigation and fertilization is not economical. So far, root distributions have been determined by taking samples, which requires a tremendous amount of work and time especially with trees.
On the line of investigations concerning the soil-plant system we

tried to determine the root system by radiometric means. For this purpose the accumulation and movement of ^{86}Rb (half-life: 18.6 d; E_γ: 1.08 MeV; E_β: 1.8 MeV) was studied using apple trees several years old. The comparison of different techniques for injecting the radioactive Rb-solution proved the "scraped bark injection" to be far more effective than leaf or branch injections.
In the following experiments the Rb-activity in the root system was determined after applying solutions of different specific activities. Investigations of the Rb translocation from the stem into the roots showed that the accumulation of Rb in the roots starts about 1-2 days after injection and reaches a maximum after about 24 days.
The actual advantage of those experiments was the autoradiographic detection of the root system. Suitable X-ray films placed into the soil during the mentioned time interval show parts of the roots at certain distances to the stem with the help of the β-radiation of ^{86}Rb.
This method yields a rough but sufficient survey of the spatial distribution of the root system of a single plant and even a whole plantation if applied to representative trees. It saves a large amount of time and work and allows economical irrigation and fertilization. Furthermore, this technique is applicable for studies concerning infiltration resistances and aeration of different types of soils.

Studies on diagnosis and therapy of dry rot in living trees - J. HANDL, W. KUHN.

After the development of a X-ray-diagnostic method to determine dry rot in living trees the influence of manganese on the infection of trees by fungi, especially by *Fomes annosus*, was studied.
It is known that the decomposition of wood in trees is more severe with *Fomes annosus* when the manganese concentration is higher. To investigate a possible correlation between Mn-content and infection, more experiments were carried out with the 250-year old Lime trees, which have been diagnosed with X-rays during the last report period. The manganese content of samples of wood from sick and healthy parts of those trees was determined by activation analysis. It showed that in most cases the Mn-content is higher in sick wood than in healthy parts. This result suggests the possibility to influence the growth of fungi and consequently the decomposition of wood, when radioactive manganese is incorporated into infected parts.
Therefore, *Fomes annosus* was grown in nutrient solutions of different concentrations of inactive and active manganese (^{54}Mn). Experiments were carried out in several series of 4 different activities each, resulting in radiation doses between 0.8 R and 8 R per day. The results indicate that the growth rate of fungi is depending upon the dose, which is a necessary supposition for the possibility of influencing the growth rate by relatively low doses. However, we also found a positive, i.e. growth-promoting effect. This is in agreement with earlier experiments concerning low-dose radiation effects on fungi.
The experiments are continued, as they are of practical importance for the therapy of dry rot and the radiation effects at low doses.

Microlocalization in plant material - S.C. VAN DE GEIJN, G. SAUER, G.P. MIX.

Calcium localization in beanfruits

The results obtained in this project during 1973 mainly concern combined application of the available methods (semiconductor detector counting, -spectrometry, microautoradiography, X-ray fluorescence) to a specific topic: the uptake of ^{45}Ca after root application to bean plants (*Phaseolus vulgaris* L. cv. Saxa) at the fruiting stage. Some interesting physiological aspects of the accumulation of calcium have been studied.
In long term experiments (3 to 5 days of treatment with ^{45}Ca-labelled solution), in some parts of the plant an important re-distribution was found over the subsequent period of 7 days, whereas in other parts the labelling level remained perfectly stable. More precisely, it was found that the countrate measured by semi-conductor detectors placed at the dorsal side of older fruits after 7 days stabilized at about 30 - 40% of the maximum countrate which is reached at the end of the labelling period, whereas the countrate at the ventral side of the fruit remained constant.
For younger fruits the dorsal as well as the ventral side showed a constant countrate. The ^{45}Ca countrate measured on the stem reduced to 15% of its maximum level, and the leaf again showed no change whatsoever.
Analyses of the spectra, obtained during different stages of the experiment showed a remarkably stable internal distribution of the radioisotope, at both sides of the fruit. A thin layer of heavily labelled material was present at 0 to 100 µm below the surface, at the position of the vascular bundle.
This was confirmed by a microautoradiographic study. The thin layer of heavily labelled cells was identified as the bundle sheath. Some of the cells were almost completely filled by small crystals, as confirmed by the observation under a polarization microscope.
The chemical nature of the crystals was determined by a treatment with weak respectively strong acids, and found to be calciumoxalate.
A first test was done subsequently to determine the distribution of the stable calcium in the considered tissue part. Samples were prepared for observation in the scanning electron microscope, equipped with an X-ray fluorescence measuring system. The embedding agent used for the light microscopic observation (methacrylate) turned out to be not fitted for this type of observations. The embedding material and consequently the tissue were seriously damaged by the bombardment with the electron beam. Nevertheless it was possible to draw a first conclusion from the obtained pictures. The majority of calcium in the tissue was indeed concentrated in the cells of the bundle sheath filled with the small crystals. The region between this accumulation tissue and the cuticula contained less calcium compared to the tissue inside this layer.
Whether, and to what extent, the mentioned cell layer interferes with calcium redistribution still has to be investigated. The dependence on growth stage of the fruit is also an unknown factor.

Cadmium in the stem of the tomato plant

Preliminary experiments have been done to investigate the lateral movement of cadmium in the stem of tomato plants (see before). The redistribution from the transport vessels to other tissue parts near to the surface is studied by measuring the β-spectrum of ^{115m}Cd and determining its maximum energy. Analysis of the results is in progress.

R E L A T E D T O S O I L - S T U D I E S

Studies on the evaporation and condensation of water vapor in soils (Desert loess) - C. BUNNENBERG, W. KUHN.

During the report period 1973 the equipment built in the year before was completed by a programme-controlled temperature regulation providing an automatic simulation of the temperature inversions in atmosphere and soil in a day-night cycle measured in the Negev Desert in Israel.
The radial heat transport in the soil column was minimized by careful insolation of the heat conducting steel container aspiring at "infinite geometry" of the soil column.
Experiments with this set-up and Negev loess soil yielded results concerning depth and speed of penetration of water vapor originating from the atmosphere under the condition of daily temperature inversions. The atmospheric vapor was labelled with tritium for 2 days while collecting it in tubes at different depths of the soil column and determining the tritium content.
At a depth of 40 cm below the surface activity was not detected until 13 days after labelling. After 27 days the tritium content above 16 cm below surface was much lower, while it was still increasing in deeper soil layers (see figure 62).
Pure diffusion was not taken into account. The results of those experiments were reported at an IAEA-symposium at Vienna, October 1973.
During a visit to Israel in September 1973, the experimental results and possibilities of corresponding field experiments in the Negev were discussed with members of the Institute of Botany of the Hebrew University, Jerusalem.
Further work is concentrated on the investigation of special parameters governing the vapor movement within soils and between soil and atmosphere. A new soil container is presently planned to increase the experimental variability of certain parameters and the measurement accuracy.

Measurement of moisture in thin loessial soil layers by a capillary porous probe - H.P. SCHÄTZLER, W. KÜHN.

After the application of the equipment for measuring low moisture contents in thin soil layers in the laboratory during an IAEA training course, experiments were carried out in the Negev Desert in cooperation with the Institute of Botany of the Hebrew University, Jerusalem, in September 1973.
They have shown that this technique is superior to neutron probes, if high spatial resolving power and sensitivity at low moisture contents (below 9%) is required. On the other hand the porous body probe can only be driven into the soil after rough-drilling, if the soil has not been cultivated for more than a year. Rough-drilling, however, may result in partially insufficient contact between soil and porous body. So, the high sensitivity and accuracy of this measuring technique can only be achieved in fairly soft soils.
Experiments in the Negev also showed that the moisture content of the top dew wetted soil layer is of special importance and interest. The contribution of this dew dependent moisture to the natural irrigation of plants cannot yet be determined. Prof. Evenari suggests to study the influence of this top soil layer in connection with the

temperature inversions in the soil-atmosphere system, which includes the continuous measurement of the moisture in the top 2-cm layer. For this purpose the development of a new measuring technique will be started this year.

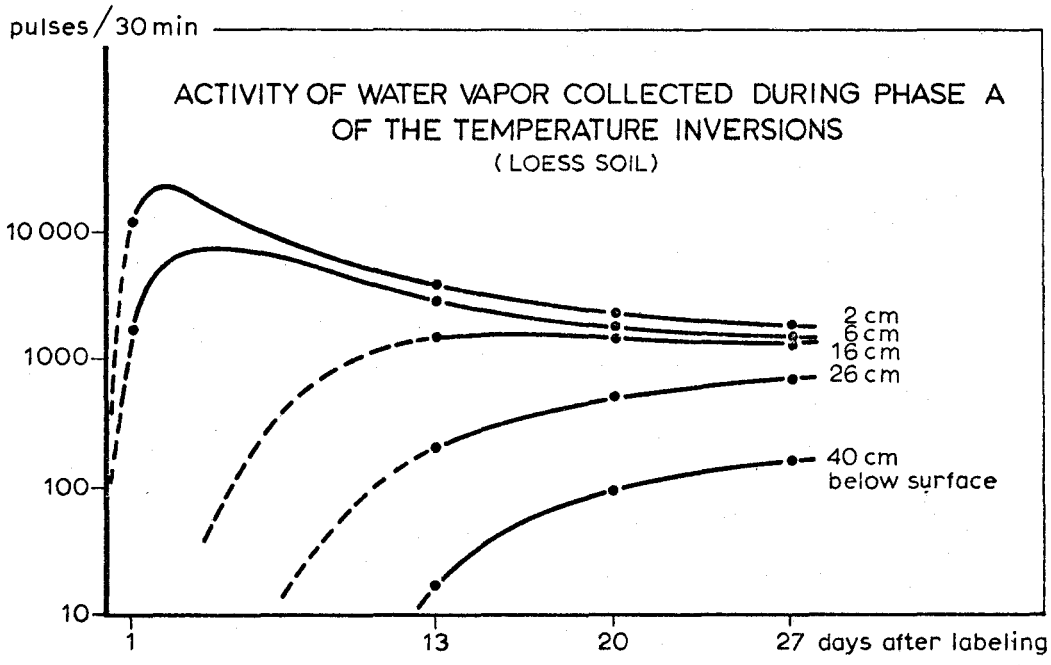


Figure 62 - The activity of the water vapor collected in tubes at certain depths of the soil column between 6 and 12 a.m. (Phase A), on different days after labelling the atmosphere.
The activity was determined by feeding the vapor into a flow-through type proportional counter with the help of a N_2-stream and measurement during 30 min.

PRACTICAL APPLICATIONS, SERVICES, COURSES

MUTATION BREEDING OF ECONOMICALLY IMPORTANT CROPS - C. BROERTJES.

During 1973 again many scientists and commercial plant breeders, mainly from the Netherlands but also from other countries, used the service of the Association: general information about the possibilities of mutation breeding, requests for literature, and irradiation of plant material. It is noteworthy that an increasing number of the persons contacting us, are academically trained plant breeders, either at Institutes or engaged at a commercial plant breeding station. This certainly will improve the scientific basis of the projects and may thus lead faster to positive results. Thirteen new projects were studied in 1973, namely with cultivars of *Alstroemeria*, *Amaryllus*, *Arum*, *Begonia*, *Dianthus*, *Chrysanthemum*, *Euphorbia*, *Gerbera*, *Gladiolus*, *Kalanchoë*, *Lycopersicon esculentum* L. and *Saintpaulia*, all but one being vegetatively propagated ornamentals. In a few of the above listed species use is made of the adventitious bud technique (*Begonia*, *Kalanchoë*, *Saintpaulia*). In *Gerbera* a propagation method has been developed in which very young buds seem to produce shoots, In *Alstroemeria* almost exclusively solid mutants are obtained after irradiation of actively growing rhizomes of young plants, unforeseen but advantageous circumstances which speeded up the breeding programme of the crop.

In 1973 several mutant varieties were released to the trade, such as *Alstroemeria* c.v.'s Harmony, Rosita, Yellow Sun and Harlequin flower colour mutants of various cultivars (partly in Broertjes and Verboom, 1974), *Achimenes* c.v. Cupido, a very compact and free flowering mutant of "Paul Arnold" and "Orion", a large flowered mutant of the same variety. Of *Chrysanthemum* four different yellow-flowered mutants of the pink flowering c.v. Beamsville Pink, were released, namely the c.v.'s Uncle Danny, Danny Boy, Danny's Pearl and Danny's cape. Of the *Dahlia* cultivar Andries Wonder (a plant with a unique flower form which resembles that of a Chrysant). three flower colour mutants were released, namely "Maarse's Purple Wonder", "Maarse's Red Brown Wonder" and "Maarse's Golden Wonder" (this is a typical example, showing that by mutation breeding very rapidly genetic variation can be obtained without changing the unique genotype). Of *Streptocarpus* two mutants were released, namely c.v. Snow-white, a dwarfy mutant of c.v. Maassens White and c.v. Albatros, an autotetraploid of number 7111, a mutant of "Maassens White". (The autotetraploid was obtained by a combined usage of the adventitious bud technique and colchicine). Of onion, two selections from irradiated material were released namely "Compas" a hard onion with a tough skin which is less susceptible to neckrot (*Botrytus allii* Munn.), and "Brunette", which is early, moreover, and higher yielding.

Furthermore, a mutant of *Dactylis glomerata* L. (Cocksfoot or Orchard-grass), obtained in 1960, was entered into the list of varieties in 1972; its main advantage seems to be a lower silicic acid content which makes it more attractive for cattle.

Promising results were reported in the same or in other crops and it is expected that mutants will soon be released in *Alstroemeria*, *Begonia*, *Chrysanthemum*, *Lilium*, *Kalanchoë*, onion (*Allum cepa* L.) and *Poa*.

FOOD PRESERVATION BY IRRADIATION - J.G. VAN KOOIJ, H.G. HEINS, D.I. LANGERAK.

Sprout inhibition of onions by irradiation.

In continuation of previous investigations, attention was paid to the influence of the irradiation dose on the internal discolouration of the product.
For that purpose comparable dryed samples were irradiated on the same day with 8, 6, 4, 2 or 0 krad.
After the storage period a slight browning of the growing point was found in 57 - 70 % of the irradiated samples.
The sprout-inhibiting effectiveness tended to increase at decreasing doses in these preliminary experiments. A further study will consider this aspect and also aim at an evaluation of the internal discolouring.

Effects of heat (10 minutes 120°C) and 5 Mrad irradiation on vitamins in feed.

In relation to the wholesomeness testing programme, the decomposition of vitamins as the result of heating and irradiation was analysed. Results for gestation-feed indicated, that sterilization by heat caused losses up to 70% for B_1 and B_6 vitamins, while radappertization reduced vitamin E by 60%. Further results demonstrated that natural sources of E-activity in feed are very sensitive to irradiation, but added λ-tocopherol was not decomposed by 5 Mrad. In order to meet the vitamin requirements of pigs the feed was enriched before treatment with appropriate amounts of those vitamins, which appeared to become deficient in the respective feeds. The investigation was carried out in cooperation with the Central Institute for Nutrition and Food Research, Zeist, the Netherlands.

Identification of irradiated products on basis of gross and/or specific bacteriological examinations.

In cooperation with the Agricultural University, Laboratory of Food Microbiology and Food Hygiene, Wageningen, the Netherlands, investigations were carried out to evaluate the usefulness of bacteriological methods as an identification procedure for the process of irradiation. The methods were applied to strawberries. Although many questions are still to be answered as far as microbiological detection of irradiation is concerned, there is a strong evidence, that this kind of technique is promising as a quick method for identifying irradiation treatment in the future, especially when compared with the prospects of other sophisticated techniques.

Consumer-attitude studies towards irradiated food.

The practical application of food irradiation depends largely on consumer-acceptance of irradiated products. By means of opinion-polls such information can be obtained. Results of an enquiry amongst a representative group of housewifes have indicated, that approx. 40% of the questionees said to have heard from this process, and their opinion about irradiation varied from "good idea" (10%), "indifferent" (45%), towards "scrupulous" (40%). Another question about willingness to participate in tests with irradiated products received a positive comment by 60% of the housewifes.

Services to institutions on behalf of food irradiation.

The "Spelderholt Institute for Poultry Research", Beekbergen, the Netherlands, carried on with its programme on the radiation-resistance of microorganisms relevant to poultry preservation.
In cooperation with the Netherlands Institute for Dairy Research the radiation-sterilization of milkblancs was studied. The migration tests with irradiated cartons according to the "Packaging and Food-utensils Regulation" (Food Law) was carried out by the Central Institute for Nutrition and Food Research.
The Institute for Fishery Products TNO, IJmuiden, the Netherlands, continued its research programme on the radiation preservation of fishfillets and other marine products.
The irradiation of microbiological samples and/or food products used in above mentioned studies was supervised by researchworkers of the Association concerned, and executed by the irradiation facilities of the Pilot Plant for Food Irradiation, Wageningen.

SERVICES TO OTHER INSTITUTIONS, MAINLY IN THE NETHERLANDS.

Velocity measurements of granualr material in an elevator system - D.J. VAN ZUILICHEM, J.G. DE SWART.

Experiments were extended by measurements of the density of the flowing material, especially in the acceleration traject, with the help of a γ-absorption technique. A paper on the subject has been presented at the second International Conference on the pneumatic transport of solids in pipes. Extention to horizontal transport measurements will be desirable in the future programme.

Measurements of density variations of flowing granular material in silo systems - D.J. VAN ZUILICHEM, J.G. DE SWART.

Experiments are continued according to planning.
For determination of direction and velocity of the grains, measurements have been performed with a pulsed X-ray apparatus. After marking some grains with contrast material, direction of the material and, if the pulse-intervals are precisely known, the velocity can be derived from the exposed photographic film.

Measurements of residence-time distributions of liquid in a rotating thin film evaporator - H. AKSE, G.J. MOCKING, J.G. DE SWART.

Liquid foodproducts (eggs, coffee essences, fruitjuices) are partially dehydrated or concentrated by evaporation in thin-film units. About 1965, "Alfa Laval" introduced for this purpose a rotating thin-film evaporator with "ultra"-short residence times. This evaporator ("Centritherm") is schematically shown in figure 63.
The liquid foodproduct enters the evaporation unit via inlet pipe (1) and is spouted on the rotating cone (2) which is heated by steam. The liquid flows down as a very thin film by centrifugal forces (film-thickness 10^{-4} - 10^{-5} m). As a result of the very high velocities the residence time in this part of the apparatus is short ($\sim$ 0.5 s). Next, the liquid is collected at the bottomplate (3) and the concentrate is finally removed via the outlet pipe (5).
The apparatus causes some problems, contrary to the assertion of an "ultra" short residence time of the liquids. This assertion is probably

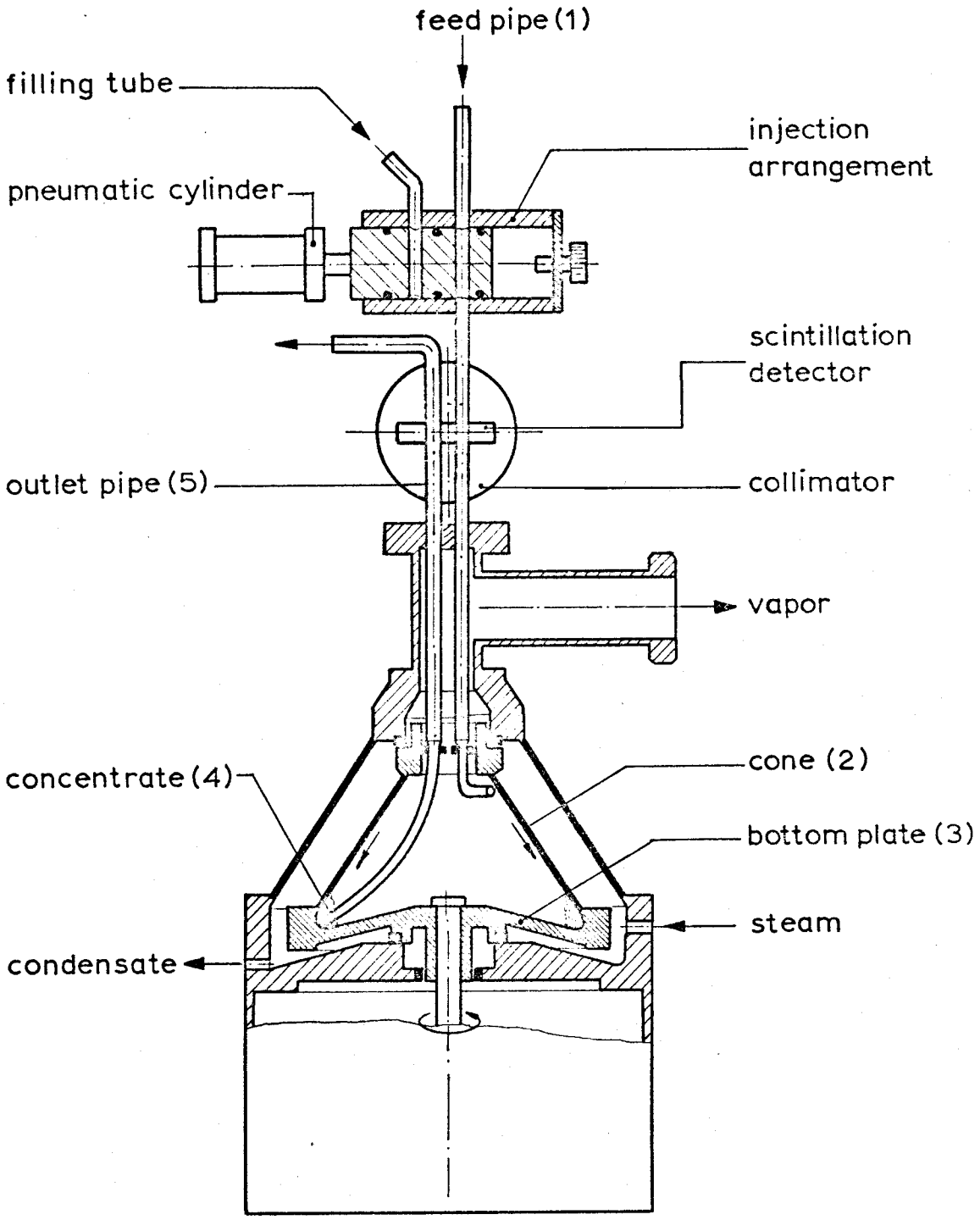


Figure 63 - Schematic representation of a centrifugal thin-film evaporator.

only based on the values of the residence times at the cone, where they are very short indeed with a very small variation of the values. However, the total residence times and their distribution may be determined by the conditions at the bottom plate of the apparatus, where the concentration is still exposed to the heating medium. This part of the apparatus must act as a perfectly mixing system with a certain number of perfect mixers.
The longer heat exposure causes extra destruction and/or loss of volatile components (flavour). This effect is mostly responsible for unwanted changes in quality and taste.
Apart from the liquid hold-up in the apparatus, which is an uncertain factor, the overall residence time and its distribution were unknown in such a process-unit. Factors which may influence the residence time distribution are: the rotational speed of the cone and the flowrate. The temperature level and the temperature difference between steamside and productside seem to be minor factors in this field.
The experiments were carried out with water as test medium. Copper-64, produced by neutron activation, is dissolved in water and brought into the continuous productstream by an injection arrangement.
The amount of the activity of each shot was about 2 mCi.
The shots form disturbances with the shapes of an impuls in the feed.
The diagram of such an impuls is shown in figure 65.
It is evident now, that its shape and its length approach a real impact-function. The response was detected by means of a collimated scintillation counter, a single channel spectrometer and a 200-channel analyser in time mode with a time base adjusted at 20 seconds over the 200 channels. The results were read out by a parallel printer.
A block diagram of the system is shown in figure 64. Diagrams, as shown in figures 65 and 66 were obtained and the residence time was determined by methods, usually applied in control-techniques. The average residence time and the residence time distribution were derived from the diagrams.
The results are presented in table 18.

Table 18 - The influence of flowrate and rotational speed on the average residence time (seconds) in the apparatus.

flowrate (10^{-5} m^3/s)	rot.speed	average res.time
	s^{-1}	s
2	25	7,6
3	25	4,9
4	25	4,8
3	15	6,3
3	25	4,9

The variation of the average residence times is only about 3 s. However, the range of residence times is not less than about 15 s, which is in contradiction with the demand for an optimal product treatment and at least unfavourable with respect to the purpose of the apparatus.
The residence time distribution in this evaporator is hardly affected by flowrate and rotational speed of the cone.

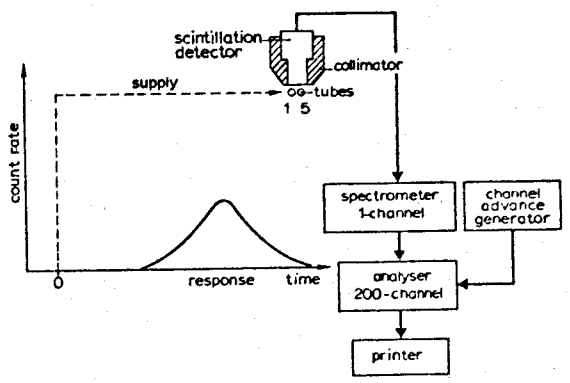


Figure 64 - Block-diagram of the experimental set-up.

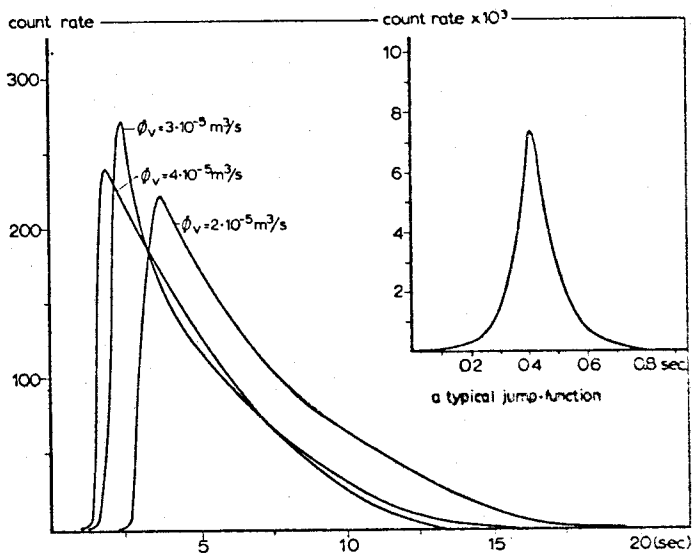


Figure 65 - Residence-timedistributions in a centrifugal thin-film evaporator (Centritherm) at different flowrates. Insert: A typical jump-function of the tracer in the inlet pipe.

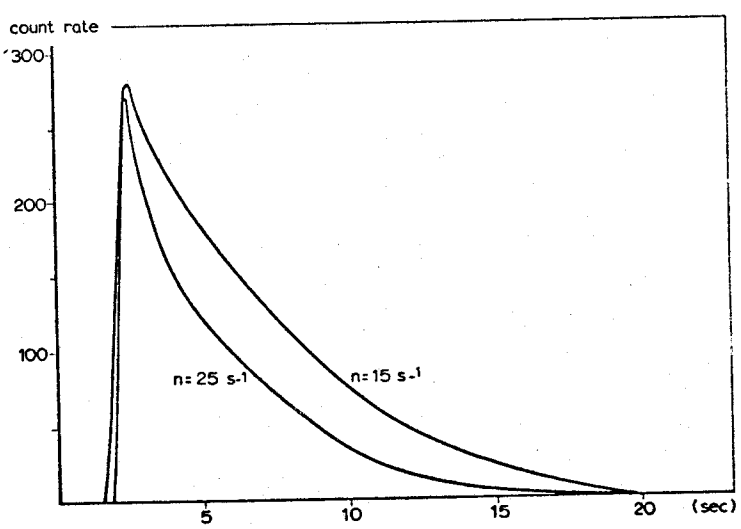


Figure 66 - Residence-time distribution in a centrifugal thin-film evaporator (Centritherm) at different rotational speeds of the cone.

Mathematical treatment of the results has shown that the flowsystem can be described as a system with four or five perfect mixers with a little tendency to a plugflow.

Moisture content measurements in plant leaves - J.G. DE SWART.

A two-channel system was built for ir. H. Challa, Centre for Plant Physiology Research, Wageningen.

A single channel γ-spectrometer with teletype read out - J.G. DE SWART.

This single channel γ-spectrometer with teletype read out has been built for the Department of Food Science, Agricultural University of Wageningen.

Measurement of the water-oil ratio in soils - P.G.C. DE JONG, J.G. DE SWART.

As part of its research programme, the Koninklijke/Shell Exploratie en Produktie Laboratorium, Rijswijk, carries out flow experiments in models of oil fields. For a proper scaling of such experiments continuous determination of the waterfraction (watercut) in the oil-water mixture of producing wells is essential.
This watercut is determined by measuring the absorption of low-energy γ-rays by the produced oil-water mixture. A strong low-energy γ-ray absorber is added either to the oil or to the water, in order to obtain sufficient precision within a reasonable measuring time.
With the assistance of the Association's Institute a series of experiments has been carried out in order to gain insight into the accuracy of the method under practical circumstances.
A 941 mCi ^{241}Am radioactive source has been used for these experiments. In the water NaI had been dissolved. For various NaI concentrations, production rates and watercuts the absorption of the γ-rays has been measured. Using Lambert-Beer's law, the relevant watercuts have been calculated. These data were compared with volumetrically obtained values.
For an oil-water mixture flowing trhough a glass tube of 0,8 cm inner diameter, the watercut could be determined within plus or minus 2 % absolute, using ten seconds counting time and a NaI concentration of 15% by weight.

Effect of quenching on the influence of wavelength-shifters in Cerenkov-counting of hard β-emitters - J.F. STOUTJESDIJK, W.F. PIETERS.

The counting efficiency of ^{32}P in water could be increased from 45 to 65% by addition of the wavelength-shifter 4-methylumbelliferone (100 mg/l), but by the presence of this compound the method becomes sensitive to chemical quenching: e.g. 20 g/l trichloroacetic acid gives a decrease in counting efficiency of 26%. Also $HClO_4$, H_2SO_4 and especially HNO_3 cause quenching. The channels ratio method proved to be suitable for quench corrections. The counting efficiency of ^{32}P in 40% H_2SO_4-solutions was 4% higher than in water, but in 90% H_2SO_4 6% lower than in water.

Determination of small amounts of fission products from tests with nuclear weapons and activated corrosion products from nuclear reactors in fish and fishery products - J.F. STOUTJESDIJK, W.F. PIETERS.

For the Ministry of Agriculture and Fisheries, fish and fishery-products will be investigated for the presence of radioactive contaminants. Preliminary experiments were performed to study the preparation of samples for counting with a new semi-conductor detector assembly. Suitable samples could be prepared by pressing with a hydrolic press 10 g of ash (from about 1 kg fish) to which 10 g of carbon black has been added, to cylinders with a diameter of 5 cm and a height of 1 cm (density 1 g/cm^3). The samples were measured with a Ge(Li)-semiconductor detector and a 4000 channel γ-spectrometer. According to preliminary experiments 10 - 50 pCi/kg of ^{51}Cr, ^{54}Mn, ^{59}Fe ^{57}Co, ^{60}Co, ^{65}Zn and ^{137}Cs could be detected. With a better leadcastle it is expected that the sensitivity of the method will be improved with a factor of at least two, whereas processing of a larger amount of ash may give a further increase in sensitivity.
The counting data will be processed by the PDP-11 computer which was delivered in November 1973.

Determination of metals in fish and fishery products - J.G. STOUTJESDIJK, W.F. PIETERS.

For the Ministry of Agriculture and Fisheries, fish and fishery-products were investigated for the presence of several metallic elements. Cr, Mn, Fe. Co, Cu, Zn and Hg were determined by the Soil Group with atomic absorption spectroscopy (AAS). Some analyses of Cr, Fe, Co, Zn and Hg were also performed by neutron activation analysis (NAA) by the Soil Group: good agreement between the two methods was found with Fe, Zn and Hg; for Cr NAA gave appreciably lower values than AAS; for Co NAA mostly gave somewhat higher values. The results of the analyses in 1973 are collected in table 19. Preparations have been made for neutron activation analysis of the samples with a 72 cm^3 Ge(Li) semi-conductor detector and processing of the data with an on-line PDP-11 computer of Digital Equipment.

Further cooperation - J.G. DE SWART, J.F. STOUTJESDIJK, W.F. PIETERS.

- With the Technical and Physical Engineering Research Service (T.F.D.L.), Wageningen, on behalf of the Department of Animal Science of the Agricultural University, Wageningen, to study the possibilities of automatic registration of electrical signals from muscles and movements of animals for correlation investigations.
- With H. de Regt, Department of Physics and Meteorology, Agricultural University of Wageningen, to study the possibilities of using positron-emitters for localization problems.
- With the Department of Virology, Agricultural University of Wageningen (Ir. P.W.L. Tas): determination of ^{125}I with a single channel γ-spectrometer and by liquid scintillations counting.
- Determination of algal growth in surface water: cooperation with Mr. W. Eenkhoorn of the Governmental Service for the IJsselmeerpolders. A method was developed to prepare samples for liquid scintillation counting with sufficient reproducibility and stability.

Table 19 - Contents of metals in fish and fishery-products determined by atomic absorption spectrometry.

Product	Sampling location	Sampling date 1973	Content in mg/kg fresh material								
			Cr	Mn	Fe	Co	Cu	Zn	As	Cd	Hg
Sole	Northsea near Texel	January	0,52	0,29	5,7	-*	-	5,8	2,7	<0,01	0,09
		April	0,32	0,11	8,9	-	0,42	5			0,09
		July	0,46	0,34	59	-	0,25	6			0,11
	Northsea near Scheveningen	January	0,59	0,24	6,7	-	-	9,7	2,5	<0,01	0,08
		April	0,33	0,13	6,9	-	0,35	5,4			0,16
		July	0,11	1,4	8,6	-	0,34	7			0,22
	Northsea near Breskens	April	0,26	0,13	6,9	-	0,35	5			0,23
		July	0,08	1,7	3,4	-	0,22	7			0,06
Pike-perch	IJssellake	January	0,77	0,21	14	-	-	4.6	0,02	<0,01	1,06
		July	-	0,08	3,4	-	0,09	6			0,83
Shrimps	Dollard	January	0,90	2,0	39	0,04	9,1	21		<0,01	0,16
		April	0,57	3,0	63	-	15,5	35			0,20
		July	0,32	2,7	25	-	8,8	21			0,06
	Northsea near Texel	January	1,1	1,6	42	0,03	6,2	23	2,6	<0,01	0,08
		April	0,41	1,6	62	-	2,9	33			0,13
		July	0,50	2,3	50	-	14,4	19			0,15
	Northsea near Breskens	January	1,1	1,7	74	0,05	8,9	22	2,9	<0,01	0,08
		April	0,98	2,4	88	-	12,8	34			0,14
		July	0,39	2,3	32	-	9,6	25			0,06
Mussels	Shallows	January	0,78	5,4	74	0,14	1,6	29	0,09	<0,01	0,13
		April	0,64	3,1	71	0,26	1,2	31			0,09
		July	1,11	12,0	240	0,32	1,2	32			0,07
	Oosterschelde	January	0,67	6,0	125	0,14	1,3	20	0,1	<0,01	0,14
		April	0,44	5,2	134	0,22	1,5	27			0,07
		July	0,70	5,9	127	0,24	1,3	18			0,04
Oyster	Oosterschelde	July	0,23	5,6	85	-	20,3	830			0,06

* - = not detectable.

Advices - J.G. DE SWART.

have been given to Drs. A.J.W. Visser, Department of Biochemistry of the Agricultural University, Wageningen, about the procuring of a nanosecond fluorescent decay measurement system, to several institutes about the purchase of liguid scintillation apparatus and to ir. J. Birnie of the Department of Physics of the Agricultural University, Wageningen about the advisability of revision and modification of an existing data aquisition system. After an extensive examination a negative advice was given.
With Prof. T.J. Schaafsma, Department of Molecular Physics of the Agricultural University of Wageningen, the labelling of virus material and algae with ^{2}H and ^{13}C for NMR-studies has been discussed.

With Drs. H. van Lonkjuysen, Institute for Cereals, Flour and Bread, TNO, Wageningen, the use of ^{14}C-monoglycerides for studying starch suspensions has been considered.

With scientists of the Deltadienst, Department for Environment Research, The Hague, the possibilities of the use of radionuclides have been discussed for the study of the pathway of phosphate movement in the river Rhine.

Work for the Coordination Commission on Radioactivity Measurements - J.F. Stoutjesdijk.

For the C.C.R.A. the annual report 1972 was prepared. As technical secretary of C.C.R.A. Stoutjesdijk was a member of a commission of the Health Council to prepare safety standards for the biosphere after nuclear accidents. This commission has met twice in 1973.

Radiation Protection - H.P. LEENHOUTS, P.H. DIGNUM.

The Radiation Protection Service group of the Association EURATOM-ITAL, authorized by the "Stralingsbeschermingscommissie Wageningen", performs the technical control of the work with radionuclides and ionizing radiation in the agricultural institutes and laboratories at Wageningen and its surroundings. Its field of control was expanded with the department of Zoology of the Agricultural University, which bought an X-ray machine.
The main activities of the group were:

The filmbadge service

In the agricultural complex Wageningen the number of workers controlled with a filmbadge dosimeter was 280; 156 of them were employed by the Association. All these radiological workers received less than the minimum detectable dose of 0,5 rem/year.

The transport and distribution of radioactive materials

The transport and distribution of radioactive materials in the working area of the group were done as usual. The most frequently required nuclides were:

^{3}H	44 mCi
^{32}P	45 mCi
^{51}Cr	1[illegible] mCi
^{203}Hg	10 mCi

Approximately two thirds of these radionuclides were used outside the Association's Institute.

The radioactive waste was collected by the group and treated by the Association's waste department. A total of 175 mCi of liquid waste was discharged to the river Rhine. The solid not destructable waste was transported to Mol (Belgium).

Radiation and radioactive contamination surveys

were performed on a regular schedule and on request. Special attention was paid to two open-air experiments. In one set up the speed of grain in an elevator system was measured using ^{64}Cu as a tracer and in another the movement of beatles was followed by detecting ^{192}Ir fixed on their back shields.

Assistance and advice

was given in cases of difficult or dangerous experiments. Special attention was paid to the transport and loading of an additional amount of ^{60}Co activity for the gamma source of the medical sterilization facility of Gammaster (Ede). The gamma source contains now 233.242 Ci of ^{60}Co.

No radiation accidents occurred which needed medical attention.

COURSES - J.F. STOUTJESDIJK.

General radioisotope course

A general radioisotope course has been organized from March 12 - 30, 1973 with 22 participants, of whom five members of the personnel of the Association, two students of the Agricultural University at Wageningen and one guest scientist from Jordan.

Health Physics course

In cooperation with the Health Physics Department of the Association a Health Physics course was organized, mainly for scientists of Institutes at Wageningen, who are responsible for radiochemical laboratories. The course was organized from April 9 to 13 during the mornings; 14 people participated, of whom two members of the Association.

Liquid scintillation courses

Two liquid scintillation courses have been organized from November 26 till December 7, and December 10 till 21, with 19 and 20 participants respectively. Among them were one Algerian, one Czechoslovakian and three Belgian participants and two members of the Association's personnel.

NEWSLETTERS

On the application of nuclear methods in Biology and Agriculture - J.F. STOUTJESDIJK.

In order to give more publicity to radiological methods suitable for agricultural and biological research a proposal has been made for issuing a Newsletter under the auspices of ESNA. A first experimental Newsletter was distributed in August and about 80 European scientists have responded favourably.

On incompatibility - A.J.G. VAN GASTEL, G.M.M. BREDEMEIJER.

Number 3 and 4 of this Newsletter were distributed during 1973.

PUBLICATIONS

BEELEN, R.H.J., A.M. FELDMANN and H.J.W. WIJSMAN. A regulatory gene and a structural gene for alaminase in *Escherichia coli*. Molec. gen. Genet. 121 (1973): 369-374.

BREDEMEIJER, G.M.M. Peroxidase activities and peroxidase isoenzyme patterns during growth and senescence of the unpollinated style and corolla of tobacco plants. Acta Bot. Neerl. 22 (1973): 40-48.

BROERSE, J.J. and K.J. PUITE. Comparisons of X-ray dosimetry for the coordination of late effects research in Europe. National and International Dose Intercomparisons IAEA-PL-479 (1973): 21-31.

BROERTJES, C. *Achimenes* en *Streptocarpus*. Nieuwe variëteiten door bestraling en colchicine-behandeling. Vakbl. Bloem. 28 (1973): 11.

BOZZINI, A. Analisi per resistenza alla carie di alcuni frumenti tetraploidi. XV Conv. S.I.G.A., Genetica Agraria (1973): 93-100.

CHADWICK, K.H. The choice of measurement wavelength for clear HX-perspex dosimetry. Dosimetry in Agriculture, Industry, Biology and Medicine. IAEA Vienna (1973): 563-568.

CHADWICK, K.H. Is dosimetry required in radiation processing. Dosimetry in Agriculture, Industry, Biology and Medicine. IAEA, Vienna (1973): 3-9.

CHADWICK, K.H., J.J. TEN BOSCH and W.F. VERHELST. Mechanism involved in the effect of temperature and light on the clear perspex dosimeter. Dosimetry in Agriculture, Industry, Biology and Medicine. IAEA, Vienna (1973): 569-576.

CHADWICK, K.H., W.R.R. TEN BROEKE and D. RINTJEMA. An intercomparison of read out systems for the clear perspex dosimeter. National and International Dose Intercomparisons. IAEA-PL-479/6 (1973): 33-40.

CHADWICK, K.H. and H.P. LEENHOUTS, The molecular target theory of cell survival and its application in radiobiology (EUR 4918-e (1973) 52 pp).

CHADWICK, K.H. and H.P. LEENHOUTS, Evidende for a dose rate effect in type B spermatogonia of the mouse at high dose rates. International Journal of Radiation Biology 23 (1973): 627-629.

CHADWICK, K.H. and H.P. LEENHOUTS. A molecular theory of cell survival. Phys. Med. Biol. 18 (1973): 78-87.

DAMMANN, W., M. ELMDUST and W. KÜHN. Kontinuierliche Bestimmung des Wasserdampfes im Boden durch Tritiummarkierung. Kerntechnik 7 (1973): 306-311.

DIRKSE, W.G. and G. SAUER. Some considerations concerning microlocalization in dehydrated and living plant material. I. Track autoradiography of soluble substances. Trans. of the IIIrd Symp. on Accum. and Transloc. of Nutr. and Regulators in Plant Organisms. Warzawa, Jablonna, Skierniewice, Brzezna, Krakow, 14-18th May (1973): 571-578.

ECOCHARD, R. and G. MERKX. A primary monosomic for chromosome 5 in the tomato. Caryologia 25 (1972): 531-536.

FRISSEL, M.J. and K.J.A. WIJNANDS-STÄB. Computer simulation of genetic manipulation as a means of controlling onion fly populations. "Transactions 7th IACA Congress on Hybrid Computation" Prague 1973.

FRITZ, A., M. BAUMER, E. ULONSKA and W. LENZ. A contribution to the analytical determination of the lysine content in barley. Nuclear Techniques for Seed Protein Improvement. Proc. Research Coordination Meeting IAEA/FAO/GSF, Neuherberg(Munich) 1972, IAEA, Vienna 1973.

FRITZ, A., E. ULONSKA, M. BAUMER and W. LENZ. Die serienmässige Bestimmung des Lysingehaltes von Getreideproteinen zum Zweck züchterischer Auslese. Bayer. Landw. Jahrbuch 50, Sonderheft 1, (1973): 145-153.

GASTEL, A.J.G. VAN. Incompatibility in higher plants. Report at the Mutation Breeding Contat Group Meeting, Grünbach. (1973).

GAUL, H., E. ULONSKA, V. LIND and H. WALTHER. Studies of selection for high protein and lysine content in barley mutants. Nuclear Techniques for Seed Protein Improvement. Proc. Research Coordination Meeting IAEA/FAO/GSF, Neuherberg (Munich) 1972, IAEA, Vienna (1973).

GEIJN, S.C. VAN DE. Some considerations concerning microlocalization in dehydrated and living plant material.
II. Beta spectrometric localization in living plants. Trans. of the IIIrd Symp. on Accum. and Transloc. of Nutr. and Regulators in Plant Organisms. Warzawa, Jablonna, Skierniewice, Brzezna, Krakow, 14-18th May (1973): 579-589.

GOTTSCHALK, W. The evolutionary qualification of some leaf mutants of Pisum. Egypt. J. Genet. Cytol. 2 (1973): 219-238.

GOTTSCHALK, W. Two highly pleiotropic genes showing a nearly agreeing action on the plant. Pisum Newsletter 5 (1973): 6-7.

GOTTSCHALK, W. The influence of the genotypic background on the action of the cochleata gene. Pisum Newsletter 5 (1973): 8-9.

GOTTSCHALK, W. and S.R. BAQUAR. Highly unstable translocations induced through irradiation in Pisum. Cytologia 38 (1973): 327-336.

GOTTSCHALK, W. and E. HASENBERG. The physiological capacity of some pea mutants in early developmental stages as related to their seed production. Pisum Newsletter 5 (1973): 9-10.

GOTTSCHALK, W. and M.M. IMAM. The yielding capacity of mutants under different climatic conditions. Ghana J.Sci. 13 (1973): 63-71.

GOTTSCHALK, W. and G. JAHR. The pollen tube growth of some Pisum mutants. Pisum Newsletter 5 (1973): 13-14.

GOTTSCHALK, W. and S. KUMAR. The yielding capacity of some pea mutants in Germany and India. Pisum Newsletter 5 (1973): 11-12.

GOTTSCHALK, W. and V. MILUTINOVIĆ. Untersuchungen zur Heterosis bei Selbstbefruchtern. I. Die Morphologie von Bastarden verschiedener Pisum-Mutanten im Vergleich zu den elterlichen Genotypen.Genetika (Beograd) 5 (1973): 59-72.

GOTTSCHALK, W. and V. MILUTINOVIĆ. Untersuchungen zur Heterosis bei Selbstbefruchtern. II. Die Samenproduktion und andere Leistungsmerkmale von Bastarden verschiedener Pisum-Mutanten im Vergleich zu den elterlichen Genotypen. Genetika (Beograd) 5 (1973): 117-134.

HARTEN, A.M. VAN. Annual Report Biology Programme Euratom 1972 (1973): 572-574.

HARTEN, A.M. VAN and H. BOUTER. Dihaploid potatoes in mutation breeding; some preliminary results. Euphytica 22 (1973): 1-7.

HARTEN, A.M VAN, H. BOUTER and B. SCHUT. Ivy leaf of potato (*Solanum tuberosum*), a radiation-induced dominant mutation for leaf shape. Radiation Botany, 13 (1973): 287-292.

HERMSEN, J.G.TH. Genetic analysis of self-compatibility in primary dihaploids from a potato cultivar. Potato Research 16 (1973): 316.

KEDINGER, M., C. MENDEL, K. HAFFEN, E. WITTENDORP et J.F. GRENIER. Survival of chick embryonic organs submitted to high hydrostatic pressures. Biomedicine, 18 (1973): 311-318.

KÜHN, W. and J. HANDL. Zur Erkennbarkeit von Fäulen in stehenden Bäumen durch Röntgendiagnose. Holzforschung 5 (1973): 27.

KÜHN, W. und H.P. SCHÄTZLER. Mass Determination of Plantations by Absorption of Gamma-Radiation. Atomkernenergie 21 (1973): 141-144.

KÜHN, W. und H.P. SCHÄTZLER. Moisture measurement in thin soil layers by absorption of gamma-rays in a capillary porous body. Soil-Moisture and Irrigation Studies II, IAEA, Vienna (1973).

LANGERAK, D.IS. and M.F.L. BRUURS. Preliminary study concerning the influence of combined heat and radiation treatment on the quality of some horticultural products. Acta Alimentaria 2 (1973): 229-243.

LAUDELOUT, H. La modélisation des processus pédologiques de drainage et d'irrigation. Hommes, Terre et Eaux, no. 7 (1973): 129-133.

LINSKENS, H.F. Activation of the ovary. Caryologia suppl. 24 (1973): 27-41.

LINSKENS, H.F. Accumulation in anthers. Proc. Res. Inst. of Pomol., Skierniewice, ser. E., 3 (1973): 91-106.

LINSKENS, H.F. and P.L. PFAHLER. In vitro germination and pollentube growth of maize (*Zea mays* L.) pollen. VIII storage temperature and pollen source effects. Planta 111 (1973): 253-259.

MONTI, L.M. and F. Saccardo. Radioinduced increase of recombination frequency in tomato. Tomato Genetics Cooperative 23 (1973): 24-25.

MOUGENOT, F. et A. GALLEZ. L'accumulation sélective du magnésium dans les sols: mécanisme et influence sur la croissance et la nutrition du ray-grass d'Italie. Ann. agron. 24 (1973): 457-464.

MÜLLER, H.P. Comparison of the amino acid composition of seed proteins with a "standard protein". Pisum Newsletter 5 (1973): 35.

MÜLLER, H.P. Content in globulins, albumins and essential amino acids in seeds of pea mutants and recombinants. Pisum Newsletter 5 (1973): 37.

MÜLLER, H.P. and W. GOTTSCHALK. Quantitative and qualitative situation of seed proteins in mutants and recombinants of *Pisum sativum*. Nuclear Techniques for Seed Protein Improvement. IAEA Vienna (1973): 235-253.

MÜLLER, H.P. and I. HENKE: Comparison between the quantitative determination of amino acids in seed proteins by microbiological methods and by an automatic amino acid analyzer. Pisum Newsletter 5 (1973): 38.

NETTANCOURT, D. DE, M. DEVREUX, A. BOZZINI, M. CRESTI, E. PACINI and G. SARFATTI. Ultrastructural aspects of the self-incompatibility mechanism in *Lycopersicon peruvianum* Mill. J. Cell Sci. 12 (1973): 403-419.

NETTANCOURT, D. DE, M. DEVREUX, U. LANERI, M. CRESTI, G. SARFATTI, E. PACINI. Ultrastructural aspects of unilateral incompatibility between *Lycopersicon peruvianum* and *L. esculentum*. Caryologia 25 (1973): 207-217.

NETTANCOURT, D. DE. Cytological aspects of self-incompatibility in *Lycopersicon peruvianum* and *Nicotiana alata*. Genetics 74 (1973): 61.

POELSTRA, P., D.W. BANNINK and J.G. DE SWART. An experimental approach to study the migration of pollutants in undisturbed soil columns. Agricultura 21 (1973): 147.

POELSTRA, P., M.J. FRISSEL, N. VAN DER KLUGT and D.W. BANNINK. Accumulation and distribution of mercury in Dutch soils. Neth. J. agr. Science 21, (1973): 77-84.

POELSTRA, P., M.J. FRISSEL, N. VAN DER KLUGT and W. TAP. Behaviour of mercury compounds in soils: accumulation and evaporation. Symposium on comparative aspects of food and environmental accumulation (FAO/IAEA/WHO), Helsinki. Sept. 1973.

ROBINSON, A.S. Increase in fertility, with repeated mating, of gamma irradiated male codling moths, *Laspeyresia pomonella* (L.). Can. J. Zool. 51 (1973): 427-430.

ROBINSON, A.S. and M.D. PROVERBS. Hybridization between geographical races of the codling moth. Can. Ent. 105 (1973): 284-290.

ROEST, S. and G.S. BOKELMANN. Vegetative propagation of *Chrysanthemum cinerariaefolium in vitro*. Scientia Horticulturae 1 (1973): 120-122.

SINNAEVE, J. and J. WIENEKE. Detailed proposal for a double labelling ion uptake experiment. Report of the joint sessions of the working group "Nuclear techniques in the study of soil-plant relationships" and the working group "Environmental pollution".

SNIEDER, D., G.W. ANKERSMIT and H.J. TER VELDE. Studies on the sterile male technique as a means of control of *Adoxophyes orana* F.R. (Lepidoptera, Tortricidae). 2. Dose response curves after irradiation of the moth with X-rays or fast neutrons. Neth. J. Pl. Path., 79 (1973): 148-155.

STROOSNIJDER, L. and J.G. DE SWART. Letter to the editor "Errors in soil bulk density and water content measurements with gamma ray attenuation". Soil Science Society of America Proceedings 37 (1973): 485.

TANG VAN HAI et J.P. ROLLAND. Cinétique de l'absorption du phosphore et du potassium par les plantes intactes d'arachides en solution diluées. Oléagineux no. 11 (1973): 517-520.

ULONSKA, E., H. GAUL, M. BAUMER and A. FRITZ. Breeding on protein quantity and quality in barley. Nuclear Techniques for Seed Protein Improvement. Proc. Research Coordination Meeting IAEA/FAO/GSF, Neuherberg (Munich) 1972, IAEA, Vienna 1973.

VERFAILLIE, G.R.M. The kinetics of chromium absorption by intact rice plants (*Oryza sativa* L. cv. Arborio). IV annual ESNA Meeting, Louvain, Belgium, 11-14 Sept. 1973.

WEBER, E. and W. GOTTSCHALK. Die Beziehungen zwischen Zellgrösse und Internodienlänge bei strahleninduzierten Pisum-Mutanten. Beitr. Biol. Pflanzen 49 (1973): 101-126.

WINTZERITH, M., E. WITTENDORP, M.E. ITTEL, R.V. RECHENMANN et P. MANDEL. Mise en évidence par trace-autoradiographie activée de la synthèse de D.N.A. nucléolaire dans le foie de rat adulte. C.R. Acad. Sc. Paris, 24 septembre 1973, t. 277 Série D, 1033-1036.

WITTENDORP, E. Nouvelles procédures ionographiques - Premières applications microdosimétriques et autoradiographiques. Thèse de Doctorat (Mention Sciences): Université Louis Pasteur de Strasbourg (24-11-1973).

ZUILICHEM, D.J. VAN, G.H. BLEUMINK, J.G. DE SWART. Slip velocity measurements by radio-tracertechniques in vertical conveying systems of different pipe diameters. Proceedings of the second international conference on the pneumatic transport of solids in pipes. "Pneumotransport 2", Guilford, Surrey, England (1973).

ZUILICHEM, D.J. VAN, J.G. DE SWART, G. BUISMAN. Residence time-distribution in an extruder, Lebensm. Wiss. Technol., 6 (1973): 184.

I N P R E S S

BECKING, J.H. Nitrogen fixation in some natural ecosystems in Indonesia. In Proceedings International Synthesis meeting Biologica Nitrogen Fixation and the Biosphere, Edinburgh, September 1973, International Biological Programme, Cambridge Univ. Press.

BECKING, J.H. Putative nitrogen fixation in other symbioses. In "Biological Nitrogen Fixation" (Ed. A. Quispel), North-Holland Publ. Co., Amsterdam.

BECKING, J.H. N_2 fixing root associations in higher plants other than Legumes. In "Dinitrogen (N_2) Fixation", vol 2 (Ed. R.W.F. Hardy), John Wiley Sons, U.S.A.

BOZZINI, A. Analysis for bunt resistance in some tetraploid wheats. Eucarpia Meet. on Genetics and Breeding of *durum* wheat, Bari (Italy) (1973).

BROERTJES, C. The production of polyploids using the adventitious bud technique. FAO/IAEA/Eucarpia Conf. on Mutations and Polyploidy, Bari (Italy), Oct. 1972.

BUNNENBERG, C., W. KÜHN. Movement of water vapor in arid loess soil under the condition of temperature inversions. Proc. Symp. on Isotopes and Radiation Techniques in Studies of Soil Physics, Irrigation and Drainage in Relation to Crop Production. Vienna, 1-5 October 1973, IAEA-SM-176/7.

CHADWICK, K.H. A standard procedure for the use of the clear perspex HX dosimeter in the dose range 1-10 Mrad. IAEA panel and Code of Practice for the Radiation Sterilization of Biomedical Products.

CHADWICK, K.H. and H.P. LEENHOUTS. A common molecular mechanism in radiobiology? Its implications in radiological protection. Presented at 3rd International Congress of the Radiological Protection Association, Washington (1973).

CHADWICK, K.H. and H.P. LEENHOUTS. Chromosome aberrations and cell death. Presented at 4th Microdosimetry Symposium, Verbania Pallanza (1973).

DONK, J.A.W.M. VAN DER. Differential RNA synthesis 24 h after self- and crosspollination in *Petunia hybrida* L.

DONK, J.A.W.M. VAN DER. The synthesis of RNA and proteins as a function of the time of pollentube-style interaction after cross- and self-pollination of *Petunia hybrida* L.

JAROS, G.G., M. SENSENBRENNER, E. WITTENDORP, R.V. RECHENMANN et P. MANDEL. Prolifération des cellules nerveuses en culture. Etude trace-autoradiographique activée de l'incorporation de thymidine ^{14}C. C.R. Acad. Sc. Paris.

FRISSEL, M.J., P. POELSTRA and N. VAN DER KLUGT. The contamination of dutch soils with mercury and a few other heavy metals. Geologie en Mijnbouw.

GASTEL, A.J.G. VAN and D. DE NETTANCOURT. The effects of different mutagens on self-incompatibility in *Nicotiana alata* Link and Otto. I. Chronic gamma irradiation. Radiation Botany.

GEIJN, S.C. VAN DE. In depth localization of beta-emitting isotopes; Dependence of the range of applicability upon maximum energy and complexity of the spectrum. Nucl. Instr. and Meth.

GEIJN, S.C. VAN DE. On the determination of electron energy losses in absorbers. A study of the ^{137}Cs spectrum. Nucl. Instr. and Meth.

GOTTSCHALK, W. and M. MILUTINOVIĆ Trisomics from desynaptic mutants. The Nucleus.

LANGERAK, D. IS. The influence of irradiation and packaging in the keeping quality of prepacked vegetables. Acta Alimentaria.

LEENHOUTS, H.P. and K.H. CHADWICK. DNA double strand breaks and chromosome aberrations. Theoretical and Applied Genetics (1973).

LEENHOUTS, H.P. and K.H. CHADWICK. The RBE-LET Relationship. Presented at 4th Microdosimetry Symposium, Verbania Pallanza (1973).

LEENHOUTS, H.P. and K.H. CHADWICK. A theoretical analysis of radiation sensitivity in cells following neutron irradiation. Presented at IAEA Symposium on the Effects of Neutron irradiation upon cell function. Munich (1973).

LIND, V. and H. GAUL. Mutagen induced mildew resistance in barley. Report,Meeting of the Mutation Breeding Contact Group, Association EURATOM-ITAL, Wageningen, Grünbach 6-8 Nov. 1973.

LINSKENS, H.F. and E. SONDHEIMER. Control of in vitro germination and tube extension of *Petunia hybrida* pollen. Proc.Kon.Ned.Akad.Wet.

MONTI, L.M., F. SACCARDO, L. TOMARCHIO. Resistenza alle tracheomicosi ed alla peronospora nel genere *Lycopersicon* ed in *Solanum pennellii*. XVII Cong. S.I.G.A., Piacenza, Genetica Agraria (1973).

MONTI, L.M. and F. SACCARDO. The modification by acute irradiation of recombination frequencies in tomatoes. FAO/IAEA/EUCARPIA Conf. on Mutation and Polyploidy, Bari (Italy) (1972).

NETTANCOURT, D. DE, F. SACCARDO, U. LANERI, E. CAPACCIO, M. WESTERHOF and R. ECOCHARD. Self-compatibility in a spontaneous tetraploid of *Lycopersicon peruvianum* Mill. Proceed. FAO/IAEA/EUCARPIA, Symp. Bari 1972.

NETTANCOURT, D. DE, F. CARLUCCIO, A.J.G. VAN GASTEL. On a possible involvement of chromosome 3 in the formation of self-compatibility mutations in *Nicotiana alata*. Proceed. FAO/IAEA/EUCARPIA Symp., Bari 1972.

NETTANCOURT, D. DE, M. DEVREUX, U. LANERI, E. PACINI, M. CRESTI, G. SARFATTI. Genetical and ultrastructural aspects of self- and crossincompatibility in interspecific hybrids between self-compatible *L. esculentum* and self-incompatible *L. peruvianum*. Theoretical and Applied Genetics.

POELSTRA, P. and M.J. FRISSEL. Report on experiments with chromium. Contribution on the meeting: heavy metals in the food chain and in the biosphere. Ispra, December 1973.

PUITE, K.J. and D.L.J.M. CREBOLDER. Energy dependence of thermoluminescent dosimeters for X-ray and dose distribution measurements in a mouse phantom. Phys. Med. Biol.

RAMANNA, M.S. and J.G. TH. HERMSEN. Unilateral "eclipse sterility" in reciprocal crosses between *Solanum verrucosum* Schlechtd. and diploid *S. tuberosum* L. Euphytica.

ROBINSON, A.S. and C.F. CURTIS. Controlled crosses and cage experiments with a translocation in *Drosophila*. Genetica.

ROBINSON, A.S. Gamma radiation and insemination in the codling moth, *Laspeyresia pomonella*. Ent. Exp. and Appl.

SACCARDO, F. and L.M. MONTI. The isolation of aneuploids originated from induced unstable chromosome aberrations. FAO/IAEA/EUCARPIA conf. on Mutation and Polyploidy, Bari (Italy).(1972).

STROOSNIJDER, L. and J.G. DE SWART. Column scanning with simultaneous use of ^{241}Am and ^{137}Cs gamma radiation. Soil Science.

TOMARCHIO, L. Fonti di resistenza e tecniche per la selezione di linee resistenti ad alcune malattie in pomodoro. XVII Cong. S.I.G.A., Piacenza, Genetica Agraria (1973).

VERFAILLIE, G.R.M. The kinetics of chromium absorption by intact rice plants (*Oryza sativa* L. cv. Arborio). FAO/IAEA/WHO Symposium on Nuclear Techniques in Comparative Studies of Food and Environmental Contamination, Otaniemi, Finland, 27-31 Aug. 1973.

WALTHER, H., H. GAUL, E. ULONSKA and K.H. SEIBOLD. Variation and selection of protein and lysine mutants in spring barley. 2nd Research Co-ordination Meeting on the Use of Nuclear Techniques for Seed Protein Improvement, Ibadan, Nigeria, 1973.

WALTHER, H. and H. GAUL. Production and selection of barleys with increased protein quantity and improved protein quality. Report, Meeting of the Mutation Breeding Contact Group, Association EURATOM-ITAL, Wageningen, Grünbach, 6-8 Nov. 1973.

WALTHER, H. Methodische Untersuchungen zur Proteinbestimmung in induzierten Gerstemutanten. 3. Arbeitstagung für Genetik der GSF, Grünbach, 24. - 25. Mai 1973, GSF Bericht.

INTERNAL REPORTS 1973

No. 134 -	H. STEGEMAN	Report on the spore group meeting of the British Spore Group, Leeds 18-19th December 1972.
No. 135 -	G. DESMET	Report of the meeting "Chromium in the food chain" Wageningen, 14-15th December 1972.
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EXTERNAL REPORTS 1973

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No. 11 -	G. DE BEKKER	Consumentenonderzoek bestraalde verse kip.
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No. 13 -	D.IS. LANGERAK R. HOVESTAD	Influence of handling, packaging and irradiation on the keeping quality of prepacked cut chicory. Season 1973.

INCOMPATIBILITY NEWSLETTER

I. BOS	Population genetics of the S-alleles of a sporophytic incompatibility system. Incompatibility Newsletter 3 (1973): 67-70.
G.M.M. BREDEMEIJER	Peroxidases in un-, self- and cross-pollinated tobacco styles. Incompatibility Newsletter 3 (1973): 55-57.
A.J.G. VAN GASTEL, M.A. BEEK and J.H. VAN DER VEEN	The effects of EMS-treatment of seeds on self-incompatibility in *Nicotiana alata.* Incompatibility Newsletter 2 (1973): 62-66.
F.A. HOEKSTRA	In vitro germination of *Compositae* pollen. Incompatibility Newsletter 3 (1973): 47.
F.A. HOEKSTRA	Exogenous factors, influencing pollen viability of *Chrysanthemum cinerariaefolium* Vis. Incompatibility Newsletter No. 3 (1973): 49.
F.A. HOEKSTRA	Respiration and vitality of bi- and tri-nucleate pollen. Incompatibility Newsletter No. 3 (1973): 52.
H.F. LINSKENS and A.W. SPANJERS	Changes of the electrical potential in the transmitting tissue of Petunia styles after cross- and selfpollination. Incompatibility Newsletter No. 3 (1973): 81-8

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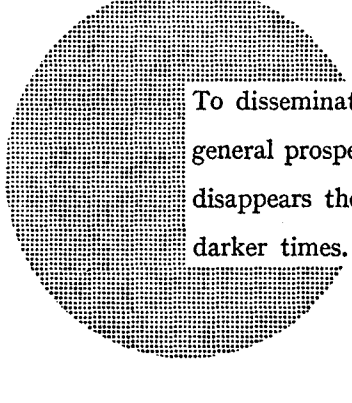

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