

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

EURATOM

Årsberetning 1974

PROGRAM BIOLOGI - SUNDHEDSBESKYTTELSE

Jahresbericht 1974

PROGRAMM BIOLOGIE - GESUNDHEITSSCHUTZ

Annual Report 1974

PROGRAMME BIOLOGY - HEALTH PROTECTION

Rapport Annuel 1974

PROGRAMME BIOLOGIE - PROTECTION SANITAIRE

Relazione Annuale 1974

PROGRAMMA BIOLOGIA - PROTEZIONE SANITARIA

Jaarverslag 1974

PROGRAMMA BIOLOGIE - GEZONDHEIDSBESCHERMING

II

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II

EUR 5332 d-e-f-i-n

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

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INDLEDNING

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Tre staters tiltrædelse af De europæiske Fællesskaber den 1. januar 1973 har gjort det nødvendigt at tilpasse programmet "Biologi - sundhedsbeskyttelse" til den nye situation, der hermed er opstået. Afsnittet "Strålingsbeskyttelse" blev tilpasset ved en afgørelse i Ministerrådet den 14. maj 1973, og kontrakter med britiske, danske og irske organer trådte i kraft i 1974. Følgelig indeholder dette bind for første gang rapporter over de fremskridt, der er gjort i de nye medlemsstater, og konkretiserer dermed den gradvise integrering af forskningen, som blev foreslået af Kommissionen i dennes udkast til en tilpasning af programmet.

For så vidt angår afsnittet "Anvendelser", et supplerende program med Tyskland, Italien og Nederlandene som deltagere, blev dette tilpasset af Ministerrådet den 2. august 1974, som besluttede, at Danmark og Irland skulle deltage i programmet samtidig med de tre oprindeligt deltagende stater.

Det må ligeledes bemærkes, at en aktiv forberedelse af et udkast til forslag om et nyt program for 1976-1980 har fundet sted i løbet af anden halvdel af 1974. Adskillige studiegrupper er mødtes og har for programmets forskellige forskningsemner foretaget en undersøgelse af den nuværende viden, de fremtidige behov og orienteringen af det fremtidige arbejde. I denne sammenhæng har der fundet en første udveksling af synspunkter sted vedrørende de store linier i et fremtidigt program under det møde, som i december 1974 afholdtes i Det rådgivende udvalg for Programforvaltning "Biologi - sundhedsbeskyttelse". (Det blev meget frugtbringende for Kommissionens tjenestegrene, for hvilke det blev muligt at tage et vigtigt skridt fremad med hensyn til forberedelsen af forslagsudkastet).

For så vidt angår selve programmet er det værd at erindre, at det består af et afsnit benævnt "Strålingsbeskyttelse" (fællesprogram) og et afsnit benævnt "anvendelser" (supplerende program), hvis forskningsområder og hvis mål kan skitseres som følger:

1. Måling og vurdering af den ioniserende stråling, som mennesket og forskellige dele af omgivelserne udsættes for:

- dosimetri, strålingsmåling og fortolkning af måleresultaterne;
- undersøgelse af transporten og ophobningen af radionukleider i mennesket og i omgivelsernes bestanddele.

2. De ioniserende strålers vekselvirkning med de biologiske systemer:

- strålingens primærvirkninger
- virkninger på generne
- kortsigtede virkninger
- langsigtede virkninger.

3. Anvendelser af kerneteknik inden for visse af den landbrugsvidenskabelige og medicinske forsknings vigtigste områder.

Dette dokument indeholder "fremskridtsrapporterne" for kontraktprogrammets enkeltprojekter og for gruppen Biologi i Ispra.

F. VAN HOECK

P. RECHT

EINLEITUNG



Durch den Beitritt der drei neuen Mitgliedstaaten zu den Europäischen Gemeinschaften am 1. Januar 1973 wurde es notwendig, das Programm "Biologie - Gesundheitsschutz" der hierdurch entstandenen neuen Situation anzupassen. Der Sektor "Strahlenschutz" wurde durch einen Ministerratsbeschluß vom 14. Mai 1973 geändert, und die Verträge mit den britischen, dänischen und irischen Institutionen traten 1974 in Kraft. Der vorliegende Jahresbericht enthält erstmals Forschungsergebnisse aus den neuen Mitgliedstaaten; die schrittweise Integrierung der Forschung, die die Kommission in ihrem Entwurf zur Anpassung des Programms vorgeschlagen hatte, nimmt damit konkrete Form an.

Der Sektor "Anwendungen", ein Ergänzungsprogramm, an dem die Bundesrepublik Deutschland, Italien und die Niederlande beteiligt sind, wurde vom Ministerrat am 2. August 1974 angepaßt, mit der Maßgabe, daß Dänemark und Irland zusammen mit den drei ursprünglichen Mitgliedstaaten teilnehmen.

Zu erwähnen ist ferner die Vorbereitung des Entwurfs eines neuen Forschungsprogramms für die Jahre 1976-1980 während der zweiten Hälfte 1974. Mehrere Studiengruppen trafen sich und untersuchten für die einzelnen Themen und Punkte des Forschungsprogramms den Stand der Kenntnisse, den künftigen Bedarf und welche Leitlinien für die Zukunft zu verfolgen seien. Desgleichen fand auf der Sitzung des Beratenden Programmausschusses "Biologie - Gesundheitsschutz" vom Dezember 1974 ein erster Gedankenaustausch über die Schwerpunkte des zukünftigen Programms statt. Die Dienststellen der Kommission gewannen daraus Anregung und Unterstützung für die weiterzuführende Vorbereitung des Programmvorschlages.

Was das Programm selbst betrifft, so sei bemerkt, daß es einen Abschnitt "Strahlenschutz" (gemeinsames Programm) und einen Abschnitt "Anwendungen" (Ergänzungsprogramm) umfaßt, deren Forschungsbereiche und Ziele nach folgendem Schema dargestellt werden können:

1. Messung und Bewertung der Belastung des Menschen und seiner Umwelt durch ionisierende Strahlungen:
 - Dosimetrie, Strahlenmessung und ihre Interpretation;
 - Untersuchung des Transports und der Anreicherung der Radionuklide im Menschen und in seiner Umwelt.

2. Wechselwirkung der ionisierenden Strahlungen mit den biologischen Systemen:
 - Primärwirkungen der Strahlungen
 - Wirkungen auf das Erbgut
 - Kurzzeitwirkungen
 - Langzeitwirkungen.

3. Anwendungen der nuklearen Techniken auf bestimmte wichtige Sektoren der agronomischen und medizinischen Forschung.

Das vorliegende Dokument enthält die "Berichte über den Fortgang der Arbeiten" an den einzelnen Projekten des Vertragsprogramms und die Berichte der Gruppe Biologie Ispra.

F. VAN HOECK

P. RECHT

INTRODUCTION

E

The accession of three States to the European Communities on 1 January 1973 made it necessary to adapt the programme "Biology - Health Protection" to the new situation which thus arose. The "Radiation Protection" part was adjusted by a decision of the Council of Ministers of 14 May 1973, and contracts with British, Danish and Irish bodies came into effect in 1974. As a result, this volume presents for the first time progress reports from the new Member States, setting out in concrete form the progressive integration of research which had been proposed by the Commission in its draft programme adjustment.

The "Applications" part, a supplementary programme in which the Federal Republic of Germany, Italy and the Netherlands participated, was adjusted by the Council of Ministers on 2 August 1974, which decided that Denmark and Ireland would participate at the same time as the three original Member States.

It must also be indicated that the active preparation of a draft proposal for a new 1976-1980 programme was effected during the latter half of 1974. Several study groups met and studied, with regard to the various research themes and subjects of the programme, the state of the art, future requirements, and the guidelines to be adopted for the future. In the same context, an initial discussion on the broad outline of a future programme was held during the meeting of the Advisory Committee on Programme Management "Biology - Health Protection" in December 1974. This discussion turned out to be most rewarding for the departments of the Commission concerned, whom it enabled to take an important step in the preparation of the draft proposal.

With regard to the programme itself, it should be mentioned that it includes a "Radiation Protection" (common programme) sector and an "Applications" (supplementary programme) sector whose fields of research and objectives can be outlined as follows:

1. Measurement and evaluation of the exposure of man and the various components of the ambient environment to ionizing radiation:
 - dosimetry, radiation measurements and their interpretation;
 - study of the transfer and accumulation of radionuclides in man and in the constituents of the environment.

2. Interaction of ionizing radiations with biological systems:
 - primary effects of radiation;
 - effects on hereditary material;
 - short-term effects;
 - long-term effects.

3. Application of nuclear techniques in certain important sectors of agricultural and medical research.

This document presents the "progress reports" for each project of the contractual programme and of the Biology Group Ispra.

F. VAN HOECK

P. RECHT

INTRODUCTION

F

L'adhésion de trois Etats aux Communautés européennes, le 1er janvier 1973, a rendu nécessaire l'adaptation du programme "Biologie - Protection sanitaire" à la nouvelle situation ainsi créée. Le secteur "Radioprotection" fut aménagé par une décision du Conseil de Ministres du 14 mai 1973, et des contrats avec des organismes britanniques, danois et irlandais prirent cours en 1974. Par conséquent, le présent volume présente pour la première fois des rapports d'avancement en provenance des nouveaux Etats membres, concrétisant l'intégration progressive des recherches qui avait été proposée par la Commission dans son projet d'aménagement du programme.

Quant au secteur "Applications", programme complémentaire auquel participaient l'Allemagne, l'Italie et les Pays-Bas, il fut aménagé par le Conseil de Ministres le 2 août 1974, qui décida que le Danemark et l'Irlande participeraient au programme en même temps que les trois anciens Etats participants.

Il convient également de signaler qu'une préparation active d'un projet de proposition pour un nouveau programme 1976-1980 a eu lieu au cours de la deuxième moitié de 1974. Plusieurs groupes d'études se sont réunis et ont étudié, pour les divers thèmes et sujets de recherche du programme, l'état des connaissances, les besoins futurs, et les orientations à adopter pour l'avenir. Dans la même optique, un premier échange de vue sur les grandes lignes d'un futur programme a eu lieu au cours de la réunion du Comité consultatif en matière de gestion de programmes "Biologie - Protection sanitaire" de décembre 1974. Il fut très fructueux pour les services de la Commission, auxquels il permit de franchir un pas important dans la préparation du projet de proposition.

En ce qui concerne le programme lui-même, il convient de rappeler qu'il comporte un secteur "Radioprotection" (programme commun) et un secteur "Applications" (programme complémentaire) dont les domaines de recherche et les objectifs peuvent être schématisés comme suit:

1. Mesure et évaluation de l'exposition de l'homme et des diverses composantes du milieu ambiant aux rayonnements ionisants:
 - dosimétrie, mesure des rayonnements et leur interprétation;
 - étude du transfert et de l'accumulation des radionucléides dans l'homme et dans les éléments du milieu.

2. Interaction des rayonnements ionisants avec les systèmes biologiques:
 - effets primaires des rayonnements
 - effets sur le matériel héréditaire
 - effets à court terme
 - effets à long terme.

3. Applications des techniques nucléaires à certains secteurs importants de la recherche agronomique et médicale.

Le présent document présente les "rapports d'avancement" par projet individuel du programme contractuel et du groupe de Biologie installé à Ispra.

F. VAN HOECK

P. RECHT

INTRODUZIONE

L'adesione di tre Stati alle Comunità europee, avvenuta il 1° gennaio 1973, ha reso necessario procedere ad un adeguamento del programma "Biologia-Protezione sanitaria" alla nuova situazione creatasi. Il settore "Radioprotezione" è stato riorganizzato con la decisione del Consiglio dei Ministri del 14 maggio 1973 e nel 1974 sono entrati in vigore contratti con organismi britannici, danesi e irlandesi. Pertanto, questo volume presenta per la prima volta delle relazioni di avanzamento dei lavori, provenienti dai nuovi Stati membri, dando forma concreta al progressivo inglobamento delle ricerche come proposte dalla Commissione nel suo progetto di riorganizzazione del programma.

Per quanto riguarda il settore "Applicazioni", il programma complementare al quale partecipavano la Germania, l'Italia e i Paesi Bassi, è stato rimaneggiato dal Consiglio dei Ministri il 2 agosto 1974 con una decisione che prevedeva la partecipazione della Danimarca e dell'Irlanda al programma a fianco dei tre Stati membri precitati.

Occorre segnalare inoltre che durante la seconda metà del 1974 ha avuto luogo una preparazione attiva di un progetto di proposta per un nuovo programma 1976-1980. Vari gruppi di studio si sono riuniti per esaminare per i diversi temi e soggetti di ricerca del programma lo stato delle conoscenze, i fabbisogni futuri e gli orientamenti da seguire in avvenire. Attenendosi allo stesso punto di vista, nel corso della riunione del dicembre 1974 del Comitato consultivo in materia di gestione dei programmi "Biologia-Protezione sanitaria", ha avuto luogo un primo scambio di vedute circa le grandi linee direttrici di un programma futuro. Tale riunione è stata molto utile ai servizi della Commissione, che hanno potuto compiere un grande passo avanti nella preparazione delle proposte.

Circa il programma, si ricorda che esso comporta una sezione "Radio-protezione" (programma comune) e una sezione "Applicazioni" (programma complementare), i cui settori di ricerca e gli obiettivi possono essere schematizzati nel modo seguente:

1. misura e valutazione dell'esposizione dell'uomo e dei vari componenti dell'ambiente alle radiazioni ionizzanti:
 - dosimetria, misura delle radiazioni e interpretazione dei risultati;
 - studio del passaggio e dell'accumulazione dei radionuclidi nell'uomo e negli elementi dell'ambiente;

2. interazione delle radiazioni ionizzanti con i sistemi biologici:
 - effetti primari delle radiazioni
 - effetti sul materiale ereditario
 - effetti a breve termine
 - effetti a lungo termine;

3. applicazione delle tecniche nucleari in alcuni importanti settori della ricerca agronomica e medica.

Il presente documento contiene le relazioni sull'avanzamento dei singoli progetti del programma contrattuale e del gruppo biologia con sede ad Ispra.

F. VAN HOECK

P. RECHT

INLEIDING

Door de toetreding van de drie staten tot de Europese Gemeenschappen op 1 januari 1973 moest het programma "Biologie - bescherming van de gezondheid" aan de nieuwe situatie worden aangepast. De sector Stralingsbescherming werd opnieuw geordend door een besluit van de Raad van Ministers van 14 mei 1973, en contracten met Britse, Deense en Ierse organisaties begonnen in 1974 te lopen.

Bijgevolg omvat dit volume voor de eerste maal rapporten over de vordering van het onderzoek, die afkomstig zijn van de nieuwe lid-staten, en waarin de geleidelijke integratie wordt geconcretiseerd van de onderzoeken, die door de Commissie in haar ontwerp tot wijziging van het programma werd voorgesteld.

De sector Toepassingen, een aanvullend programma waaraan Duitsland, Italië en Nederland deelnamen, werd op 2 augustus 1974 opnieuw door de Raad van Ministers geordend : de Raad besloot dat Denemarken en Ierland tegelijkertijd met de drie al eerder deelnemende staten aan het programma zouden deelnemen.

Er moet tevens worden vermeld dat tijdens de tweede helft van 1974 het ontwerp-voorstel voor een nieuw programma 1976-1980 intensief werd voorbereid. Een aantal studiegroepen zijn bijeengekomen en hebben voor de verschillende thema's en onderzoekonderwerpen van het programma een studie gemaakt over de stand van de kennis, de toekomstige behoeften, en de richtlijnen die voor de toekomst moeten worden goedgekeurd. Uit hetzelfde oogpunt, heeft tijdens de vergadering van het raadgevend comité op het gebied van het programmabeheer "Biologie - bescherming van de gezondheid" van december 1974 een gedachtenwisseling plaatsgevonden over de grote lijnen van een toekomstig programma. Deze gedachtenwisseling was heel nuttig voor de diensten van de Commissie die hierdoor een belangrijke stap vooruit konden zetten bij de voorbereiding van het ontwerp-voorstel.

Wat het programma zelf betreft, moet erop gewezen worden dat dit een sector Stralingsbescherming (gemeenschappelijk programma) omvat en een sector Toepassingen (aanvullend programma), waarvan de gebieden en doelstellingen als volgt in een schema kunnen worden samengevat :

1. Meting en evaluatie van de blootstelling van de mens en verschillende componenten van het omgevingsmilieu aan ioniserende straling :
 - dosismeting, stralingsmeting en interpretatie hiervan;
 - studie van de overdracht en accumulatie van de radionucliden bij de mens en bij de milieucomponenten.

2. Interactie van de ioniserende stralingen met de biologische systemen :
 - primaire effecten van stralingen
 - effecten op het erfelijk materiaal
 - effecten op korte termijn
 - effecten op lange termijn.

3. Toepassingen van de nucleaire technieken op bepaalde belangrijke sectoren van het agronomisch en medisch onderzoek.

Dit document omvat de "rapporten over de vordering van het onderzoek" per individueel project van het contractueel programma en van de groep Biologie te Ispra.

F. VAN HOECK

P. RECHT

Mitglieder im Jahr 1974 des Beratenden Programmausschusses
"BIOLOGIE - GESUNDHEITSSCHUTZ"

Members in 1974 of the Advisory Committee on Programme Management
"BIOLOGY - HEALTH PROTECTION"

Membres en 1974 du Comité consultatif en matière de gestion de programmes
"BIOLOGIE - PROTECTION SANITAIRE"

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FORSCHUNGSTÄTIGKEIT ANWENDUNGEN LANDWIRTSCHAFT

RESEARCH ON APPLICATIONS IN AGRICULTURE

RECHERCHES RELATIVES AUX APPLICATIONS AGRONOMIQUES

MUTAGENESE, BODEN-PFLANZEN BEZIEHUNGEN,
STRAHLENANALYSE, LEBENSMITTELKONSERVIERUNG

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

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

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

Nummer van het contract: 094-72-1 BIAN

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

Algemeen onderwerp van het contract:

APPLICATIONS OF NUCLEAR METHODS IN AGRICULTURE AND BIOLOGY

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

Algemene omschrijving van de uitgevoerde werkzaamheden:

In the soils and plant part of the 1974-programme, the following results were obtained:

- Information was obtained on adsorption, desorption, decomposition and leaching rates of the herbicide BAM in different types of soils. Mathematical models considering these aspects were worked out and compared to experimental results.
- Further progress was made with respect to the development of a suitable solid synthetic growth medium which, better than nutrient solutions, simulates the normal root environment in soils. An experimental set-up with overall, but separate control of, respectively, the environments of the root and aerial parts of intact plants, was planned and its building started. Maize plants were grown for the purpose of collecting basic data (1) for a mathematical model describing the relation between the growth of roots and shoots and the nutrient absorption and (2) about rates of growth, transpiration and ion absorption.
- In a study of the kinetics of ion uptake by intact plants from (soil) solutions, emphasis was put on nitrogen and various ratios between NH_4^+ and NO_3^- . Uptake of manganese was also considered in relation to chloroplast activity.
- Distinct behaviour of ^{134}Cs and ^{137}Cs was observed in double labelling experiments with young tomato-plants. This apparent discrimination is not due to methodological errors, but also could not be related to any biological effect.

In the field of mutation breeding, incompatibility and mutagenesis, the following topics were considered:

- By the combination of adventitious bud technique and mutation breeding of vegetatively propagated ornamentals again several interesting mutants were obtained.
- *In vitro* incubation of pediceal-segments of *Chrysanthemum* yielded adventitious plantlets, which, apart from the interest of the method for vegetative propagation, produce solid, non-chimeral mutants after irradiation of the explants.
- The gene responsible for chlorophyll aberration in mutant C₁₁, obtained after irradiation of tomato plants, is located on chromosome 4. Results in this respect for mutants C₆ and C₁₂ are not conclusive.
- Results on the induction by γ -irradiation of self-compatibility in a self-incompatible dihaploid *Solanum tuberosum* L. clone confirm earlier observations (characteristic induced by radiation, of genetic nature, without permanent changes in the S-locus); also chimerism cannot account for the results.
- Research on the mutation spectrum induced by mutagenic treatments at the S-locus of pollen mother cells of *Nicotiana glauca* Link and Otto again showed that neither X-rays nor fast neutrons generate new S-alleles. The increase in pseudo-self-compatibility (seed-set) by chronic γ -irradiation results from its effect on the pistil of the flower. Other data suggest that a relationship exists between gene dosage and stage of occurrence of the incompatibility reaction and that complementation fully explains the manifestation of self-compatibility in pollen-part mutants. Further progress has been made with respect to identification of the S-bearing chromosome and the role of peroxidase isoenzymes in the incompatibility reaction.
- Other research on incompatibility, concerning the induction of new S-alleles in different species and metabolic processes in pollen, is in progress.
- Concerning the X-ray irradiation-dose fractionation effect on *Saintpaulia*-leaves, it was concluded that transport of a radiation-induced protective agent is not involved. Metabolic activity, as well as the time interval between detaching and irradiation play a role. It was demonstrated that the epidermal cells of the petiole of *Saintpaulia*-leaves, at the time of irradiation, are in the G₁-stage of the cell cycle.

In the food-irradiation part of the programme, the following results may be mentioned:

- It was shown that selected sensitive microbial strains show a progressive adaptation to irradiated glucose media. This adaptation or resistance is lost when cultivated in either non-irradiated medium or in irradiated glucose conserved for some period of time. It was also found that sulphhydryl groups in the cells gradually increase with repeated passage through irradiated media and decrease when grown in non-irradiated medium.

- Spores of *Bacillus subtilis* ATCC 6633 were much more radiation resistant when loaded with Li^+ , Na^+ , K^+ and Mg^{2+} ; Ca^{2+} , Ba^{2+} , Sr^{2+} , Co^{2+} , Ni^{2+} , La^{3+} and Al^{2+} increased the heat resistance, but not the radiation resistance.
The earlier observed synergistic effect of a combined radiation and heat treatment on metal loaded spores was confirmed and the sensitizing mechanism of pre-irradiation is probably located in the cortex of the spores.
- Practical applications of the food irradiation procedure have been further extended to a certain number of different foodstuffs of economic importance.
Attention was also paid to legislation, marketability and international trading aspects.
- A total diet feeding test for the purpose of studying wholesomeness of irradiated food showed no negative effects of irradiated feed on the growth, reproductive function, growth rates and fattening of the offspring. Results for the offspring in the "autoclaved" feed-group were inferior to those obtained for the control and "irradiated" feed treatments.

The programme on radiation genetic control of insect pests progressed in the following way:

- Using low doses of X-rays, four chromosome translocations and two inversions have been induced in *Hylemya antiqua* Meigen. The translocations showed reduced fertility, although to a smaller extent than initially expected. Homozygosing of rearrangements is now giving very encouraging results.
During inbreeding experiments a stock of heterozygous triploid larvae was obtained; its possible use in genetic control is still speculative.
Further efforts in this field of research aimed at induction of new rearrangements, of visible mutants and at testing of fertility in population cages.
- With respect to the work on *Adoxophyes orana* F.v.R., less competitiveness of irradiated sperm was confirmed. Differences in the heredity of chromosomal aberrations induced in, respectively, irradiated male and female moths can not unequivocally be explained by the timing of irradiation with respect to meiosis. Again in cage experiments and small field trials, the behaviour of sterile and semi-sterile moths has been studied.
- In research on genetic control of the two-spotted spidermite, *Tetranychus urticae* Koch it was found that the R.B.E. for induction of dominant lethals in spermatids (1.4) is much lower than R.B.E. values for similar material given by literature. Fast neutron and X-ray irradiations of oocytes have been compared: at low doses of neutrons the percentage of recessive lethals is reduced. Homozygous lines characterized by structural chromosome mutations were isolated and further tested for viability, stability and competitiveness. Males, irradiated with 4 krad X-rays are as competitive as untreated males; this observation is related to repair of the irradiation damage within a 15 day period after treatment.

The following aspects of the methodology part of the programme are worthwhile mentioning:

- Further improvement of the freeze-drying process of plant material for the purpose of microlocalization of absorbed elements.

- Microlocalization of Cd by β -spectrometry using semiconductor detectors, during its lateral transport in tomato-stems. Also the interpretation of the in depth distribution of Ca in *Phaseolus vulgaris* L. has been improved.
- In research on the effect of gamma radiation in recycling of liquid waste, it was found that a dose of 300 krad clearly improves floc formation in both laboratory and full scale experiments. Data about variation in electrophoretic mobility and hydration tend to give a preliminary explanation of the fundamental process involved.
- Measurements of density gradients and velocity in flowing granular material.
- Measurements of ensilage velocity in cattle-food silos.
- Measurements of moisture transport in cattle-food pellets.
- Developments aiming at automation of operation, data collection, recording and analysis in several experimental set-ups.
- Improvement of Cherenkov-counting of β -emitters: quenching and wavelength shifters; concentration of H_2SO_4 .
- Improvement of determination of heavy metals and radioactive corrosion products in fish and fishery products.

The programme for 1974 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 working-groups of the European Society of Nuclear methods in Agriculture (ESNA);
- on pollution, radioactive and other, with the Biology group at Ispra and institutes in the Netherlands, Belgium and Germany;
- on 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 crops, protein improvement, disease resistance) and incompatibility in higher plants in the Mutation Breeding Contact Group;
- cooperation to projects concerning the testing of irradiated food, wholesomeness testing set up by the Organization for Economic Cooperation and Development (OECD) and the International Atomic Energy Agency (IAEA).

In this respect also collaboration exists with institutes in the Netherlands, Denmark, Belgium.

- research on genetic control of insect pests, coordinated in Section VII

of the TNO working group "integrated control of Insect Pests" and in the joint European Working Group of the "Organisation Internationale de la lutte biologique" (OILB). Cooperation within projects of the IAEA and of the entomology programme of the Biology Division.

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Changes in the Scientific Staff

Ir. D. Snieder and ir. S.C.E. Romkes have left the Institute to accept teaching and research duties elsewhere.

New members of the scientific staff are:

ir. W.W.A. Bergers, ir. H. Breteler, dr. F.M. Engels, Miss dr. C.H. Hänisch ten Cate, ir. H. Siebering and ir. P.W.F. de Vrijer, all from The Netherlands.

Temporary members (post-graduate fellows) responsible for particular aspects of the programme: mrs. ir. H.M.G. Ebbens-Groot, ir. F. van Dorp, mrs. M. van Duyvendijk-Matteoli, dr. P. Mix and ir. C. Petit.

Several guest-workers have spent 6 to 12 months at the Institute.

Resultaten van het project No. 3

Hoofd van het team en wetenschappelijke medewerkers:

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

Titel van het project:

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

Beschrijving van de resultaten:

The study of the ionic composition of the soil solution in function of time and place has been retarded due to difficulties with the ion specific micro-electrodes. In the course of the year the experimental approach (continuous withdrawal of the equilibrium solution at very low rates - about 1 ml per 24 hours-) has been applied to ion exchanger mixtures. The experiments will be continued as soon as the ion-specific micro-electrodes become available.

The research on the composition of a mixture of synthetic resins suitable for long term plant growth experiments has been continued. A mixture used for experiments with seedlings of Pinus radiata (D. Don) and (Picea glauca (Moench Voss) (Mc Fee, 1966, ammonium and nitrate as nitrogen sources for Pinus radiata and Picea glauca, Ph.D. Thesis submitted to the Cornell University) revealed nutrient deficiencies when used for barley (Hordeum vulgare L. cv. Aramir). The mixtures used for growth experiments with maize (Zea mays L. cv. Ona) are described in tables 1 and 2. Mixtures I, II and III caused serious phosphate deficiency symptoms almost immediately after transplantation and after two weeks plants stopped growing. Mixtures IV, V and VI with higher anion contents appeared suitable for the first three weeks of growth, whereafter growth retardation occurred, although some plants overcame this barrier and developed rapidly. This was especially the case on mixture VI which revealed to be the best medium of this series. Figure 1 compares the results of mixture VI with those of a growth experiment using a Hoagland Arnon I nutrient solution. Although the synthetic mediums were saturated with technical degree chemicals and the agraperlite contains some impurities, we suspected the growth retardation to be due to deficiencies of micro-nutrients. We, therefore, decided to prepare the other mediums as well with as without micro-nutrients, in amounts as indicated in table 2. Mixtures I to VII were all composed of a weak acid and a strong base exchanger. As this results in a rather high pH value of the equilibrium solution we also tried out mixtures of a weak acid and a weak base resin (table 2). The growth retardation occurring with the mediums VII, VIII, and IX suggests that a too high cation exchange capacity might be the reason. A new experiment with plants growing in small beakers (capacity of 250 ml) was started; four plant species (Hordeum vulgare L. cv. Aramir, Zea mays L. cv. Ona, Spinacea oleracea L. cv. Winterreus, and Pisum sativum L. cv. Allround) were used and a potting soil was used for comparison.

The physical properties of the media used were almost equal to those reported in the annual report 1973. Maize, pea and spinach growing on mixtures without micro-nutrients died within 5 weeks; barley showed restricted growth with regard to its length but formed three times as much tillers, indicating boron deficiency. The results (table 3) clearly show the necessity of extra addition of micro-nutrients. Three further conclusions may be drawn:

1. a strong acid and strong base resin mixture (XV) is not suited
2. generally differences between the other mediums were less significant.
3. after six weeks of growth all barley and maize plants (also those plants on the potting soil) showed phosphorus deficiency, except the plants on medium XVII, a weak acid-weak base mixture. Maize on medium XVII was growing much better after six weeks than on the other media. (It should be remarked that pea and spinach revealed to be very sensitive to aeration and both species were hampered by a too long photo-period inducing early flowering).

Experiments using a mixture of a weak acid exchanger and a weak as well as a strong base exchanger are planned for next year.

A computer simulation programme, describing the relation between the growth of shoots and roots and the nutrient absorption is being prepared. The model itself is divided in different sub-models, which can be handled independently from each other (figure 2). The output of one sub-model or experimental data can be used as input for the next one. The sub-models of the programme describing the geometry of the rooting medium and the three dimensional distribution of the roots over the medium are already completed. The data of a growth experiment with maize (see project no. 6 of the application programme) will be used to extend the programme as given in the flowchart (figure 2).

Table 1

Synthetic resins used.

Trade name	properties + active group	Capacity me/g dry resin	Average size mm
Dowex 1 = Dowex SBR	<u>Bs</u> styrene DVB -N ⁺ - (CH ₃) ₃	3.5	0.55
Dowex WGR	<u>Bw</u> epoxy-amine -N(R) ₂ - NHR -NH ₂	8.5	0.52
Dowex 50W = Dowex HCR-W	<u>As</u> styrene DVB -SO ₃ ⁻	4.8	0.56
Dowex CCR 2	<u>Aw1</u> acrylic -COOH	10.5	0.47
Amberlite IRC 50	<u>AW2</u> acrylic -COOH	10.3	0.47

Bs = strong base anion exchanger
Bw = weak base anion exchanger
As = strong acid cation exchanger
Aw = weak acid cation exchanger

1 and 2 indicate different trade marks

Table 2. Resin mixtures used

Mixture	Resin types see table 1	Inert material	Me per liter mixture						pH
			Ca	Mg	K	NO ₃	SO ₄	H ₂ PO ₄	
I ⁻	Aw2 + Bs	Agraperlite	1440	180	180	160	20	20	
II ⁻	Aw2 + Bs	"	1080	135	135	120	15	15	
III ⁻	Aw2 + Bs	"	720	90	90	80	10	10	
IV ⁻	Aw2 + Bs	"	1280	160	160	320	40	40	8.5
V ⁻	Aw2 + Bs	"	960	120	120	240	30	30	8.6
VI ⁻	Aw2 + Bs	"	640	80	80	160	20	20	8.3
VII ⁺ and ⁻	Aw2 + Bs	Agraperlite	1100	150	120	250	30	50	8.5
VIII ⁺ and ⁻	Aw2 + Bw	"	1100	150	120	250	30	50	6.3
IX ⁺ and ⁻	Aw1 + Bw	"	1100	150	120	250	30	50	6.3
X ⁺ and ⁻	Aw2 + Bs	Agraperlite	400	50	50	100	20	15	8.0
XI ⁺ and ⁻	Aw2 + Bs	Gravel	400	50	50	100	20	15	8.0
XII ⁺ and ⁻	Aw2 + Bs	Agraperlite	260	30	40	120	30	20	7.9
XIII ⁺ and ⁻	Aw2 + Bs	Gravel	260	30	40	120	30	20	7.9
XV ⁺ and ⁻	As + Bs	Agraperlite	260	30	40	120	30	20	4.0
XVI ⁺ and ⁻	Aw1 + Bs	Agraperlite	260	30	40	120	30	20	6.9
XVII ⁺ and ⁻	Aw1 + Bw	"	260	30	40	120	30	20	5.6

⁺: micro-nutrients added
to mixture VII 7.2 me Fe, 3.6 me Mn, 3.6 me Zn, 3.6 Me Cu and 3.6 me Mn as Amberlite IRC50
and 5.2 me B as Dowex I per liter mixture
to mixture VIII and IX—the same but Fe.Mn,Zn,Cu and Mo—as Dowex 50W
to mixture X to XVII 3.6 me Fe, 1.8 me Mn, 1.8 me Zn, 1.8 me Cu,
and 1.8 me Mo as Dowex 50 W and 2.6 me B as Dowex 1
per liter mixture

⁻: no micro-nutrients added

Table 3. Length, Total Transpiration, Fresh weight and Dry weight per plant at 63 days after transplantation.

Mixture	Length cm.				Tot. Transp. per Plant ml				Fresh weight g				Dry weight g			
	ZM	HV	PS	SO	ZM	HV	PS	SO	ZM	HV	PS	SO	ZM	HV	PS	SO
X-	37	47	14	2	226	761	423	88	2	11	3	0	.5	1.7	.8	.1
X+	68	57	29	21	922	1176	1037	493	33	20	14	3	6.0	4.1	3.1	.7
XI-	28	37	3	1	330	846	162	82	3	17	0	0	1.2	2.8	.0	.0
XI+	65	51	26	18	996	918	910	409	32	16	14	4	5.8	3.1	2.9	.8
XII-	30	40	12	0	188	450	453	87	2	5	4	0	.5	.9	.9	.0
XII+	63	64	29	13	660	1004	989	415	24	16	14	2	4.0	3.6	3.3	.5
XIII-	30	36	3	0	276	763	145	98	5	15	0	0	.8	2.4	.1	.0
XIII+	66	53	25	15	632	831	863	385	27	15	13	3	4.7	3.0	2.9	.6
XV-	39	35	10	2	242	219	284	144	2	1	1	0	.4	.2	.2	.0
XV+	41	33	8	0	267	218	244	144	2	1	0	0	.5	.2	.2	.0
XVI-	35	32	8	0	179	208	293	93	1	1	1	0	.3	.4	.4	.0
XVI+	67	58	31	21	820	914	1027	456	29	18	14	5	5.4	3.2	3.1	.8
XVII-	33	44	10	0	110	269	282	90	1	3	4	0	.3	.7	1.0	.0
XVII+	81	55	28	22	1089	923	1020	411	58	17	18	8	10.1	2.7	4.4	1.2
Potting soil	64	60	30	16	563	661	917	312	20	6	16	2	5.0	2.0	3.8	.6

- without micro-nutrients
+ with micro-nutrients

ZM = Zea mais
HV = Hordeum vulgare
PS = Pisum sativum
SO = Spinacea oleracea

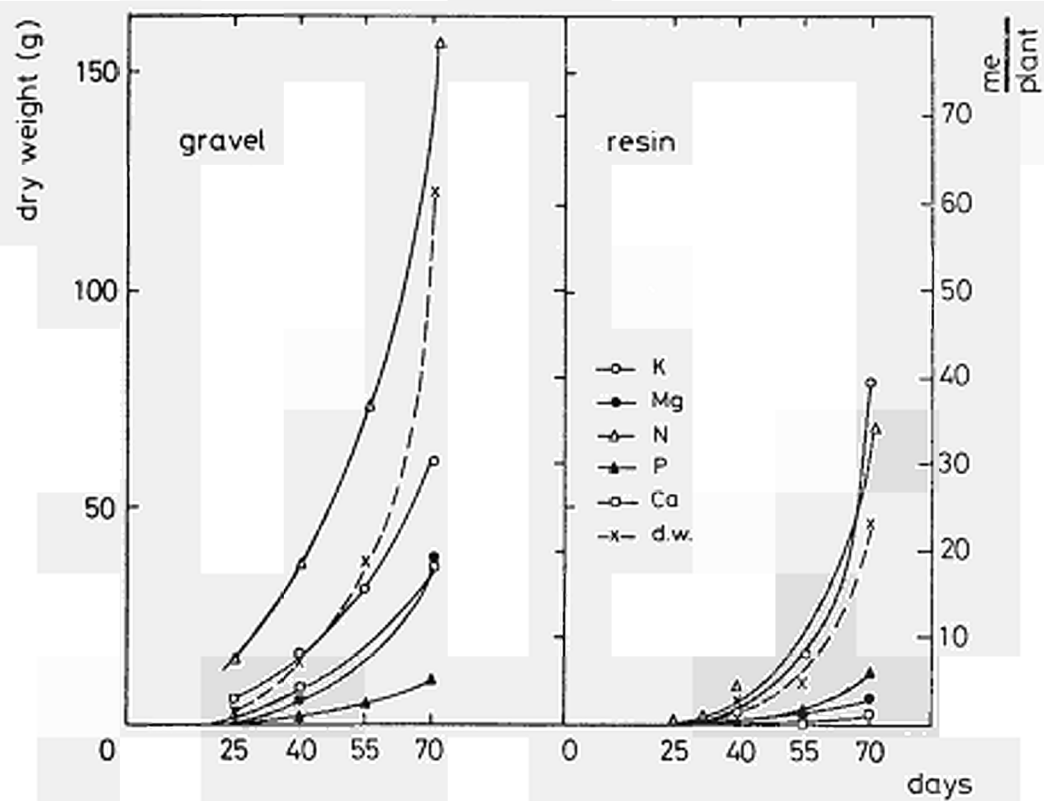


figure 1 : Ionic composition of maize on nutrient solution and synthetic resin mixture VI.

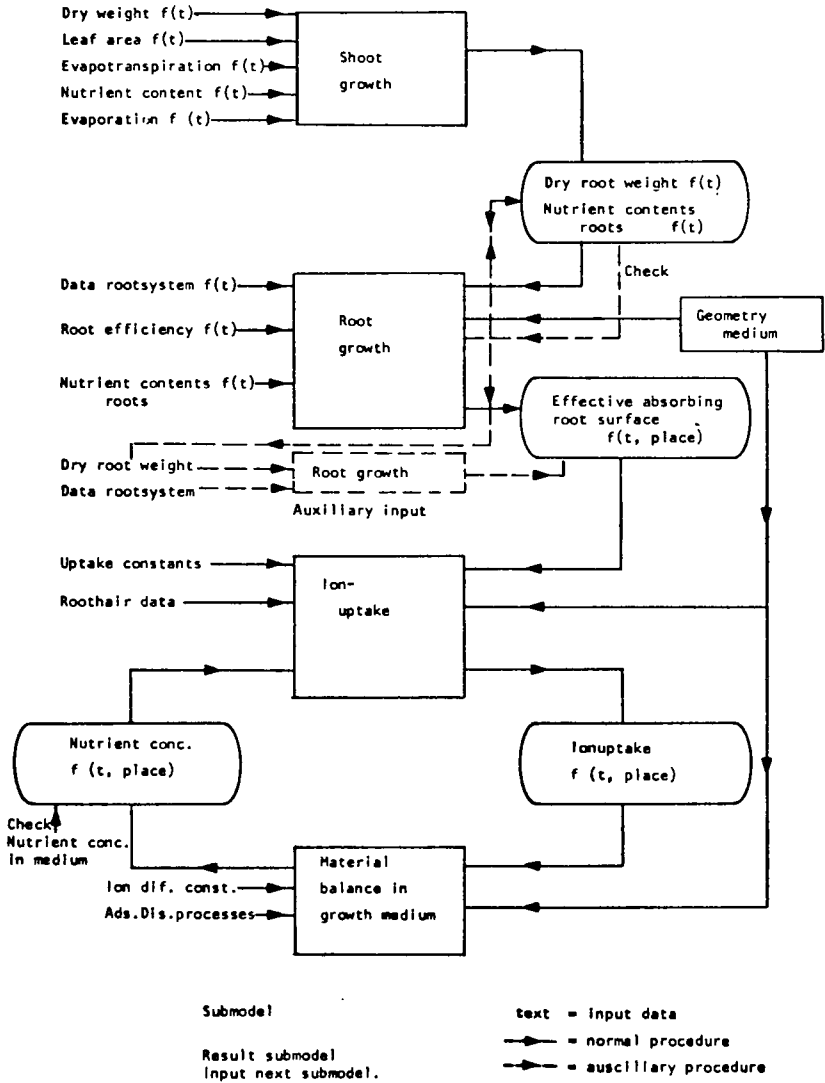


figure 2 : Flowchart computer simulation programme.

Publication 1974

F. VAN DORP, J. SINNAEVE : Synthetic ion-exchanger resins as a growth medium for plants in ion uptake studies.
Presented at the annual ESNA meeting Bucharest Sept. 1974.

Resultaten van het project No. 4

Hoofd van het team en wetenschappelijke medewerkers:

M.J. Frissel, P. Poelstra, J.P. Rolland and H. Siebering.

Titel van het project:

Transport of pesticides (insecticides, fungicides) and herbicides in soils.

Beschrijving van de resultaten:

State of Progress

There is a growing interest in guidelines for the determination of ecological parameters which control the fate of pesticides and herbicides in the soil. Which properties a manufacturer should determine to obtain a licence for his product? This subject was studied as part of a joint programme of the TNO-CNB working group (CNB = Side effects of pesticide and related compounds).

The problem is situated in the fact that not always the same characteristics have to be determined. E.g. leaching of a compound which decomposes within a few weeks is hardly of concern. Also a high or low vapour pressure may influence the characteristics to be analysed. Model studies can be used to obtain an insight in the overall behaviour of pesticides, provided that the data required in the model are available.

In cooperation with Leistra of the Laboratory for Insecticide Research, a calculation was set up for three comparatively well-studied pesticides: the herbicide bromacil (5-bromo-3-sec. butyl-6-methyluracil), the nematocidal soil fumigant *cis*-1, 3-di-chloropropene, and the herbicide propyzamide (N-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide). It appeared that there are several gaps in the availability of basic data. Vapour pressure and water solubility are given only for 25°C. Rates of decomposition of the three compounds in soil are reasonably well-known, although the great variation in decomposition rates as for *cis*-1,3-dichloropropene, makes the selection of a representative value for a particular situation difficult.

There are clear indications that chemisorption of pesticides in soil occurs; information on the rates is scarce. For part of the pesticides, information about adsorption on soils is available and compilations of adsorption data have been published. However, discrepancies between values determined in the laboratory and values required in the field can be expected. Rolland showed that data for Bam, measured in the laboratory with the slurry technique, overestimates the mobility of Bam in a rather dry soil column. Complications in adsorption behaviour, like an increase in adsorption strength with time and comparatively slow desorption, will play an important role, to these discrepancies, particularly for soils that dry out occasionally. Siebering has started a study of the behaviour of mono-methyl-bromide with the mentioned model as base.

Table 1 shows the results of calculations as they were made with the evaluated data. The table shows nicely that various properties lead to various ecological processes to be studied.

Rolland especially investigated the fate of Bam, both by experiments and by simulation studies. For a correct use of herbicides, quantitative insight in the behaviour of pesticides in soil-water systems is necessary. This is the more true for herbicides which are water soluble and chemically rather stable and which may be leached to "deep" ground water. Therefore the transport of 2,6-dichlorobenzamide (Bam) through representative soil types was investigated. Bam is a biologically active and rather stable decomposition product of 2,6-dichlorobenzonitrile or Dichlobenil. Tritiated water was used to evaluate the diffusion and dispersion parameters. Four soils were used in the experiment: a sandy soil, a humic sandy soil, a clay soil and a loess. An automatic rain simulator supplied "rain" to undisturbed columns. CaCl_2 , KCl and NaCl were added to keep salt concentration at a level of 0.005 N. After a steady state flow was attained in the columns, the "rain" was labelled with tritiated water for about 55 days. Thereafter, the tritium plug was leached by unlabelled "rain". Every day, the effluent was sampled and tritium activity was measured by liquid scintillation.

During the tritium-leaching experiment 4000 μg of 2,6-dichlorobenzamide (corresponding to 4 kg active material per ha), dissolved in 10 ml water, were applied to the surface of each column. Leaching occurred and every day effluent samples were taken for analysis by Gas Liquid Chromatography (Varian Acrograph equipped with a tritium foil (3A) electron capture and a 1.9 meter long glass column filled with Supelcon GP 4% SE-30/6% SP-2401 on 100/120 Supelcon AW DNCS).

The adsorption isotherms of the soils were determined in separate experiments. Fifty g of air-dry soil were mixed for 24 hours with 50 ml of a solution containing known amounts of ^{14}C -Carbonyl labelled Bam (kindly supplied by Philips Duphar B.V.). Blanks consisting of a solution of Bam alone were included in each determination. Adsorption rate experiments were performed according to the above described procedure.

Results and discussion

The results of the leaching experiments with tritiated water were analysed with the existing simulation model for tritium. Little spreading occurred in the sandy soil. The tritium concentration of the effluent remained lower than the concentration of the influent suggesting some exchange between tritium and hydrogen present in the column.

The humic sandy soil, the clay and loess soils showed more spreading, which increased with time. Probably non-instantaneous processes played a role. The adsorption isotherm for Bam appeared to be linear over a wide range of initial concentrations (0.5 to 21 ppm). Table 2 shows the capacities of the various soils to adsorb 2,6-dichlorobenzamide from solutions and the effect of organic matter on the adsorptive abilities.

Leaching experiments with Bam.

The results of the leaching experiments are shown in fig. 1. Calculated curves were based on:

- a. the dispersion data obtained with tritiated water were valid for Bam too;
- b. the adsorption occurred instantaneously and can be described by adsorption isotherms which were determined separately.
- c. ignorance of irreversible adsorption.

The experimental leaching curves of the four soils show the tailing effect, which is so typical for all leaching experiments with herbicides; it is most pronounced for the clay soil.

In the calculated curves tailing is missing. The best fit between calculation and experiment was obtained with the sandy soil, (dispersion distance 0.5 cm and distribution coefficient between soil and water $0.22 \text{ cm}^3 \text{ g}^{-1}$). Agreement was somewhat less good for the humic sandy soil (disp. dist. 1.5 cm and distribution coeff. varying between 0.365 and $0.04 \text{ cm}^3 \text{ g}^{-1}$). For the loess soil, there was a time-discrepancy of about 5 days between calculated and experimental maximum concentration of Bam. For the clay, a dispersion distance of 6 cm and a distribution coefficient varying with depth from 0.6 to $0.11 \text{ cm}^3 \text{ g}^{-1}$ predicted a maximum peak concentration 4 days later as found by experiment. The explanation of these differences could be that the surface area in contact with the herbicide, being a function of the pore size distribution and pore water velocity, failed to attain equilibrium resulting in a large longitudinal spreading. It can be concluded that an instantaneous equilibrium in clay and loess does not seem to exist and that adsorption, which in solution is complete within a few hours, takes much more time in soil. A more complex model, taking into account the adsorption and desorption rate and irreversible adsorption was developed. It was based on:

$$\frac{\delta C}{\delta t} = D_0 \frac{\delta^2 C}{\delta x^2} - V \frac{\delta C}{\delta x} - \frac{\rho}{v} \frac{\delta s}{\delta t}$$

where C is the concentration of Bam ($\mu\text{g ml}^{-1}$)
 D_0 is the apparent diffusion coefficient ($\text{cm}^2 \text{ day}^{-1}$)
 x is the linear distance in the direction of flow (cm)
 t is the time (day), ρ the bulk density (g cm^{-3}), V the flux (cm day^{-1})
 v is the volumetric water content ($\text{cm}^3 \text{ cm}^{-3}$) and
 s is the source term including adsorption, irreversible adsorption and decomposition ($\mu\text{g g}^{-1}$).

The reversible adsorption and desorption is expressed by:

$$\frac{ds_{\text{ads}}}{dt} = k_A (KC-s) \text{ or } \frac{ds_{\text{ads}}}{dt} = k_D (KC-s)$$

where K is the distribution coefficient
 k_A and k_D are respectively the adsorption and desorption rate coefficients (day^{-1}) and
 s is defined by $s = KC$ for equilibrium condition.

Both the decomposition and irreversible adsorption rate were introduced as first order rate reactions and better agreement was obtained between calculation and experiment. A drawback of such models is that, in practice, the transition from irreversible to reversible adsorbed compounds is varying with time and therefore assumptions concerning relations between desorption time spans and amounts, respectively reversibly or irreversibly bound, remain rather arbitrary.

Table I. Computed material balance of pesticides in soil.

Compound	Soil type, layer, period	Fraction of the dose removed by:					Fraction of dose as residue in soil
		decomposition	chemisorption	volatilization	transport to subsoil	uptake by plants	
bromacil	orchard, sandy loam, 0-100 cm 3 years	0.63	0.03	0.007	0.05	0.29	< 0.001
cis-1,3-dichloropropene	humic sand, 0-100 cm, Sept./Nov.	0.19	0.19	0.56	0.04	-----	0.02
cis-1,3-dichloropropene	sandy loam, 0-100 cm, Sept./Nov.	0.30	0.30	0.40	0.001	-----	< 0.001
propyzamide	loamy sand, 0-20 cm, May/Oct.	0.64	0.05	0.22	<0.001	0.03	0.06

Table 2. Soil characteristics and adsorption data for 2,6-dichlorobenzamide in four soils.

soil type	depth	bulk density	org. material	adsorbed at an equilibrium conc. of 1 mg per ml
	cm	g cm ⁻³	weight percent	mg per g
sand (sandy soil, subsoil)	100-190	1.49	0.01	0.02
humic sand (plaggept)	0- 30	1.44	15.0	0.365
	30- 90	1.58	1.0	0.01
clay	0- 25	1.20	10.0	0.60
(fluventric, eutrochrept)	25- 80	1.40	7.0	0.11
loess	0- 30	1.42	6.0	0.27
(typic hapludalf)	30- 90	1.44	4.0	0.04

Publications - 1974

- FRISSEL, M.J. and P. REINIGER: Transport of herbicides, partly volatile, in the simulation monograph "Simulation of accumulation and leaching in soils". Pudoc Wageningen 1974, p. 70-84 and p. 109-114.
- FRISSEL, M.J. Computer simulation models for the behaviour of herbicides in soils. Compilation of discussions EWRC Symposium "Herbicides and the Soil". Weed Research.
- LEISTRA, M. and M.J. Frissel. Computations on the material balance of pesticides in soil. Accepted for publication in "Environmental Quality and Safety", George Thieme Verlag, Stuttgart. Expected to come out April 1975.
- ROLLAND, J.P. and M.J. FRISSEL: Leaching of Bam in soil columns and evaluation of the results by simulation. Accepted for publication in "Environmental Quality and Safety", George Thieme Verlag, Stuttgart. Expected to come out April 1975.

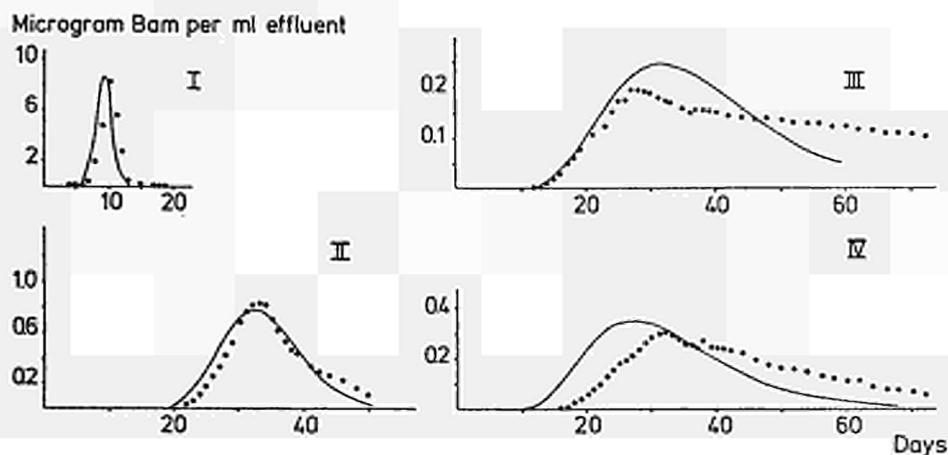


Fig. 1 - Leaching curves of Bam.

Experimental points: ● Calculations: —

- I Sandy soil, dispersion distance 0.5 cm, distribution ratio soil/water $0.02 \text{ cm}^3 \text{ g}^{-1}$.
- II Humic sandy soil, dispersion distance 1.5 cm, distribution ratio soil/water varying from 0.365 to $0.01 \text{ cm}^3 \text{ g}^{-1}$.
- III Clay soil, dispersion distance 6 cm, distribution ratio soil/water varying from 0.6 to $0.11 \text{ cm}^3 \text{ g}^{-1}$.
- IV Loess soil, dispersion distance 6 cm, distribution ratio soil/water varying from 0.27 to $0.04 \text{ cm}^3 \text{ g}^{-1}$.

Resultaten van het project No. 6

Hoofd van het team en wetenschappelijke medewerkers:

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

Titel van het project:

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

Beschrijving van de resultaten:

The development of a growth chamber for special purposes, as mentioned in the previous annual report, has been started this year and the air conditioning equipment for controlling the climatic parameters has been ordered. The set-up will become operational in the course of 1975. The aim is to determine complete nutritional balances for plants, which grow in solid substrates (soils or synthetic resins) under controlled and reproducible conditions with emphasis on the characteristics of the root development. This committed us to realize a separate and independent temperature control of the solid medium and an adapted management of the water content of the profiles in order to extend the research possibilities.

As the water movement in natural soils is bidirectional in a vertical plane (downward in case of heavy precipitation, upward by capillary rise in case of dry weather) a double control system has to be applied.

The first one maintains a constant water level in the soil cylinder (ranging in depth between 13 and 70 cm); the other one simulates rainfall on top of the column (programmed as required by the experiment). The second system can also be used for fertilizer application at different stages of growth or for the addition of pollutants or radioactive tracers. The double water management system also permits determination of the evapotranspiration per cylinder, each containing one growing plant. The temperature of the soils can be varied between 5 and 25°C, independent from the temperature of the air surrounding the leaves. The top layer of the soil (upper 5 cm) is brought at the atmospheric temperature (a small time effect is observed, as well as additional heating caused by the irradiance in the cabinet); the bulk soil (from 10 to 75 cm) is at the selected temperature of the soil and the layer in between (5 to 10 cm) is a transition phase characterized by a temperature gradient as a function of the depth. The temperature cycle of the soil as function of the temperature of the air flowing around the cylinder is shown in figure 1 (preliminary result). The root system is studied at harvest time by pushing the profile out of the cylinder. The soil is completely washed away to study overall root development or disks are cut to study penetration.

The reactions of the shoot with the atmosphere determine the overall metabolic activity of the plant. Besides temperature, humidity and visible light (300-700 nm) also the carbondioxide consumption (or release) and the long wave radiation (700-3000 nm) directly acting on the rate of transpiration are controlled. The carbondioxide consumption is measured

by infra-red gas analysis in an open (with addition of fresh air) or a closed system (without addition of fresh air but with direct CO₂ injection to maintain a selected CO₂ concentration between 50 and 2000 ppm).

The contribution of the long wave radiation is quantified by regulating the temperature of the wall of the growth chamber. The interrelationship between the temperatures of the air (t_a), of the leaf (t_l) and of the wall of the growth chamber (t_{wall}) and the transpiration rate (T) at different relative humidities and for two realistic combinations of the resistances of the air (r_a) and of the leaf (r_l) are illustrated by figure 2. This shows to what extent the transpiration can be varied by acceptable modifications of the temperature of the leaf. Similar graphs were worked out in function of other temperatures, vapour deficits of the air and irradiances as applied to this equipment. The described approach will enable us to collect the integrated data required for an experimental test of the simulation model (see the related project No. 3).

A long term growth experiment with maize plants (*Zea mays* L. cv. Ona) was carried out. The plants were grown in columns (diameter 9 cm, height 75 cm) identical to those to be used in the described growth chamber. The columns were filled with gravel and a Hoagland-Arnon nutrient solution was used. The purpose of the experiment was to obtain basic data needed for a first version of the simulation model, and to determine the capacities of a maize plant with regard to rate of development of roots and shoot, transpiration and ion absorption. The data characterizing shoot and root development, available at this moment, are given in tables 1 and 2.

Mineral analysis of the plant material is in progress. Considerable variation of the plant data is observed. This is due to failing air circulation and heterogenic illumination. We expect these variations to be much smaller in the described set-up. Detailed analysis of the root development (table 2) illustrates the regular growth of the primary roots, the important volumetric development of secondary roots between the third and seventh week of growth, the very explosive growth of the tertiary roots in the same period and the continued extension of the secondary roots between the seventh and ninth week combined with an apparent loss of importance of the tertiary roots after fruit initiation. Root elongation rates were determined through a transparent lucite wall. The mean elongation rate of the primary roots was 10,6 mm per 24 hours (range 0-20 mm/day) and 4,0 mm per day for secondary and tertiary roots (range 0-15 mm/day).

Table 1 - Data characterizing the shoot development of *Zea mays* L. cv. Ona - average values.
(Gravel culture with a Hoagland-Arnon nutrient solution).

Age (days)	Fresh weight		Dry weight		Root/Shoot ratio	Height (cm)	Number of leaves	Leaf surface (cm ²)	Total transpiration (l)	Transpiration rate (ml.cm ⁻² .day ⁻¹)
	Root (g)	Shoot (g)	Root (g)	Shoot (g)						
25	16.3	17.1	0.795	1.515	0.52	57	7	330	0.18	0.046
35	34.0	63.8	1.760	5.610	0.31	86	8	1040	0.86	0.073
46	94.9	193.6	6.165	18.715	0.33	100	12	2740	2.49	0.067
60	114.7	370.3	11.030	48.445	0.23	133	13	3100	5.34	0.043
84	178.5	629.1	15.400	115.285	0.13	146	13	2980	10.59	0.058

Table 2 - Data characterizing the root development of *Zea mays* L. cv. Ona - average values.
(Gravel culture with a Hoagland-Arnon nutrient solution)

I = primary roots; II = secondary roots; III = tertiary roots.

Age (days)	Mean diameter (cm)			Length (cm)			Surface (cm ²)			Volume (cm ³)			% Volume		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
25	0.125	0.038	0.015	880	5560	4720	263	508	277	6.7	4.2	1.3	55	34	11
35	0.123	0.033	0.016	980	13590	20000	338	1429	1010	10.4	12.7	4.2	38	47	15
46	0.169	0.052	0.018	910	20675	303050	424	3233	16460	23.9	46.7	75.6	16	32	52
60	0.181	0.099	0.020	884	31787	146910	503	9986	9230	24.5	249.7	46.2	8	78	14

Publications - 1974.

SINNAEVE, J., F. VAN DORP, M.J. FRISSEL, P. POELSTRA.
 Motivation and description of an experimental approach
 to the study of plant-soil relationships.
 Report to the "Commissie Technische Toerusting Klimaatruimten TN0".

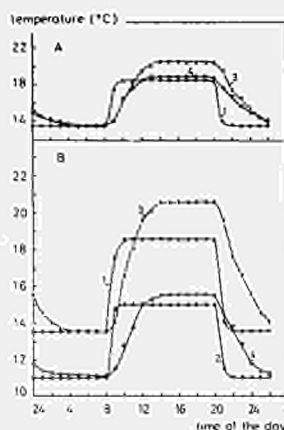


Fig. 1 - Temperature cycle of a soil column
 (illumination: 10,000 lux; wind speed 10 on sec⁻¹)
 A: day temperature (9h - 20 h) 18°C (overall);
 night temperature (20h - 8 h) 13°C (overall)
 B: day temperature leaves (9 h - 20 h) 18°C C
 day temperature roots (9 h - 20 h) 15° C
 night temperature leaves (20 h - 8 h) 13°C C
 night temperature roots (20 h - 8 h) 10° C
 (1) Air temperature leaf environment;
 (2) Temperature of the air flowing around the volume;
 (3) Soil temperature at 3 cm depth;
 (4) Soil temperature at 50 cm depth (below water table)

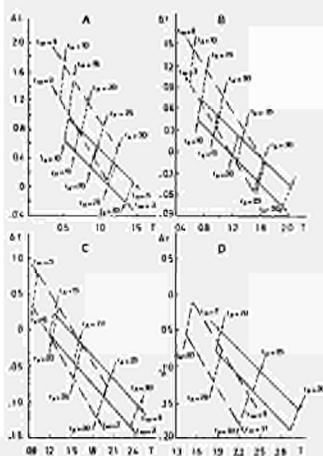


Fig. 2 - Relation between $t_a - t_w$ (°C) and transpiration T [$\text{g H}_2\text{O}\cdot\text{dm}^{-2}\cdot\text{h}^{-1}$],
 at a relative humidity of 80% (A), 70% (B), 60% (C) and 50% (D) in
 function of the temperature of the air (t_a) and the difference
 between the temperatures of walls and air ($t_w = t_{\text{wall}} - t_a$) for
 two combinations of r_a and r_s .
 ($r_a = 1.0$; $r_s = 1.0$ - - - - -; $r_a = 0.5$; $r_s = 1.0$ - - - - -)

Resultaten van het project No. 7

Hoofd van het team en wetenschappelijke medewerkers:

G. Verfaillie, H. Breteler.

Titel van het project:

Study of the kinetics of ion uptake by intact plants

Beschrijving van de resultaten:

Nitrogen uptake.

The following results only concern methodological trials carried out in an ordinary growing chamber in the prospect of a study of the relations existing between the ion uptake and the nitrogen metabolism in plants. Nitrogen having no radioactive tracer, suitable methods had to be selected for monitoring the nitrate and ammonium concentrations in nutrient solutions. However, continuous monitoring of the nitrogen content of a nutrient solution might be more difficult to perform than that of radioactively labelled ions. Therefore, special care has been taken for obtaining experimental plants that present the highest possible standard of reproducibility. Two varieties of millet (Pennisetum typhoidium L. cv TIF and BDY) have been successfully cultivated in a phytotron on large volumes of nutrient solution of which the pH was kept constant. Each variety has been grown on two different nutrient solutions, one devoid of NH_4^+ (Hoagland-Arnon I) and another, recommended by the IRAT (Institut de Recherches Agronomiques Tropicales et des Cultures Vivrières) at Paris, in which the ratio $\text{NH}_4^+/\text{NO}_3^-$ is equal to 1/2. For each treatment, pH5 and pH6.5 have been used. Both varieties grew normally without presenting any variation in their phenological or physiological behaviour toward the various treatments. Therefore, for millet, Hoagland-Arnon I formula could be retained as basic nutrient solution, the $\text{NH}_4^+/\text{NO}_3^-$ ratio being varied by partial substitutions of NH_4NO_3 to the nitrates of the major cations. Up to the age of 6 weeks, millet plants are suitably handled in the set-up for kinetic studies. Three plants can be used for each run and the weight of their fresh roots exceeding 100 grams produces a rate of ion uptake quite sufficient to be followed by the continuous exhaustion method required by the 'kinetic' set-up. However, no suitable technique for continuous control of the nitrate concentration has been found up to now. This concentration must be measured by separate analysis of samples taken from the nutrient solution during the kinetic runs. Even then, the Kjeldahl method is not suitable because of the inaccuracy of the titration of the resulting ammonia when the solution samples are small as they owe to be (1 ml). The analysis must be done with a nitrate selective ion electrode using the method of known addition or by spectrophotometry at 220 nm. Both techniques need further investigations in 1975 with special attention for their respective accuracy and sensitivity toward small decrease of the nitrate concentration.

Maize plants (Zea mays L. cv CIV-Z 'Prior') have also been cultivated in the same climate controlled room. Reasonably reproducible standard plants have been obtained although further improvements of the germination technique are still needed. The determination of the NH_4^+ uptake rate has been experimented by measuring the NH_4^+ concentration of the nutrient solution using the indophenol blue colorimetric method which seems to be reliable and suitable for large series of solution samples. The use of an Orion ammonia selective electrode is now in study. Excised roots of maize grown in a modified Hoagland-Arnon medium ($2\text{mN NO}_3^- + 1.4\text{ mN NH}_4^+$) showing negligible NH_4^+ uptake when transferred to 1 mN NH_4^+ solutions, the trials were made with roots of plants that had been deprived of nitrogen for at least three days. Variation in plant age (10 to 40 days) had relatively little effect on the NH_4^+ absorption during 4 hours and on its steady state that was reached after about 1 hour. As an indication for internal limiting factors, the effect of glucose incubation on subsequent NH_4^+ uptake was tested. Over the whole period of 4 hours, glucose pretreatment (2 percent, overnight) was significantly effective and resulted in about 50 percent increase of the steady state rate of uptake.

Resultaten van het project No. 8

Hoofd van het team en wetenschappelijke medewerkers:

J. Sinnaeve, S.C. van de Geijn, M.J. Frissel, G.R.M. Verfaillie

Titel van het project:

Ion uptake by roots from diluted solution

Beschrijving van de resultaten:

1-Ion uptake experiments

The double labelling experiments, described in the ESNA Annual Report (1972, Budapest, September 26-29) to investigate the reliability of double labelling procedures were evaluated and five additional experiments were carried out. As described by Sinnaeve and Wieneke (Detailed proposal for a double labelling ion uptake experiment, 1972, Annual ESNA Meeting, Budapest) four weeks old tomato plants (Lycopersicon esculentum, Mill. cv. Murette VF), grown in strictly controlled conditions on a ten times diluted Hoagland-Arnon I nutrient solution, with 0,5 mM KCl per litre were used. The nutrient solution was cesium free to avoid the existence of a stable cesium pool within the plants. At the day of the absorption experiment, the plants were transferred to absorption vessels containing the same nutrient solution as before, but three cesium isotopes (stable cesium and two of its radioisotopes, ^{134}Cs and ^{137}Cs) were added. The labelling procedure of all experiments carried out is given in table 1. All samples were counted with a Germanium-Lithium drifted detector connected to a multichannel analyzer with a resolution of 1.5 keV. The excellent results obtained with this gamma spectroscopy equipment are illustrated in figure 1. The peak areas were computed by Gaussian fitting and base line correction with a PLP 11/40 computer. The fitting procedure was derived from the GASPAN programme developed by Barnes (Gaspan - an advanced computer code for the analysis of high resolution gamma ray spectra, IEEE Semiconductor symposium, Washington 1968). The reliability of the counting and computation procedure has been determined experimentally by repeated counting and peak area computation. The standard deviations of the ^{134}Cs peak area, the ^{137}Cs peak area and the ratio $^{134}\text{Cs}/^{137}\text{Cs}$ for a sample with an activity ratio equal to 10 were respectively 1.1%, 1.4%, and 2.2%; for a sample with an activity ratio equal to 1/50 they were 4.8%, 0.6% and 5.1%. The results are generally expressed as a quotient of two quotients:

$$Z = \frac{X}{Y} = \frac{\frac{^{134}\text{Cs in the plant}}{^{137}\text{Cs in the plant}}}{\frac{^{134}\text{Cs in the solution}}{^{137}\text{Cs in the solution}}}$$

We expect Z to be equal to 1. With S_a and S_b being estimates of the standard deviation in a and b ($X = \frac{a}{b}$), the standard deviation of X is computed according to

$$S_x^2 = \left(\frac{a}{b} \right)^2 \cdot \left(\frac{S_a^2}{a^2} + \frac{S_b^2}{b^2} - \frac{2 S_{a\bar{b}}}{a\bar{b}} \right)$$

Application of this formula gives a standard deviation S_z equal to 6% for an activity ratio 10 and 10% for an activity ratio 1/50, the figures being corrected for the variation in the biological material, by expressing the results per gram dry material, and for the correlation between X and Y. The Z value for the roots of treatments A showed a significant deviation from the expected value. The higher concentrations of the radioisotopes, applied in treatments B diminished the observed discrepancy. An analysis of the concentration factors, i.e. the ratio of the concentration of the isotope in the organ (cpm/g) and the concentration of the same isotope in the solution (cpm/ml) can give a more distinct idea of the behaviour of both isotopes (table 2). The concentration factors of both isotopes in the stem are nearly equal, resulting in a Z value close to 1, but in the roots, enormous differences are observed when the concentrations of both isotopes in the nutrient solution are very different (treatments A.1. and A.4.). We observe a much higher concentration factor of the less concentrated isotope, indicating a preferential immobilization. Additional experiments were conducted to evaluate the influence of the duration of the absorption period. Short absorption periods (less than 60 minutes) provoked Z values very different from 1; longer absorption periods resulted in a decrease of the deviation. These results were partly confirmed by the analysis of the sap of detopped plants treated in parallel. Really extreme activity ratios were obtained by labelling with one cesium isotope and determining the peak areas of the isotope added and of the other cesium isotope which is always present as an impurity (treatments D, E and F). The results confirmed the influence of the ratio of the activities but some disagreements were observed. The distinct behaviour of the cesium isotopes in double labelling experiments could not be explained. It must however be emphasized that we do not have a straight forward proof for the existence of any biological effect.'

2- Interlab test

During the last years, the question has been raised to know whether an isotopic discrimination could occur in biological processes. Because such an effect would infringe the validity of the isotopic tracer technique widely used in our kinetic studies, it was relevant to determine objectively the degree of liability of the methods used to measure isotopic ratios in nutrient solution and plant materials. Therefore, we organized, an interlaboratory test in the frame of the ESNA collaboration. This test consists in a simulation of double tracing experiment with ^{134}Cs and ^{137}Cs where nutrient solution and plant materials were replaced by solid sources obtained by evaporation of drops of series of prepared solutions containing well defined activity ratios of both isotopes.

Seven $^{134}\text{Cs}/^{137}\text{Cs}$ activity ratios have been used :
 0.9 - 1 - 1.1 - 8 - 8.8 - 1/8 and 1/8.8, each of them being represented by 5 replicates. The 10 laboratories who accepted to measure the sources did not know the partition of the r values in advance. By statistic analysis of the measured values, it has been possible to determine all the parameters affecting the variance of the results. The conclusions of the test were given in details during the annual ESNA meeting at Bucharest in september 1974 and can be summarized as follows:

- Unexpected variance can be obtained in the measurement of the activity ratio of two isotopes when this ratio departs much from unity. The variance could not be attributed to biological discrimination (Table 3).
- Besides the variance due to random errors, very significant systematic errors also occur which, in the present test, favors ^{137}Cs against ^{134}Cs (negative values in table 4).
- The dispersion of the results as well as the systematic errors increase when the single isotopic ratio departs more and more from unity, and influence more the ratios of ratios than the single ratios self.
- The highest variances and systematic errors occur when the activity of one isotope (^{137}Cs) is measured by the X-ray.
- F test applied to the variance analysis of the results shows that in the total sum of square deviations 72 percent are due to ratio level only with a significance probability $P \ll 0.01$. The laboratory or methodological effect amounts non significantly to 10.5 percent and the residue of 17.5 percent represents the experimental random errors. It is thus advisable that, for only pure methodological reasons the isotopic activities ratio used in double labelling biological experiment vicinates unity as much as possible..

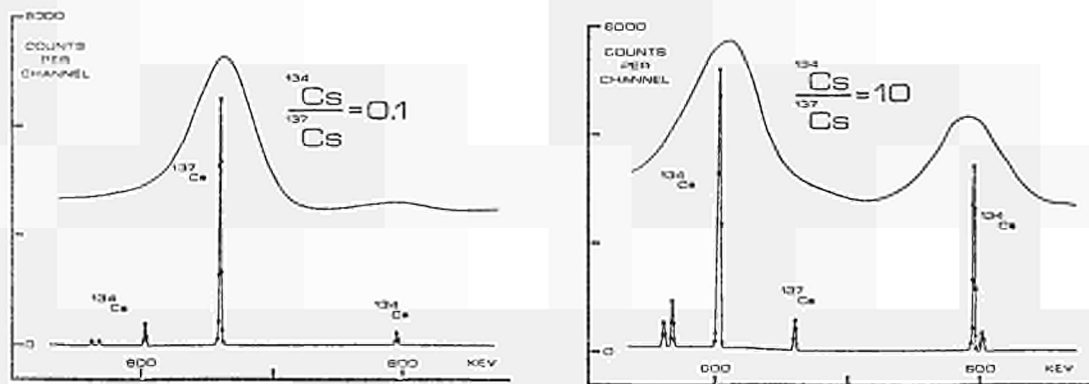


Fig. 1 - Gamma spectra of a mixture of Cs isotopes.
 On the left $^{134}\text{Cs}/^{137}\text{Cs} = 0.1$; on the right $^{134}\text{Cs}/^{137}\text{Cs} = 10$.
 The upper curve has been determined with a Na I (TL) detector, the lower one with a Ge-Li detector.

Publications 1974

- FRISSEL, M.J., report from collaborators in a joint project of ESNA. Some absorption and translocation experiments in tomato plants Lycopersicon esculentum Mill. cv, Marette VF to check the reliability of radioactive tracing methods as an international joint project of ESNA. Int. Hort. Congres, Warszawa, 1974.
- SINNAEVE, J., Ion uptake by roots from dilute solutions using two radioisotopes of the same element. Presented at the Annual ESNA Meeting, Bucharest, September 1974.
- VEEN, H., and FRISSEL, M.J., Simulation of hormone transport in petiole segments of COLEUS. Submitted for publication.
- G. VERFAILLIE. ESNA Interlab Test. Presented at the ESNA joint group meeting, Hannover, February 1974.
- G. VERFAILLIE. Contribution to the ESNA joint experiment on Isotope Concentration Effects. Results of the ESNA Interlab Test. Presented at the 5th Annual ESNA Meeting. Bucharest, September 1974.

Table 1 - Experimental conditions of the absorption experiments.

Treatment	Stable cesium mg/l	¹³⁴ Cs μCi/l	¹³⁷ Cs μCi/l	Activity ratio ¹³⁴ Cs/ ¹³⁷ Cs	S.A. ¹³⁴ Cs μCi/mg	S.A. ¹³⁷ Cs μCi/mg
A.1.	0.3	0.05	2.5	0.02	0.167	8.333
A.2.	0.3	0.5	5.0	0.10	1.667	16.677
A.3.	0.3	5.0	0.5	10	16.667	1.667
A.4.	0.3	2.5	0.05	50	8.333	0.167
A.5.	3.0	0.05	2.5	0.02	0.017	0.833
A.6.	3.0	0.5	5.0	0.10	0.167	1.667
A.7.	3.0	5.0	0.5	10	1.667	0.167
A.8	3.0	2.5	0.05	50	0.833	0.017
A.9	30.0	0.05	2.5	0.02	0.0017	0.083
A.10.	30.0	0.5	5.0	0.10	0.017	0.167
A.11.	30.0	5.0	0.5	10	0.167	0.017
A.12.	30.0	2.5	0.05	50	0.083	0.0017
B.1.	0.3	2.5	25	0.10	8.333	83.333
B.2.	0.3	5	50	0.10	16.667	166.67
B.3.	0.3	50	5	10	166.67	16.667
B.4.	0.3	25	2.5	10	83.333	8.333
C	3.0	0.05	2.5	0.02	0.017	0.833
D.1.	3.0	5	traces	-	1.667	-
D.2.	3.0	traces	5	-	-	1.667
E	3.0	0.05	2.5	0.02	0.017	0.833
F.1.	3.0	traces	5	-	-	1.667
F.2.	3.0	traces	25	-	-	8.333

Table 2 - Concentration factor of both cesium isotopes in root and stem

stable Cs mg.l ⁻¹	treatment	¹³⁷ Cs cpm.ml ⁻¹	¹³⁴ Cs cpm.ml ⁻¹	concentration factors in the material			
				¹³⁷ Cs root	¹³⁴ Cs root	¹³⁷ Cs stem	¹³⁴ Cs stem
0.3	B.2.	580	41	9.1	8.9	14.3	14.3
0.3	B.1.	304	21	13.8	14.8	16.5	17.5
0.3	A.2.	75	5	19.0	19.2	20.5	20.2
0.3	B.3.	54	365	13.5	13.4	19.0	18.7
0.3	A.1.	38	0.5	19.2	39.2	18.5	19.1
0.3	B.4.	28	198	18.3	17.9	21.8	21.4
0.3	A.3.	7	47	37.9	29.9	27.4	27.1
0.3	A.4.	0.8	25	93.0	30.6	29.2	26.8

Table 3 - Interlab test.

Coefficients of variation in percent of mean values affecting single activity ratios C_r and double ratios C_R .

Functions	Sources of data	Single ratio levels	
		r close to one	r far from one
C_r	Best	1.1	2.0
	Worst	3.8	12.1
	All lab.	2.2	7.0
C_R	Best	1.7	3.0
	Worst	5.5	17.4
	All lab.	3.8	10.9

Table 4 - Interlab test.

Systematic errors in percent of the true values affecting single activity ratios E_r and double ratios E_R and the probability levels of their significance.

Functions	Sources of data	Single ratio levels	
		r close to one	r far from one
E_r	Best	- 0.3 (P=0.3)	- 3.3 (P=0.01)
	Worst	+ 2.1 (P=0.05)	- 5.8 (P=0.001)
	All lab.	- 0.5 (P=0.5)	- 2.8 (P=0.001)
E_R	Best	+ 0.4 (P=0.4)	- 3.4 (P=0.08)
	Worst	- 1.5 (P=0.3)	- 7.9 (P=0.02)
	All lab.	- 0.7 (P=0.1)	- 5.3 (P=0.001)

Legends of the tables: r : single activity ratio $A^{134}\text{Cs}/A^{137}\text{Cs}$.
 R : ratio of activity ratios $r_1/r_2 = 1.1$
 r close to one : $r = 0.9 - 1$ or 1.1
 r far from one : $r = 8 - 8.8 - 1/8$ or $1/8.8$

Resultaten van het project No. 9

Hoofd van het team en wetenschappelijke medewerkers:

G. Desmet, M. van Duyvendijk-Matteoli, A. de Ruyter.

Titel van het project:

Uptake and release of ions by subcellular structures, mainly chloroplasts and mitochondria.

Beschrijving van de resultaten:

According to the experimental results in project No. 9 of the radiation protection programme Cd^{++} intervenes in the Mn controlled enzymatic reactions and increases the concentrations of both Mn and Zn in plant leaves. Attention was therefore paid to Mn content and metabolism in spinach (*Spinacea oleracea* L. var. Verbeterd Breedblad). Plants were grown on a continuously flowing nutrient solutions with different Mn concentrations. At the lowest Mn^{++} concentration ($5 \cdot 10^{-9}$ M) stunted growth was observed although the aspect of the leaves remained quite normal. Chloroplasts were isolated from the leaves of the plants grown at the different manganese leaves. Optimal electron transport was observed in chloroplasts containing approx. 1 μg Mn per mg chlorophyll. Chloroplasts isolated from plants grown at the usual Mn^{++} concentration (10^{-5} M) already suffer from a slight manganese intoxication and have a decreased capacity of energy conservation.

Resultaten van het project No. 10

Hoofd van het team en wetenschappelijke medewerkers:

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

Titel van het project:

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

Beschrijving van de resultaten:

The use of the adventitious bud technique, namely the development of adventitious plantlets on detached leaves, has proven to be a powerful tool in mutation breeding because usually a high percentage of solid mutants is obtained. It has been demonstrated in a number of crops such as *Achimenes*, *Begonia*, *Streptocarpus* and others. Research on 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. A few (new) experiments are under way to test whether or not autotetraploids produce a higher mutation frequency and whether or not the application of DTT (dithiothreitol) changes the ratio of gene mutations / chromosome aberrations in vegetatively propagated material in favour of gene mutations (Broertjes). 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 look more and more promising, either as a tool in plant breeding or as a (fast) propagation method (Roest and Bokelmann). The plants so far produced (*Chrysanthemum*) are being investigated to know whether adventitious shoots develop from one cell and consequently are or are not of interest for mutation breeding (c) (Broertjes and Roest).

a. Of three pink-flowering *Achimenes* cultivars (Tarantella, Repelsteeltje, Little Beauty) autotetraploids were produced in 1973. These (very pretty) tetraploids attract much attention and obtained a Certificate of Merit. One of them, an autotetraploid of "Tarantella" has in the meantime been introduced into commerce under the name of "Tango", being another proof that the production of autotetraploids is very easy when the adventitious bud technique is available. The diploid and autotetraploid material is being used to test the phenomenon (observed earlier in *Saintpaulia* and *Streptocarpus*) that autotetraploids can produce extremely high mutation frequencies. Leaves of diploid "Tarantella", "Repelsteeltje" and "Little Beauty" were irradiated and produced only a few mutants. Leaves of the respective autotetraploids were irradiated in 1974 on a large scale with X-rays or fast neutrons in order to test the hypothesis.

DTT (dithiothreitol) is attracting interest because it seems to protect against the formation of chromosome aberrations (or to induce a very efficient repair mechanism for chromosome breaks). It would be interesting to know in vegetatively propagated species (where most of the mutants certainly are based upon chromosome breaks) whether or not DTT prevents the formation of drastic mutants and what the result will be on mutation frequency and spectrum (with X-rays and with fast neutrons). The cv. "Cupido" has been used for this purpose (Cupido now has a Certificate First Class and mutants of such an excellent variety may be of commercial interest). The results so far are promising, in the sense that 0,5% DTT solution during 2 hours at room temperature, applied to petioles of detached leaves, very effectively protects against radiation.

A dose of 4 krad of X-rays, for instance, almost lethal for unpretreated leaves, yields 100% survival and production of rhizomes when pretreated, whereas even a dose of 8 krad of X-rays is not lethal.

b. The development of adventitious bud techniques in crops which do not spontaneously produce adventitious buds.

The adventitious bud formation of *Chrysanthemum morifolium* Ram. cultivars Super Yellow and Bravo was investigated *in vivo* and *in vitro*

In vivo, adventitious shoots were exclusively regenerated on rooted detached leaves of the cultivar Bravo. Adventitious shoot formation was observed on callus at the base of the petiole or on root callus, 2-6 months after leaf excision, with an average of 3-4 shoots per leaf.

It was observed that leaves from stock-plants taken at the positions 1, 2, 8 and 9 (the leaf in top position being 1) generally showed a higher regeneration ability than leaves from the intermediate positions. The leaves of some stock-plants produced more adventitious shoots and more readily than leaves of other plants. The development of adventitious shoots could further be improved by detaching leaves from plants in a young developmental stage, which were grown up under long day conditions in the greenhouse.

Exposure to light of the base of the petiole and the upper part of the root system appeared to be favourable for shoot formation compared with exposure of the base of the petiole and the upper part of the root system to continuous darkness (soil). A weekly mineral nutrition with "pokon" (N-, P- and K-components) turned out to be favourable for the production of adventitious shoots.

The addition of β -indolebutyric acid (IBA), on lanolin or talc basis, immediately after leaf detaching, stimulated shoot formation and proved to be more effective than β -indoleacetic acid (IAA) and α -naphthaleneacetic acid (NAA). A separate addition of the cytokinin 6-benzylaminopurine (BA) or a combined addition of a cytokinin and an auxin did not further improve adventitious shoot formation.

In vitro, both cultivars regenerated shoots on petals and explants of the leaf, the pedicel and the flower head (capitulum), when they were exposed in a growth cabinet to a photoperiod of 16 hours (fluorescent tubes supplemented with incandescent light), a temperature of 20°C and a relative humidity of 80%. Petals, capitulum- and leaf-explants developed, usually on callus, the first adventitious shoots 3 weeks after incubation, whereas explants of the pedicel yielded a direct regeneration of the first adventitious shoots, which emerged, over the whole length of the explant, 10 days after incubation.

The formation of adventitious shoots, as affected by some factors, was analysed using pedicel-explants of both cultivars. Shoot formation of "Super Yellow" was not influenced by the explant-length in the range of 0.5, 1.0 up to 1.5 cm, whereas "Bravo" developed an increasing number of shoots at a higher explant-length. The vitamins and the macroelements KNO_3 and NH_4NO_3 of the medium of Murashige & Skoog considerably stimulated shoot morphogenesis of both cultivars. The vital importance of sucrose, with optimum concentrations of respectively 3 and 5% for "Super Yellow" and "Bravo", and a cytokinin, was demonstrated. Of 4 cytokinins BA proved to be most efficient for adventitious shoot formation of the cultivar Bravo. In the presence of BA

10^{-6} g/ml, IAA enhanced the regeneration of shoots at concentrations of 10^{-8} and 10^{-6} g/ml for "Super Yellow" and "Bravo", respectively. The shoot regeneration of both cultivars was further stimulated by a temperature of approximately 20°C and a variation of light and darkness. Finally, 11 cultivars, representing different main groups of varieties, readily initiated adventitious shoots on pedicel-explants on the medium of Murashige & Skoog, supplemented with agar 0.8%, sucrose 4% and BA 10^{-6} + IAA 10^{-7} g/ml.

The development of the shoots was almost completed 2-3 months after incubation. Shoots with a length of at least 0.4 cm were then excised from the explants (up to 85 shoots from 1 pedicel-segment) and transferred to a medium of Murashige & Skoog, completed with agar 0.8%, sucrose 2% and IAA 10^{-7} g/ml. Adventitious roots were initiated within 2 weeks after transfer and consequently plantlets were produced approximately 3 months after incubation *in vitro* of pedicel-segments.

It was estimated that according to this procedure one flowering plant can yield at least 1.5 million plantlets after one year. Apart from this aspect of vegetative propagation, this method is also useful for storage. When a rapid build-up of clonal plant material is not urgently required, pedicels which already initiated adventitious shoots, can be stored for 3 months at least at 2°C in continuous darkness. In a later stage, however, a rapid multiplication can become desirably and realized at 20°C (Roest & Bokelmann, 1975, *Scientia Hort.* (in prep.)).

c. Significance of adventitious bud techniques *in vivo* and *in vitro* for mutation breeding.

In vivo adventitious buds produced on detached irradiated leaves of cv. Bravo, proved to be of a chimeral nature in the majority of cases. They developed on callus, obviously from more than one cell, formed at the base of the petiole.

Via *in vitro* culture 1300 flowering plants of the cultivar Bravo were obtained, which originated from explants of the leaf, the pedicel and the capitulum. Before incubation the explants were irradiated with different doses of X-rays, ranging from 0-2000 rad, to determine the radiosensitivity, the mutation frequency and thus the optimum dose. The results can be summarized as follows: Pedicel-segments regenerated the highest number of adventitious shoots and, moreover, they developed faster as compared to young flower heads or leaves. The mutants produced by irradiating the various explants were almost exclusively of a solid, non-chimeral nature. In addition, histological observations suggested that single epidermal cells were involved in the initiation of the adventitious shoots on pedicel-explants. The optimum dose for mutant production was approximately 800 rad X-rays. Rather often, more than one phenotypically identical mutant was found, which always was derived from the same explant. They probably originated from a multi-apical meristem formed by a single mutated cell.

Publications - 1974

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Eucarpia-FAO-IAEA Conf. on Mutations and Polyploidy,
Bari 1972: 29-35 (1974).
- Broertjes, C. The development of (new) *in vivo* and *in vitro* techniques of significance for mutation breeding of vegetatively propagated plants. First FAO/IAEA Research Coordination Meeting on Improvement of Vegetatively Propagated Crops and Tree Crops through Induced Mutations, Ohmiya-Tokai, Japan, 1974 (in press).
- Broertjes, C., S. Roest and G.S. Bokelmann. Mutation breeding of *Chrysanthemum morifolium* Ram. using *in vivo* and *in vitro* adventitious bud techniques.
Euphytica (in prep.), 1975.
- Broertjes, C. Meeting of the Mutation Breeding Contact Group, Wageningen.
External Report No. (in press).
- Roest, S. and G.S. Bokelmann. Vegetative propagation of *Chrysanthemum morifolium* Ram. *in vitro*.
Scientia Hort. (in prep.), 1975.

Resultaten van het project No. 11

Hoofd van het team en wetenschappelijke medewerkers:

A. Ringoet, G. Merkk.

Titel van het project:

Research on the nature of induced and spontaneous mutations.
Induction and nature of unstable chlorophyll mutants in tomato
(*Lycopersicon esculentum* Mill.).

Beschrijving van de resultaten:

The main object of the research in 1974 was the localization of the mutated chlorophyll genes in the mutants C₆ and C₁₂, obtained at an earlier stage in this research. For this purpose a series of trisomics have been crossed with the mutated plants, which produced the F₁. If the F₂ segregates in 3 : 1 for green and yellow plants respectively, it can be concluded that the chromosome which is present three times in the trisomic used for producing the F₁, is not the chromosome on which the chlorophyll gene is located. Deviations from the 3 : 1 ratio may indicate that the trisomic plant has the chlorophyll gene bearing chromosome three times.

In all F₂ progenies of the mutant C₆ a 3 : 1 ratio for green and yellow plants respectively was found. Therefore, the C₆ (chlorophyll) gene could not be localized.

The gene responsible for the chlorophyll aberration in mutant C₁₁ is located on chromosome 4 (see Annual Report 1973).

Because of the difficulty to make the F₁ cross and to obtain enough seeds from all trisomic crosses, the experiments with C₁₂ were not conclusive.

Due to other priorities, this programme will be discontinued in 1975.

Resultaten van het project No. 12

Hoof van het team en wetenschappelijke medewerkers:

A.J.G. van Gastel

Titel van het project:

Induction of self-compatibility in dihaploid Solanum tuberosum L.

Beschrijving van de resultaten:

After chronic γ -irradiation and self-pollination of a self-incompatible dihaploid Solanum tuberosum L. a number of berries with seeds were obtained. Genetic analysis of plants (and progenies) derived from the mutated seeds indicated that the self-compatibility character is radiation-induced (no self-compatibility in the control series) and of a genetic nature (self-compatibility present in I_1 and I_2). The data of 1974, which are not yet completely analyzed, confirm our earlier conclusion that no permanent changes on the S-locus had been induced. Moreover, the data show that chimerism cannot account for the fact that not all flowers of a self-compatible plant set seeds upon selfing.

Resultaten van het project No. 13

Hoofd van het team en wetenschappelijke medewerkers:

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

Titel van het project:

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

Beschrijving van de resultaten:

a. Mutation spectrum analysis at the S-locus of *Nicotiana glauca*
Link and Otto

Pollen mother cells were exposed to acute doses of X-rays or fast neutrons and, at anthesis, the pollen produced was used to pollinate non-irradiated plants of the same S₂S₃-clone. The results (fig.1) show that fast neutrons are slightly more effective than X-rays for inducing self-compatible pollen-part mutations but that neither fast neutrons nor X-rays have the capacity to generate new S-alleles. An unexpectedly high fraction of the SC-mutations induced does not display the centric fragment which usually characterizes such mutations. The ratio of fragment to non-fragment SC-mutations of fast neutrons is identical to that of X-rays. A possible use of permanent SC-mutations for production of F₁ hybrid seed is proposed (fig.2). It has been found that chronic irradiation, although ineffective for inducing SC-mutations, considerably stimulated seed-set upon selfing (VAN GASTEL and DE NETTANCOURT (1974). Radiation Botany 14: 43-50); the experiments were carried out in such a way that it was not possible to determine if the seed-set induced by chronic irradiation resulted from an effect on the pollen or on the stylar component of the self-incompatibility reaction. The data of 1974 indicate that the increase in pseudo-self-compatibility (seed-set) essentially resulted from an effect of irradiation on the pistil of the flower. Experiments in which PMC's were treated with EMS did not yield S-mutations (see Annual report 1973). This year experiments have been carried out to establish whether or not the absence of S-mutations could be due to the fact that the EMS did not reach the pollen mother cells. It was clear that EMS did not have an effect (as measured by the pollen sterility) upon buds with PMC's at the meiotic stage, but that a significant effect (pollensterility increased to 70%) could be found at the premeiotic stages. Therefore, one reason for the absence of mutations might have been the fact that the stage treated was not the most sensitive one. In a preliminary experiment pollen mother cells were treated with a base analogue (bromodeoxyuridine). Base analogues are known to induce changes in the DNA (new specificities?).

Further more, tetraploid individuals, diploid pollen-part mutants and their advanced progenies were studied. The incompatibility reaction in the homozygous tetraploid did not always occur in the style. Such results, suggest that tetraploidy may shift the site of inhibition from the style to the stigma or that a relationship exists between gene dosage and stage of occurrence of the incompatibility reaction.

Among the inbred progeny of the self-compatible heterozygous tetraploid, self-incompatible individuals have been detected. Therefore one may wonder if the competition theory really provides the only possible explanation to the self-compatibility in tetraploids. Results obtained in progenies of pollen-part mutants suggested that competitive interaction does not play a role in the manifestation of the self-compatibility in pollen-part mutants, complementation being the only explanation.

b. The analysis of factors and mechanisms involved in the generation of new S-alleles

In order to elucidate the mechanism operating when inbred plants of Lycopersicon peruvianum Mill. generate new S-alleles (DE NETTAN-COURT et al, Proc.R.Soc.Lond.B., in press) the same S_1 and S_2 alleles were placed in several different genetic backgrounds to ascertain the influence of such backgrounds on the frequency and specificity of spontaneous constructive mutations at the S-locus. The same material will permit the detection of an eventual relation between new specificity and the identity of the S-alleles which have been associated, one generation earlier, to S_1 and S_2 (Edström theory). The S_1S_2 genotypes in new genetic backgrounds were submitted, in 1974, to obligate inbreeding. Only 48 seeds were obtained from 1014 pollinations after heat shocks and hormone treatments. These seeds have been sown and the resulting populations are being submitted to detection tests and to ancestry tests to find out if a new specificity is present and if the new specificity is identical to one of the S-alleles which had been associated, one generation earlier, to S_1 and S_2 .

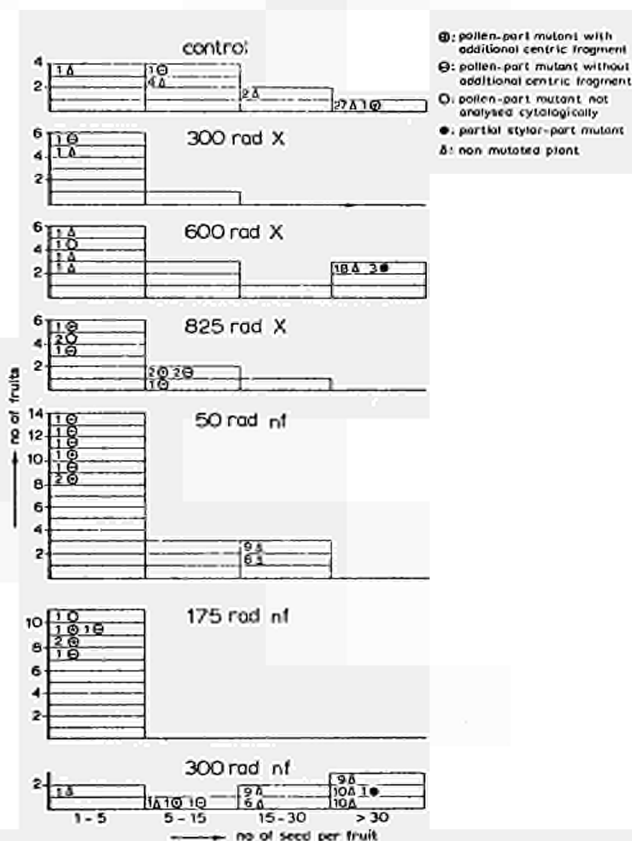


fig 1: Distribution of seed set and distribution of mutated and non-mutated individuals after crosses between S_2S_3 ♀ and irradiated S_2S_3 ♂ (*Nicotiana glauca*)

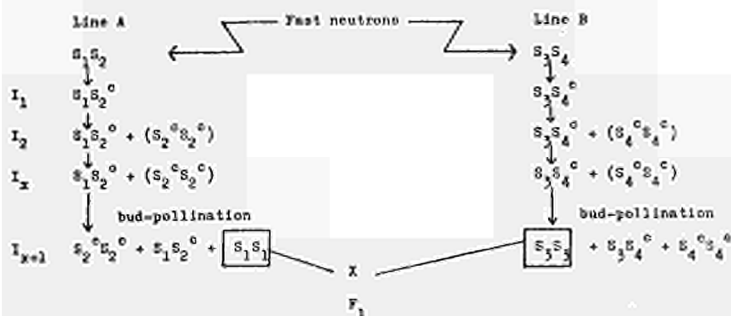


fig 2: Inbreeding scheme of self-compatible S-heterozygotes and production of self-incompatible S-homozygotes for constitution of F_1 hybrid seeds.
 S^o = permanent mutation conferring compatibility to the pollen which carries it. If the self-compatibility character originates from a competition effect mediated by a free centric fragment, the inbred progenies will only consist of self-compatible S-heterozygotes.

Publications - 1974

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- BREDEMEIJER, G.M.M. and GASTEL, A.J.G. van. Incompatibility in flowering plants. Internal report No. 148 (1974).
- GASTEL, A.J.G. van, and NETTANCOURT, D. de. The effects of different mutagens on self-incompatibility in Nicotiana glauca Link and Otto. -I Chronic gamma irradiation. Radiation Botany 14, 43-50 (1974).
- GASTEL, A.J.G. van, and NETTANCOURT, D. de. The effects of different mutagens on self-incompatibility in Nicotiana glauca Link and Otto-II Acute irradiations with X-rays and fast neutrons. Heredity (in press).
- NETTANCOURT, D. de, DEVREUX, M., CARLUCCIO, F., LANERI, U., CRESTI, M., PACINI, E., SARFATTI, G. and GASTEL, A.J.G. van. Facts and hypothesis on the origin of S-mutations and on the function of the S-gene in Nicotiana glauca and Lycopersicon peruvianum. Proc.R.Soc. (Lond) B. (in press).

Resultaten van het project No. 14

Hoofd van het team en wetenschappelijke medewerkers:

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

Titel van het project:

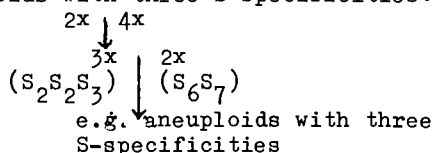
Establishment of linkage relationships with the S-locus of self-incompatible plants and identification of the S-bearing chromosome.

Beschrijving van de resultaten:

Identification of the S-bearing chromosome (*Nicotiana alata* Link and Otto)

In the progeny of a triploid x diploid cross, aneuploids can be expected. Among these, the plants with $2x + 1$ chromosomes are the most suitable for the identification of the S-bearing chromosome, because all such plants which are self-compatible can be suspected to carry the S-bearing chromosome as the additional chromosome. However, SC-aneuploids with $2x + 2$, $2x + 3$, ... chromosomes can also be used, since all these plants must at least display the S-bearing chromosome three times. The chromosome which is present three times in all SC plants must be the S-bearing chromosome. We have chosen, however, a slightly different approach because it was found that competitive interaction which is essential for the method described above, does not always confer self-compatibility to heterogenic diallelic pollen grains. Our method is based on the fact that all aneuploid plants displaying three different S-specificities in the style should be trisomic for the S-bearing chromosome.

Production of aneuploids with three S-specificities:



Two aneuploids have been cytologically analysed (Table 1).

In plant C191A-22 (figure 1) and C191A-26 (figure 2) chromosome no. 1, 2, 4, 5, 8, 9 and chromosome no. 2, 4, 6, 8, 9 are respectively present three times (classification: Carluccio et al). Since chromosome no. 2, 4, 8 and 9 are present three times in both plants one of them must contain the S-bearing chromosome.

Table 1. Cytological analysis of two aneuploids with three S-specificities

No of the aneuploid	chromosomes present three times								
	1	2	3	4	5	6	7	8	9
C 191A-26		X		X		X		X	X
C 191A-22	X	X		X	X			X	X

Publications - 1974

CARLUCCIO, F., NETTANCOURT, D. de and GASTEL, A.J.G. van.
On a possible involvement of chromosome 3 in the
formation of self-compatibility mutations in Ni-
cotiana alata Link and Otto. In: Mutation and Po-
lyploidy, Proc. Joint Session, EUCARPIA-IAEA-FAO,
Bari: 41-50 (1974).

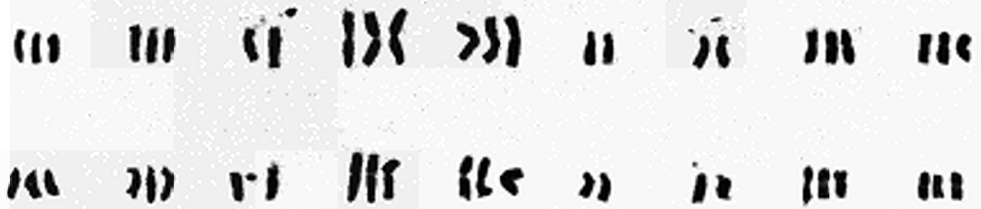


Fig. 1 : Karyotype of the aneuploid C191A-22

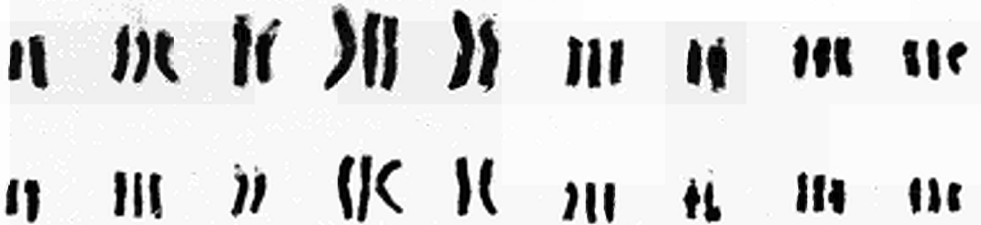


Fig. 2 : Karyotype of the aneuploid C191A-26

Resultaten van het project no. 15

Hoofd van het team en wetenschappelijke medewerkers:

G. Bredemeijer, A.J.G. van Gastel, F.A. Hoekstra.

Titel van het project:

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

Beschrijving van de resultaten:

Peroxidase in Incompatibility research

Pollination and subsequent pollen tube growth in Nicotiana alata cause an increase in the activity of several stylar peroxidase isoenzymes. In order to establish whether these changes play a role in the inhibition of incompatible pollen tube growth the peroxidase isoenzyme composition in the various parts of the style were examined by means of starch gel electrophoresis. The results are presented in fig. 1. Peroxidase isoenzyme no. 8 which had been in the past, suggested to be necessary for compatible pollen tube growth increases only in stylar parts which have already been passed by the pollen tube tips (see fig. 1; cross-pollinated t = 69 and 93 hrs). This means that this increase is rather a consequence than a prerequisite of compatible pollen tube growth. Concerning peroxidase 10 it can be observed that after pollination, a wave of activity arises in segment 1 and is then transmitted to the basal end of the style. The changes in the activity of peroxidase 10 are independent of the type of pollination performed.

During the first day after pollination both compatible and incompatible pollen tubes grow in segment 1, which has a very low peroxidase 10 activity. Thereafter, tips of compatible pollen tubes grow in front of the activation wave of peroxidase 10, whereas tips of incompatible pollen tubes grow behind this front in a zone with a high peroxidase 10 (fig. 1, t = 45, 69 and 93 hrs). This fact indicates that peroxidase 10 is directly or indirectly involved in the inhibition of incompatible pollen tubes. A few preliminary results appear to support, at the moment, the hypothesis of pollen tube rejection by peroxidase 10. In the first place it has been demonstrated that peroxidase 10 is able to inhibit and stop pollen tube growth in vitro and in the second place, peroxidase 10 has a very low activity in self-pollinated immature styles in which the pollen tube growth is not inhibited.

Oxidative phosphorylation in respiring binucleate and trinucleate pollen (in collaboration with G.M. Desmet)

In order to elucidate the mechanism of energy conservation in respiring but non-germinating bi- and trinucleate pollen in humid air (RH = 97%) - information which may be of interest in relation to the incompatibility reaction - mitochondria have been isolated.

At first, preparations were made from germinating binucleate Nicotiana alata Link et Otto and Typha latifolia L. pollen, to gain some experience in the techniques of isolation. The composition of the medium, in which pollen was germinated, turned out to be of importance for the oxidative capacities of the isolated mitochondria. Best mitochondrial activity was obtained by germinating the pollen in 300 $\mu\text{g/ml}$ $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 200 $\mu\text{g/ml}$ $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 100 $\mu\text{g/ml}$ KNO_3 , 0.2 M saccharose and 100 $\mu\text{g/ml}$ H_3BO_3 . In this way, leakage of U.V.-absorbing material out of the pollen grains into the medium was considerably reduced. Figure 2 shows the respiratory and phosphorylating activities of isolated mitochondria from pollen of Typha latifolia L., equilibrated in humid air and germinated for 3 hours (A), 1 hour (B) respectively, and ungerminated (C). Oxygen uptake was measured polarographically by the oxygen electrode method, NADH serving as substrate. Other substrates such as succinate and malate/pyruvate also gave good results. The antibiotic oligomycin acts as an inhibitor of both ATP formation and oxygen uptake, without uncoupling. No accelerated oxygen uptake of the oligomycin inhibited system occurred upon renewed ADP gift (figure 2 (A)). 2-4 Dinitrophenol stimulates oxygen uptake by uncoupling respiration and phosphorylation. At proper concentrations of DNP, the maximum capacity of the electron transport chain (ETC) of the isolated mitochondria can be established. Mitochondria, isolated from ungerminated but reconditioned pollen of Typha latifolia L. showed no stimulation of oxygen uptake upon addition of ADP or DNP (figure 2 (C)). The capacity of the ETC, however, considerably improved during germination in vitro. It can be concluded that ungerminated binucleate Typha pollen, normally respiring in humid air (RH = 97%) of 30°C at about 0.07 $\mu\text{mol CO}_2/\text{mg.h}$, does not carry out oxidative phosphorylation. This phenomenon was also established for the binucleate pollen of Nicotiana alata Link et Otto and Narcissus poeticus L.

For the trinucleate pollen of Compositae, best germination in vitro and optimal reduction of leakage could be realized with the following germination medium: 0.93 M saccharose, 500 $\mu\text{g/ml}$ $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, and 100 $\mu\text{g/ml}$ H_3BO_3 . Unfortunately, the preparations of mitochondria from Cosmos bipinnatus Cav. and Aster tripolium L. were not as excellent as from Typha. After 10 minutes of germination, mitochondria were isolated and tested for oxidative and phosphorylative activity. The maximum respiratory control (RC) ratio's that could be obtained amounted to 1.5 - 2. On the other hand, a proper concentration of DNP substantially increased oxygen uptake. The DNP-stimulated state rate divided by the steady state rate amounted to 4-5, indicating the ETC to be well developed after 10 minutes of germination. Isolations from ungerminated but reconditioned pollen, showed a slight stimulation of oxygen uptake by ADP and a considerable stimulation by DNP. Foregoing suggests ungerminated trinucleate Compositae pollen, normally respiring in humid air (RH = 97%) of 30°C at about 0.40 $\mu\text{mol CO}_2/\text{mg.h}$, to be capable of oxidative phosphorylation. Isolation of mitochondria out of intact cells and experiments in vitro, the integrity being involved, may lead to wrong results. To avoid this, an alternative method has been developed, dealing with the phosphorylative activity of respiring pollen, indeed, but leaving the grains intact. The procedure was based on penetration of oligomycin and DNP in the ungerminated pollen, prior to incubation in humid air. Diethylether as carrier, turned out to cause no damage to vital processes. Gas exchange of the respiring pollen was followed by gas chromatography. Table 1 shows the effect of oligomycin on the CO_2 evolution and O_2 uptake.

Table 1.

Effect of oligomycin (100 $\mu\text{g}/\text{ml}$ ether) on respiratory properties of bi- and trinucleate pollen in humid air (RH = 97%) at 30°C. CO_2 and O_2 given in $\mu\text{mol}/\text{mg}\cdot\text{h}$.

Species	- oligomycin		+ oligomycin			
	CO_2	O_2	CO_2	%contr.	O_2	%contr.
<u>Trinucleate</u>						
<u>Cosmos bipinnatus Cav.</u>	0.48	0.47	0.07	16	0.05	10
<u>Tanacetum vulgare L.</u>	0.30	0.34	0.06	19	0.03	10
<u>Aster novi Belgii L.</u>	0.44	0.49	0.07	15	0.05	10
<u>Chrysanthemum spec.</u>	0.49	0.56	0.08	16	0.04	7
<u>Binucleate</u>						
<u>Narcissus poeticus L.</u>	0.069	0.071	0.068	99	0.068	96
<u>Typha latifolia L.</u>	0.066	0.077	0.067	102	0.079	103

The binucleate pollen of Narcissus poeticus L. and Typha latifolia L. did not change the oxygen uptake as a result of addition of oligomycin. In contrast, pollen of Cosmos bipinnatus Cav., Aster novi Belgii L., Tanacetum vulgare L., and Chrysanthemum spec. considerably reduced oxygen uptake, indicating the trinucleate types to have a high rate of ATP production. These observations support the earlier results, obtained with isolated mitochondria. The different rates, at which bi- and trinucleate pollen produce ATP during incubation under humid conditions (RH = 97%), allow for more detailed studies concerning the energy consuming processes, especially the protein and RNA metabolism.

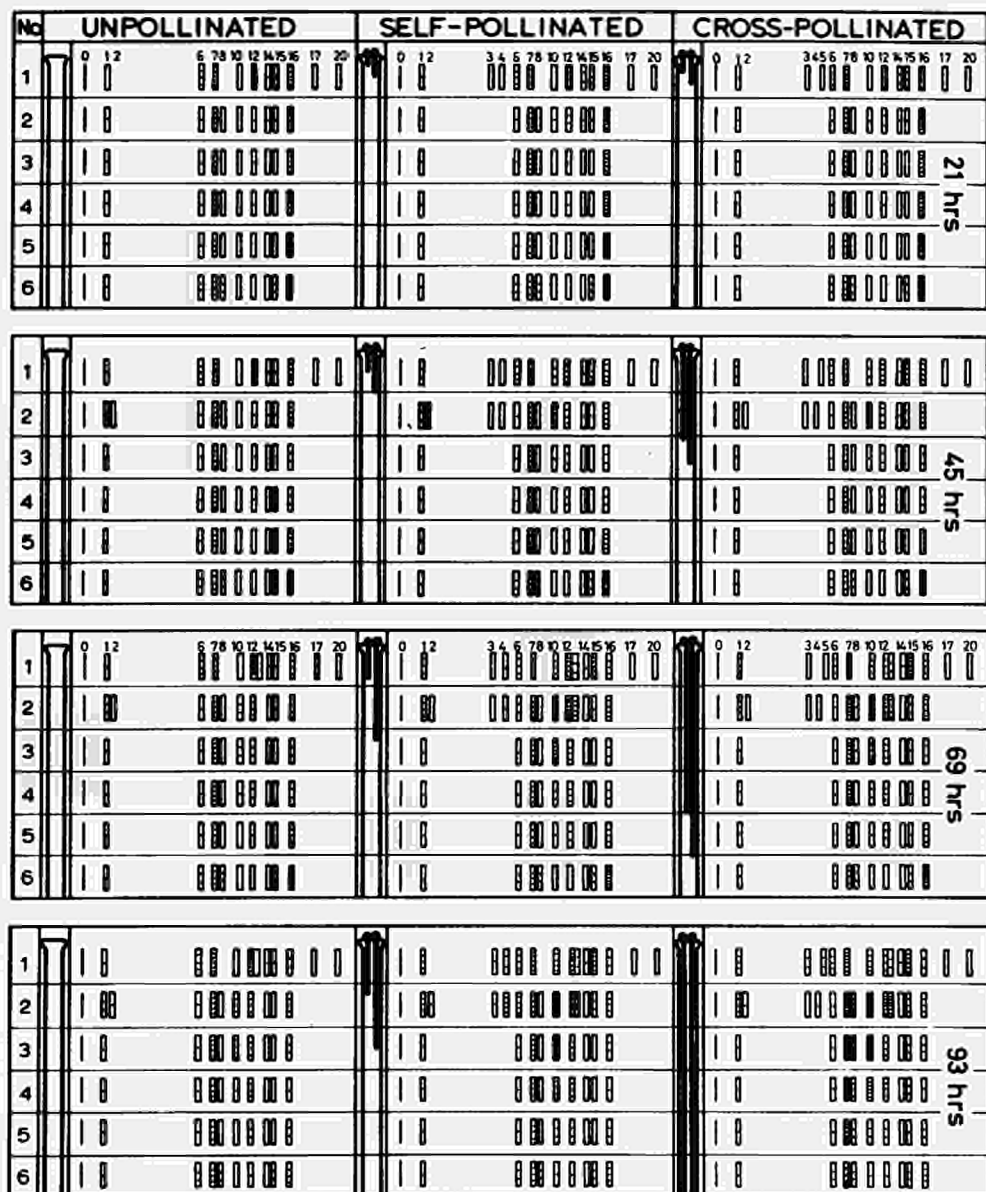


Fig. 1. Distribution of cationic peroxidase isoenzymes in un-, self-, and cross-pollinated styles of *Nicotiana glauca* at various times after pollination. Minimum and maximum pollen tube lengths out of at least 20 styles are presented. The degree of hatching of the peroxidase isoenzyme bands approximates the staining intensity. No hatching means that a band on the gel was just visible. 0 is origin.

Publications - 1974

- BREDEMEIJER, G.M.M. (1974): Peroxidase activity and peroxidase isoenzyme composition in self-pollinated, cross-pollinated and unpollinated styles of *Nicotiana glauca*.
Acta Bot. Neerl. 23: 149-157.
- BREDEMEIJER, G.M.M. and A.J.G. van GASTEL (1974): Zelf-incompatibiliteit in hogere planten.
Vakbl. Biol. 54: 346-352.
- BREDEMEIJER, G.M.M. and A.J.G. van GASTEL (1974): Incompatibility in flowering plants. A meeting organized by the Royal Society of London, April 25, 1974. (Internal Report 148)
- HOEKSTRA, F.A. and J. BRUINSMA: Respiration and vitality of binucleate and trinucleate pollen.
Physiologia Plantarum (in press).
- HOEKSTRA, F.A. and J. BRUINSMA: Viability of Compositae pollen: germination in vitro and influences of climatic conditions during dehiscence.
Zeitschrift für Pflanzenphysiologie (in press).

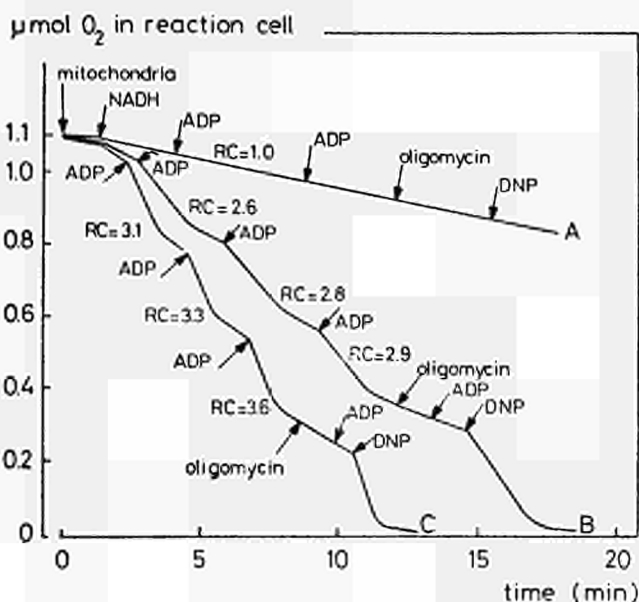


Fig. 2. Oxidative and phosphorylative activities of mitochondria, isolated from *Typha latifolia* L. pollen, germinated *in vitro* for 3 hours (A), 1 hour (B) and 0 hour (C). Reaction mixture: 3,8 ml medium containing 0.5 M mannitol, 0.01 M TES pH 7.2, 0.0005 M Na₂-EDTA, 0.3% bovine serum albumine, 0.005 M MgCl₂ and 0.005 M KH₂PO₄; 0.4 ml mitochondrial suspension, 3 µmol NADH and 0.5 µmol ADP each time, 5 µg oligomycin and 10⁻⁴ M DNP. The respiratory control ratio's are also given. O₂-uptake during the reaction given as µmol O₂/min. per 100 mg dry pollen.

Resultaten van het project No. 16

Hoofd van het team en wetenschappelijke medewerkers:

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

Titel van het project:

The radiation dose-fractionation effect in *Saintpaulia*.

Beschrijving van de resultaten:

Leaves of *Saintpaulia ionantha* H. Wendl. c.v. Utrecht show, when exposed to a low initial radiation dose, a higher radioresistance against a second X-ray dose, given 8 to 24 hours after the first one, in comparison to untreated leaves. 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, radiation stimulation were investigated.

Moreover, the problem of the G₁- or G₂-stage of epidermal cells of leaf petioles has been studied by cytochemical methods.

As for the metabolic activity of the leaves the influence of temperature was further studied. The optimum time interval for a maximum dose-fractionation effect seemed to be somewhat longer for 10° than for 20°C. The fact, that the dose-fractionation effect lasts for a longer time when the leaves are stored, during the time interval, at a lower temperature, has been confirmed.

Experiments carried out formerly seemed to indicate that there was no relation between the dose-fractionation effect and a possibly altering gas-environment inside the sealed polyethylene bags, in which the leaves normally are stored during the experiments. This statement proved to be a correct one.

Experiments, in which plants were irradiated and excised leaves were kept on water during the time interval between irradiations, proved that the dose-fractionation effect was clearly present in both cases.

Another way to find out whether the dose fractionation effect has a metabolic origin was by applying a SH-compound named dithiothreitol (DTT). From literature it is known that DTT is an effective protector against ionizing radiation in living cells and that it significantly decreases all types of chromosomal breakage. The objective of using DTT on *Saintpaulia* leaves was to see whether or not a pretreatment with DTT can give protection against a high X-ray dose, just as the low dose of 500 rad is able to do.

Experimental results demonstrated that a DTT-pretreatment is equivalent to the protective effect of 1 - 1,5 krad. Results of an experiment in which the fractionation effect was investigated after a treatment of 500 rad and DTT are not yet available.

The question whether or not transport is involved in the development of the dose-fractionation effect, was brought to a conclusion. Experiments were

carried out, in which petioles of leaves were irradiated with high X-ray doses, with (24 h before) or without pretreatment of the leaf blades with 500 rad X-rays. These experiments demonstrated that transport was absent: differences in radiosensitivity between leaves with pretreated leaf blades and with unpretreated leaf blades were not observed. Apart from these results it was demonstrated that the radiosensitivity increases as a greater part of the leaves receives irradiation (whole leaf versus petiole).

Another fact to be mentioned is the stimulation of the production of adventitious plantlets at the base of the leaf petiole by doses of radiation in the $\frac{1}{2}$ - 3 krad range. This radiostimulation amounts on the average to 10 - 30% above the control-value. Concerning the dose, at which maximum stimulation occurs and the influence of storage of the leaves upon the stimulation, inconclusive results have been obtained.

Radiostimulation occurred very frequently in the experiments carried out, but was not always present. Reasons for the sometimes observed absence of the stimulation are unknown at present.

Concerning the radiosensitivity of the leaves it has been demonstrated by many, though not by all, experiments, that the radiosensitivity of the leaves decreases with a longer time interval between detaching and irradiation (from 0 h up to 8 or even 24 h ("time-effect")). There are no clear indications, at this moment, that this time-effect and the dose-fractionation effect have a parallel course with time in the 0 - 24 h period after detaching. Two other experiments have made it unlikely that the time-effect is part of the dose-fractionation effect. In one experiment the dose-fractionation effect was still present after a 24-h storage of the leaves inside sealed polyethylene bags in darkness. The fractionation effect was practically identical for leaves, irradiated on the plant and for leaves, irradiated when detached in absence of any storage period. This time-effect on radiation sensitivity seems to be a complicating factor in measuring the effect, caused by dose-fractionating.

During 1974 the growth medium for the leaves has changed from a soil-peat medium to watercultures, as this way of culturing proved to be just as good and three times faster than the "soil-method".

The Feulgen reaction on DNA was used to study changes occurring in nuclei of epidermal cells of Saintpaulia petioles. A morphological change in shape of the nuclei has been found closely related to the age of the leaves.

Cytofluorometric measurements, performed on epidermal strips stained with Feulgen reagens, by means of UV illumination, reveal that all the nuclei of one strip contain the same amount of DNA, thus indicating that the nuclei are at the same stage of the cell cycle (G_1 , S, G_2 or M). No variation in DNA content between cells of petioles of different ages have been observed. To investigate the stage of the cell cycle in epidermal cells, pollen mother cells have been chosen as a reference for the DNA content. In premeiotic nuclei the DNA content has reached its maximum at the end of the S-stage of the cell cycle. The anthers of Saintpaulia were prepared under experimental conditions comparable to those for the epidermal strips of the petioles. Only those cells exhibiting a minimum of DNA condensation (early Leptotene) were measured. The results show that the DNA quantity of the epidermal cells is half the DNA content of premeiotic cells, thus indicating the G_1 -phase for epidermal cells.

A still better reference was found within the epidermis of excised leaves, cultured for 5 days on aerated water. At this time some cells in the immediate vicinity of the wound are dividing again.

The results show that the DNA content of nuclei is half the DNA content of profase nuclei. Statistical calculations on the values obtained from the experiments lead to the conclusion that epidermal cells of the petiole of a leaf are in the G_1 -stage of the cell cycle.

Publications - 1974.

ENGELS, F.M. (1975) Cytofluorometric detection of the cell cycle stage of epidermal cells of *Saintpaulia* petioles. (In preparation).

Resultaten van het project No. 17

Hoofd van het team en wetenschappelijke medewerkers:

Geneticist, CH.H. HÄnisch ten Cate

Titel van het project:

The control for plant breeding purposes of mutagenesis and the induction of directed mutations (provisory title)

Beschrijving van de resultaten:

Because the responsible geneticist has not yet been recruited, no research was done on the principal subject of this project. In relation to the methodology for induction of haploids, using plants cultivated respectively on ^{15}N and ^{14}N containing solutions and thermal neutron irradiation after selective pollination, dosimetric measurements have been done in the thermal column of the Barnreactor (collaboration K.J. Puite). The γ -contamination of the neutron flux being rather high (860 rad over the irradiation period), its specific effect on this type of plant material has to be studied in a preliminary experiment.

Resultaten van het project No. 18

Hoofd van het team en wetenschappelijke medewerkers:

C. Broertjes.

Tital van het project:

Organization and coordination of applied mutation breeding.

Beschrijving van de resultaten:

During 1974 again many scientists and commercial plant breeders used our service and asked for general information about the possibilities of mutation breeding and existing literature resulting in the beginning of a new project when the prospect seemed positive, scientifically speaking. Thirteen new projects were started in 1974, namely with *Amaryllus*, *Begonia*, *Bouvardia*, *Canna*, cucumber, *Liatris*, *Rosa*, *Saintpaulia*, *Sinningia*, *Streptocarpus*, tomato, *Tulipa* and *Watsonia*, all but two being vegetatively propagated ornamentals.

The total number of projects, started since 1959 has now surpassed 200. Very promising results have been obtained with a combined cross-breeding and mutation breeding programme, using the adventitious bud technique, in *Begonia*. By a well-planned procedure yellow flowered mutants (winter-flowering potplants) were obtained of which one or two have been introduced into commerce (Doorenbos, J. and J.J. Karper: X-ray induced mutations in *Begonia x hiemalis* to be published in *Euphytica*, 1975). Among thousands of mutants only very few chimeras were found. (Similar results have been obtained in the USA by Mickelsen).

No other mutants have, to our knowledge, been introduced into commerce in 1974, except an autotetraploid of *Achimenes* cv. *Tarantella* (under the name "Tango") having darker pink and larger flowers as well as a more compact growth habit.

The Mutation Breeding Contact Group had a successful meeting at Wageningen, The Netherlands. For the first time several scientists from Denmark and Great Britain joined the group. The discussions were mainly concentrated upon the new 5-years programme of the Biology Division of the Commission of the European Communities (see External Report).

Publications-1974.

- Broertjes, C. and H. Verboom. Mutation breeding of *Alstroemeria*.
Euphytica 23, 39-44 (1974).
- Broertjes, C. Tetraploïde, rose-bloeiende *Achimenes*-nieuwigheden.
Vakbl. Bloem. 29 (39): 21 (1974).
- Broertjes, C. Mutation breeding of vegetatively propagated plants.
Manual on Mutation Breeding; 2nd revised edition, chapter ..
(in press).
- Broertjes, C. Mutation breeding of vegetatively propagated plants.
19th Internat. Hort. Congress, Warsawa, Poland, 1974 (in press).

Resultaten van het project no. 19

Hoofd van het team en wetenschappelijke medewerkers:

S.C.E. Romkes, J.H. Becking.

Titel van het project:

Influence of irradiated medium on microorganisms.
Uptake and localization of cytotoxic compounds, induced by
irradiation of food.

In the course of an investigation into the cytotoxic effects of irradiated media, some hundreds of strains have been isolated from tropical fish, Sardinella cameronensis. These strains have been cultured in synthetic medium, containing irradiated glucose. The most sensitive strains have been grown in chemically defined medium under conditions of controlled pH, temperature and aeration in a fermentor. By repeatedly passing these sensitive strains through irradiated glucose medium at increasingly higher doses of irradiation an adaptation process could be observed. This adaption is very slow and requires many passages through irradiated medium for a two-fold increase in resistance to develop. The resistance does not increase monotonously at each subsequent step but develops rather with leaps and bounds.

In previous reports it was mentioned that the selected strains did not seem to be very consistent in their growth rate, when exposed to irradiated medium. As the growth rate in irradiated medium is a measure of the sensitivity of the strain, this posed serious problems. It was found, however, that this feature is an integral part of the adaptation process and is due to the loss of resistance. The acquired resistance is completely lost if the strain is cultured in an unirradiated medium and is completely or partially lost in medium that has lost all or some of its toxicity due to the passage of time. The toxicity of irradiated glucose decreases steadily with time. It was found that the concentration of sulphhydryl groups in the cell increases gradually after several passages through irradiated medium and decreases in the event that the strain loses its resistance by growth in unirradiated medium. Further confirmation of previous results was sought in a series of short experiments. Due to the expiration of the temporary employment contract (post-graduate fellowship) of the responsible scientist (S.C.E. Romkes), research was temporarily stopped. Recruitment of his successor is in progress.

Resultaten van het project No. 20

Hoofd van het team en wetenschappelijke medewerkers:

H. Stegeman.

Titel van het project:

The radiation and heat resistance of bacterial spores.

Beschrijving van de resultaten:

Metals play important roles in formation and in final properties of bacterial spores. The aim of this study is to get more information about the basic role of metals in relation to the radiation and heat resistance of bacterial spores.

As mentioned in earlier annual reports, spores with a high manganese content which were obtained by increasing the manganese content of the sporulation medium or reducing the calcium content, were more resistant to irradiation. Therefore, investigations were started to know in which way manganese may play a role in formation of spores with increased resistance to irradiation. First spores of *Bacillus subtilis* ATCC 6633 were loaded with manganese by suspending the spores in 0.02 M $MnCl_2$ and incubating this suspension for 18 hours at 60 °C. The heat resistance increased considerably by this treatment. Table 1 shows the effect of loading with manganese and some other cations on the radiation and heat resistance. It was surprising to find the high radiation resistance of Li^+ , Na^+ , K^+ and Mg^{2+} loaded spores. Other cations studied such as Ca^{2+} , Ba^{2+} , Sr^{2+} , Co^{2+} , Ni^{2+} , La^{3+} and Al^{3+} , were able to increase the heat resistance, but the radiation resistance was unaffected. The synergistic effect of a combined radiation and heat treatment of metal loaded spores remained. See figures 1 and 2.

Elutions of metals from spores with HCl at pH 1.0 - 1.5 gave spores having a decreased heat resistance and an increased high synergistic effect of a combined radiation and heat treatment. See figure 3. Elutions at lower pH resulted in spores with a reduced synergistic effect. See figure 4.

Release of dipicolinic acid from spores was only measured after an hydrochloric acid treatment below pH 1.0.

Reloading the spores having a high or reduced synergistic effect with metals gave again spores with the initial heat resistance and synergistic effect. The above results suggest that the sensitizing mechanism of pre-irradiation is probably located in the cortex of the spores and related with the contraction of the thick peptidoglycan layer in this cortex. Further investigations will be orientated towards the role of the cortex in radiosensitization of bacterial spores to heat.

Table 1 - Radiation and heat resistance of cation loaded *B. subtilis* spores.

Cation	Radiation Resistance		Heat Resistance	
	D ₁₀ -value (krad)	95% confidence interval	D _{90°C} -value (min)	95% confidence interval
non-treated	161	156<D ₁₀ <166	2,6	2,4<D<2,8
LiOH-pH10	243	222< " <263	-*	
NaOH-pH10	202	196< " <209	21,8	19,3<"<24,9
KOH-pH10	209	203< " <215	19,4	16,1<"<24,6
CaCl ₂	166	160< " <172	17,8	16,3<"<19,6
MnCl ₂	183	165< " <204	12,1	11,3<"<13,0
MgCl ₂	202	197< " <208	13,1	11,8<"<14,7

*non-exponential survival curve.

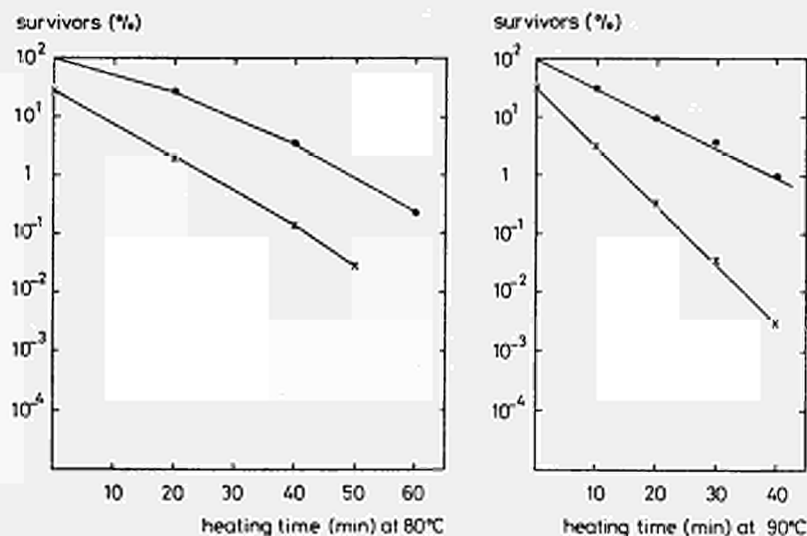


Fig. 1. Effect of pre-irradiation with 100 krad on heat resistance of untreated *B. subtilis* spores. ● unirradiated. x irradiated.

Fig. 2. Effect of pre-irradiation with 100 krad on heat resistance of calcium-loaded *B. subtilis* spores. ● unirradiated. x irradiated.

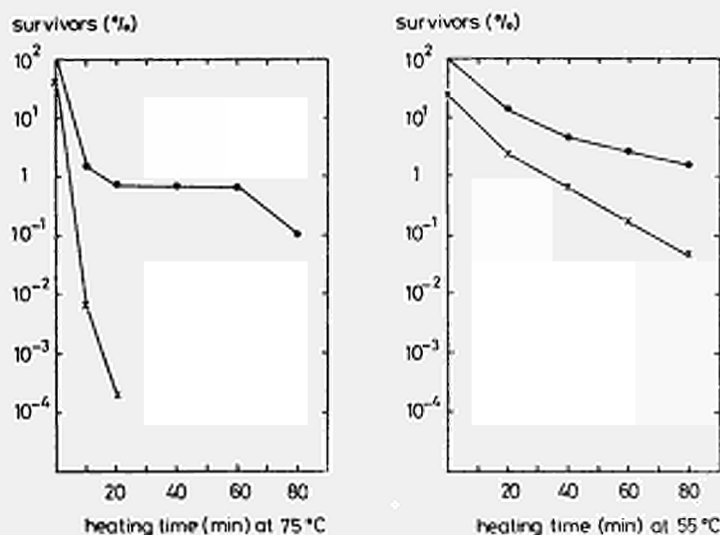


Fig. 3. Effect of pre-irradiation with 100 krad on heat resistance of acid-treated (pH 1.5) *B. subtilis* spores. ● unirradiated. x irradiated.

Fig. 4. Effect of pre-irradiation with 100 krad on heat resistance of acid-treated (pH 0.8) *B. subtilis* spores. ● unirradiated. x irradiated.

Resultaten van het project no. 24

Hoofd van het team en wetenschappelijke medewerkers:

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

Titel van het project:

Coordination of food irradiation research.

Beschrijving van de resultaten:

I. Studies to promote practical application of food irradiation

1.1 Legislation of food irradiation.

Considerable progress has been achieved in the field of legislative procedures. Petitions for the irradiation of 'Endive' and 'Base coat from vegetable protein' were prepared in order to request a category II application, which means the irradiation of experimental batches. (See also Annual Report Project no. AA 26/65). In previous petitions, which have resulted in clearances of irradiated products in either category II or III, the clearance was obtained on wholesomeness data emerging from animal feeding tests, which demonstrated toxicological safety of those products. In case of the endive petition evidence of toxicological safety was approached by means of an analysis of possible radiochemical events. The acceptance of an evaluation based on an identification of radiolysis products by the Commission of Food Irradiation is an interesting development in the Dutch policy on legislation. The irradiation dose for endive to be applied on the prepacked product is max. 100 krad.

The petition for 'Base coat from vegetable protein' is requested for a specific item of the group of coatings for a reduction of the microbial count, and especially the Enterobacteriaceae, which can be achieved with 150 Krad. Irradiation appears to be an adequate alternative of fumigation. The clearance of spices in category II obtained in 1971, was again extended for the irradiation of a batch of 4000 kg.

Favourable results from pilot plant experiments with irradiated onions received proper attention from the side of industry. For further developments towards commercial practice a petition for clearance in category III of irradiated onions with the purpose of sprout inhibition was forwarded to the Dutch Commission of Food Irradiation. The evidence of toxicological safety was based on feeding tests carried out in Canada and Japan, and furthermore on comparison of chemical composition of onions and that of potatoes, strawberries, mushrooms and endive. Clearance of onions in category III is to be expected in 1975.

At the end of 1974 negotiations were initiated for the preparation of category II petitions for irradiated fillets of cod, haddock and plaice, and for irradiated peeled prepacked potatoes. The willingness demonstrated by the Dutch Authorities, to accept the extrapolation of wholesomeness and chemical data on one commodity to others of similar chemical composition when considering petitions for clearance appears an important step towards the overall clearance of irradiation as a process.

1.2 Marketability test with irradiated chicken.

The first experiment of a series of planned activities for the commercialization of irradiated poultry was carried out. The principle of the test was the distribution of irradiated chicken in bulk by means of a commercial distributor, followed by a quality judgement on successive days during a 10 days storage period by qualified personnel of the participating organisations. Preliminary results indicate almost unanimously the acceptability of irradiated chicken as an adequate alternative when a local market for this type of product fails. The irradiation induced reduction of total plate count appeared sufficient to keep the number of microorganisms - although post irradiation growth occurred - during the storage period of this marketing test always lower than the initial count of the non-irradiated product.

1.3 International trading of irradiated items.

A trial shipment of irradiated Thai onions was evaluated technologically. Half of the consignment, carried on deck, was rejected upon arrival because of rot; the second half, which was transported in a chilled room, showed no difference between irradiated and non-treated onions. However, after a sprout testing period of 3 weeks at ambient temperature, sprouting in non-irradiated onions was almost complete, while external sprouting in irradiated onions hardly occurred. The overall result of this sea-borne shipment was negatively influenced by the fact that irradiation was not applied at the optimal time after harvest.

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

The Institute for Fishery-products T.N.O. has continued its programme on the irradiation of fish-fillets and shrimps to the stage of semi-practical application.

The Spelderholt Institute for Poultry Research has continued studies on the radio-resistance of Enterobacteriaceae in fresh and frozen poultry. The necessary irradiation dose for a feasible shelflife extension of chilled fresh poultry appears to be sufficient for obtaining products free from non-sporeforming pathogens.

The Institute for Processing and Storage of Agricultural Products continued studies on low energy electron-beam irradiation of potatoes with the aim of sprout inhibition.

Several activities related to food irradiation were developed by private industries, which needed our attention and guidance.

Resultaten van het project no. 25

Hoofd van het team en wetenschappelijke medewerkers:

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

Titel van het project:

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

Beschrijving van de resultaten:

I. Reproductive function of Groot Yorkshire sows

The study of the effects of irradiated and autoclaved feed on reproductive function were continued during 1974 according schedule. The parent generation animals have produced their second litter. Upon weaning of F_{1b} the parent generation was discarded. At this time the sows have completed a period of 13 months on the respective diets. From clinical observations made during that period no disturbances were observed in the health of the pigs of the control and the two experimental groups, that might be attributable to the feeding of irradiated or autoclaved feed. The total amount of test diets supplied during the total life span of the sows amounted to about 1100 kg per pig.

Performance data of the F_1 and the first offspring of F_2 are recorded in table 1.

Table 1.

Comparison of reproductive function of the 3 Feedgroups of sows.

Feed-group	Gene-ration	Number of young born			Number of piglets weaned		Averaged weight at		Pre-weaning losses %
		alive	still	births	male	fe-male	at birth	at 49 days	
			normal	grossly-abnormal			grammes		
Auto-claved	F_{1a}	69	7	-	31	34	1369	15361	6
	F_{1b}	90	4	-	31	25	1418	13181	38
	F_{2a}	75	5	3	29	32	1458	14734	19
Irra-diated	F_{1a}	82	11	-	36	31	1250	16224	13
	F_{1b}	77	3	-	38	20	1309	16217	25
	F_{2a}	38	13	18	16	15	1459	15490	18
Con-trol	F_{1a}	71	4	-	29	31	1349	14743	16
	F_{1b}	79	7	-	31	40	1391	16841	10
	F_{2a}	80	9	?	25	38	1383	15058	21

During pregnancy of the first offspring of the second generation a SMEDI-infection occurred amongst the sows, which resulted in severe abnormalities in the progeny of especially the groups of sows fed on irradiated and control diet. In case of the control feed group the number of grossly-abnormal youngs could not be recorded because of intra-uterine partial resorption of foetuses. The averaged pre-weaning loss of the 3 offsprings of the feed - group 'autoclaved' is 23%, while the figures for the feed-groups 'irradiated' and 'control' are 19 and 16 resp. During the lactation period the piglets were weighed at 5, 12, 21, 36 and 49 days of age to permit growth rates to be compared between the 3 feed-groups for individual youngs of the same litter. Table 2 presents the average growth of piglets from all the litters belonging to the same feed-group.

Table 2.

Comparison of growth rates of piglets for the various periods between two weighings in grammes per day for 3 offsprings.

Feed-group	0 thr. 5 days			6 thr. 12 days			13 thr. 21 days			22 thr. 36 days			37 thr. 49 days		
	F _{1a}	F _{1b}	F _{2a}	F _{1a}	F _{1b}	F _{2a}	F _{1a}	F _{1b}	F _{2a}	F _{1a}	F _{1b}	F _{2a}	F _{1a}	F _{1b}	F _{2a}
Auto-claved	153	114	169	184	201	238	281	215	214	271	227	264	412	357	379
Irra-diated	137	129	160	179	179	237	225	288	238	295	274	235	550	513	478
Con-trol	123	151	175	196	209	232	263	254	223	287	304	261	385	504	404

The results of table 2 show a tendency towards a reduced growth rate of the piglets at the end of the lactation period for the feed - group 'autoclaved'. The 49 days weight of the youngs from the 'auto-claved' feed-group is in the order of magnitude of 10% lower than that of the piglets from the 'irradiated' and 'control' feed - groups. The reduced growth rate of the piglets from the 'autoclaved' feed-group is also manifested during the post-weaning period and at the onset of the fattening. The general health and survival of the youngs from the 'autoclaved' feed-group appeared not optimal. Their resistance to normal stress conditions like changing of pens, dietary regimen, castration, was in general lower than that of the piglets from the other two feed-groups. This lack of resistance was very clear demonstrated in case of the F_{1b} litters, which showed an almost 100% mortality after weaning.

II. Fattening experiment with F_{1a} growing pigs

A fattening test comparable to a 90 days feeding test was carried out with the offspring of the first generation. The animals of the 3 feed-groups were weighed at monthly intervals to permit growth rates and feed conversion to be compared between the two experimen-tal groups and the control group.

Feed conversion data are collected in table 3.

Table 3.

Figures for feed conversion of the growing pigs from the 3 feed - groups for 4 fattening periods.

Feed - group		Fattening periods (days)			
		0 thr. 35	35 thr. 60	60 thr. 95	95 thr. 111
Auto- claved	male	3.46	2.94	3.38	3.10
	female	3.74	3.65	2.95	3.57
Irra- diated	male	2.56	3.34	2.62	3.61
	female	2.73	2.68	3.10	3.67
Control	male	2.71	2.54	3.27	3.10
	female	2.78	2.45	3.59	3.09

Fattening pigs of the 'autoclaved' feed group needed about 10% more feed per kg weight gain than the pigs from the 'irradiated' and 'control' feed-groups.

III. Wholesomeness studies of radappertized ham

This chronic toxicity study involves the feeding of ham incorporated into the standard diets of rats during two years. The ham and lean meat was obtained from the pigs of the fattening experiment (mentioned in paragraph 2 of this report), and the green product was processed by means of radappertization and autoclaving. The protocol of this feeding test includes 6 groups of animals to be studied concomitantly viz. one positive control group, and 5 groups with ham in the diet. The feeding test was started in April 1974, but terminated in July following due to an unintentional Hg-contamination of the standard diet.

The feeding test was started again in the month of November. Final results are to be expected in spring 1977.

Resultaten van het project no. 26

Hoofd van het team en wetenschappelijke medewerkers:

D.Is. Langerak

Titel van het project:

Preservation of fruits and vegetables by means of ionizing radiation.

Beschrijving van de resultaten:

In 1974 the experiments on the storability of strawberry yoghurt using irradiated strawberries have been continued. A number of experiments have been carried out with prepacked cut red cabbage and endive.

In collaboration with Sprenger Institute and S.N.U.I.F., irradiation experiments were done with onions to prevent sprouting in relationship to the commercialization.

I. Strawberry yoghurt

A. Recovery medium tests for yeast flora.

By studying the influence of irradiation on the yeastflora of deep frozen strawberries the problem arised that counting of yeast on the recovery medium was impossible because of mould growth.

The following 4 media were compared with the aim of finding a suitable recovery medium for yeast without mould growth.

- a. The yeastmedium according to Mossel;
- b. The synthetic medium for yeast according to Ayers, Rupp and Johnson.
- c. Potato Dextrose Agar (P.D.A.)-medium, pH 5.5;
- d. P.D.A.-medium, pH 3.5.

The media b, c and d were used with and without the following additions: 1. oxytetracycline (antibioticum), 2. calciumpropionate (fungicide), 3. difenyl (fungicide).

Besides these additions the influence of double pouring of the growth medium on mould and yeast growth was also investigated (anaerobic environment).

The mould growth was measured by the covered surface in percentage of the total surface. The data were statistically compared by means of the 'Signtest'.

The experiments showed that additions to the media did not induce significant differences in recovery of yeast. Bacteria growth was prevented on all media with oxytetracycline and on P.D.A.- medium with pH 3.5.

Mould growth occurred on all media without a fungicide. This growth was not suppressed by an anaerobic environment (double pouring).

The mould growth was clearly delayed by an addition of the fungicide calcium propionate. However, a complete prevention was not reached. The fungicide working of difenyl did not appear. Comparing tests between all media with calciumpropionate and oxytetracycline addition, showed no differences. In further examinations P.D.A.-medium, pH 3.5 + calciumpropionate was used.

B. Storability experiments of yoghurt with irradiated deep frozen strawberries.

In collaboration with a research laboratory of a dairy industry 2 experiments concerning the influence of irradiation on the keeping quality of strawberry yoghurt packed in plastic cups, were carried out.

Preliminary sensoric tests proved that irradiation as compared to heat pasteurization improved the texture of the fruits in the yoghurt.

II. Prepacked cut red cabbage

In a number of experiments the influence of such factors as processing, packing, irradiation and acetic-acid on the overall quality of cut red cabbage has been investigated.

After cutting the product was for periods varying between 0-60 sec dipped in water and 0-5 sec centrifuged; then packed in polythene bags without perforations. The irradiation dose was varied from 0 to 200 krad. The product was stored at 10°C. The experiments showed that a short dipping of 1 to 2 sec in tapwater gave less brown-discolouration of the cutting surface than non-dipping. To prevent visible desiccation a centrifuging time of 2 to 3 sec (900 x g) was preferable.

In the beginning of storage, the influence of irradiation on the brown-discolouration was small. However, after 4 days the colour of the irradiated product remained better than the colour of the non-irradiated product. Dipping in an acetic-acid solution of 1% did not further improve the colour.

It appeared from microbiological examinations that a dose of 50 krad reduced the total viable count with 1-2 decimals. A dose of 100 krad gave a reduction of 3 - 5 decimals. The influence of irradiation on the visible decay was obvious. The shelf life was increased by about 75%, as compared to the control.

The Enterobacteriaceae were reduced with 3 decimals at 50 krad. A dose of 100 krad eliminated them almost entirely ($< 10 \text{ g}^{-1}$).

The count of aerobic spores was very low (2.5 g^{-1}) and did not increase during storage. After cooking irradiation did not influence unfavourably the colour, odour and taste of the product.

III. Prepacked cut endive

A. Control of aerobic and anaerobic spores.

After prepackaging the product was irradiated with 0 and 100 krad gamma rays and stored at about 10°C.

The endive was inspected for:

- a) total viable count incubated on Plate Count Agar-medium;
- b) aerobic spores [Bacillus] also incubated on PCA-medium; however the suspension was preheated during 15 min. at 80°C for killing the vegetative bacteria;
- c) anaerobic spores [Clostridium] by means of the M.P.N. technique in culture tubes.

Differential Reinforced Clostridial Medium according to Gibbs and Freame (1965) modified by Mol and Timmers (1970), was used. The observations showed that, by 100 krad irradiation, the total viable count was reduced with 2,5 - 4,5 decimals.

The initial number of aerobic spores was $< 10^2 \text{ g}^{-1}$ and did not increase during storage. The reducing effect of 100 krad irradiation was less than 1 decimal.

The initial number of anaerobic spores was $< 5 \text{ g}^{-1}$ and also did not increase during storage. No effect of irradiation was observed. Therefore spoilage due to aerobic and anaerobic spores in prepacked vegetables is very small.

B. Further experiments in relation to practical application.

The product was prepacked in non-perforated polythene bags (0.02 mm) by a commercial central-packing station. The samples were taken from the normal day production.

The following treatments of the endive were compared:

- a) transported without cooling after prepackaging to the Institute and irradiated the same day;
- b) stored at 0-1°C for approx. 10 hours after prepackaging in the central-packing station, in the night transported by normal trade-transport (non-cooled) to a Supermarket and irradiated on the next day.

The irradiation dose amounted to 100 krad gamma rays.

After irradiation the endive was stored for 2 hours at 20°C (simulating transport to shops) and afterwards at about 10°C (simulating refrigerated display case).

The endive was tested on quality and microbiologically inspected for:

- a) total viable count; b) enterobacteriaceae; c) aerobic spores (Bacillus).

The experiments showed that the initial quality and a careful processing are important for the keeping quality; bruised leaves discoloured quickly, prepackaging immediately after cutting partially prevented the discolouration.

Irradiation one day after prepackaging did not influence the colour unfavourably provided the product was cooled immediately after cutting.

The initial total viable count was about 10^7 g^{-1} and increased up to 10^8 g^{-1} in 2 days at 10°C (rotting).

An irradiation dose of 100 krad reduced the total viable count with 3-4 decimals and the shelf life was lengthened by approx. 100%.

The Enterobacteriaceae count was about 10^6 g^{-1} and increased up to 10^8 g^{-1} in 3 days. Irradiation reduced this count with 4-5 decimals.

The count of aerobic spores (Bacillus) was also in the commercial endive very low, $< 10^2 \text{ g}^{-1}$. During storage this count did not increase. The reduction by 100 krad irradiation was $< 1/2$ decimal. In these experiments the development of micro-organisms was not delayed by the short cooling of appr. 10 hours at 0-1°C.

IV. The irradiation of onions, feasibility study (collaboration H.G. Heins).

The feasibility of the irradiation of onions on commercial scale was studied by a working group. Supplementary experiments were executed during the storage period 1973-1974, together with a study regarding investment and cost of running a commercially operated plant with an annual capacity of 20.000 tons, however, to be handled within four weeks.

Results are:

1. No significant differences were found in sprout inhibition in the low dose range of 1,2 to 4 krad.
2. 10 Differences races of onions, grown, harvested and irradiated (4 krad) under comparable conditions, showed great equivalence in quality after storage.
3. Complete mechanical handling (harvesting and transporting) did not damage the bulb irradiated.
4. Based on this and previous year's results, a commercial irradiation of onions is considered to be feasible up to and including the fourth week after harvesting. Beyond this 4 weeks period losses because of less effective sprout inhibition can occur, depending of climatical factors during the growth of the crop.
5. The calculated cost of running appeared to be acceptable. The final decision concerning the construction of a commercial plant with the above mentioned capacity is anticipated for 1975.

Publications

External Reports

D.Is. Langerak, R. Hovestad, A.A.M. v.d. Valk, J.C.M. Valetin: Het gistvrij maken van aardbeien bestemd voor vruchtenyoghurt door middel van gammastralen (1973). External Report no. 17. Association Euratom - ITAL.

D.Is. Langerak, R. Hovestad, C.B.G. Daamen: Vergelijkende test van verschillende voedingsbodems voor gisten t.a.v. de schimmelgroei (1974). External Report no. 19. Association Euratom -ITAL.

R. Hovestad, D.Is. Langerak: Onderzoek naar de besmetting en groei van aerobe bacteriën, aerobe en anaerobe sporenvormers in voorverpakte gesneden andijvie bij 12°C (1974). External Report no. 20. Association Euratom -ITAL.

D.Is. Langerak, R. Hovestad, J.M. Bankers: Invloed van verwerking, verpakking en bestraling op de houdbaarheid van gesneden rode kool (1974). External Report no. 21. Association Euratom-ITAL.

In Press

D.Is. Langerak:
The influence of irradiation and packaging on the keeping quality of prepacked cut endive, chicory and onions.
Acta Alimentaria.

Resultaten van het project No. 27

Hoofd van het team en wetenschappelijke medewerkers:

Alan S. Robinson

Titel van het project:

Genetical control of *Hylemya antiqua* Meigen by structural chromosome mutations.

Beschrijving van de resultaten:

1. Genetic data on radiation induced structural chromosome rearrangements.
Using low doses (500 r and 1000 r X-rays) of radiation, four chromosomal translocations (T) and two inversions (I) have been induced in *Hylemya antiqua* Meigen. Genetic information relevant to their use in a control programme has been collected; it is felt that all the rearrangements with the exception of I¹ offer potential for genetic control. The data are presented in Table I. Both males and females carrying the translocations showed reduced fertility, although the reduction was not as great as might be expected. A possible cause of this is the survival to the larval stage of a certain proportion of "lethal" duplication/deficiency zygotes. This has been shown to have occurred in T5 and T6. For the inversions, crossing-over is necessary for there to be a reduction in fertility and as only the female carriers exhibited reduced fertility it was concluded that crossing-over does not occur in *Hylemya antiqua* males. The segregation ratio for the rearrangements is not statistically different from the theoretical value of 1:1 except in one case. Females carrying I₂ have been identified which produce a distorted segregation ratio. However, unexpectedly the distortion is not restricted to one direction i.e. sometimes more inversion heterozygotes are produced and sometimes more normal individuals. Experiments are underway to determine the cause of this distortion and to try to capitalize on it, if possible. The homozygosing of the rearrangements is now giving very encouraging results, although as yet a homozygous line has not yet been established. However, in the two translocation lines so far tested viable fertile homozygous individuals have been identified by a combination of fertility measurements and cytological screening. For example, fully fertile matings, in both translocations, have been observed in which all the progeny were heterozygotes; this indicates that the mating was between a translocation homozygote and a wildtype individual. Expansion of the experimental technique must yield matings between translocation homozygotes from which a homozygous line can be established. Only one inversion has been tested for homozygous viability and it was lethal.

1.1. Triploid Stock

During inbreeding of the T6 a mating between a translocation homozygote and a wild type was identified by being fully fertile and producing all heterozygous larvae; however, all the larvae were also triploid i.e. 3N. The mating was fully fertile and it is hoped that a triploid stock can be reared. Its possible use in genetic control is speculative at the moment.

2. Field Experiments

2.1. Population cages.

In the 1974 season preliminary releases were made with a cyclic three chromosome translocation which had a fertility of 50%; only heterozygotes could be released as the homozygote is inviable. Two small field cages were utilized and into one cage, heterozygotes were released in a 1:1 ratio with wild-type insects. The other cage contained the same total number of wild-type insects. In the cage with the translocation the fertility at the first generation was 60%, in the control cage it was 94%. Many eggs were oviposited on the onion bulb and there was no evidence of a second generation. The reason for this was that the release of the flies was too late in the season relative to the stage of development of the crop i.e. the onions were too large when the first instar larvae emerged.

2.2. Inbred field material.

For the isolation of visible mutants approximately 200 pupae were collected from a small private onion garden in Wageningen. The flies from these pupae were mated and eggs were collected from individual females. Flies descending from individual females were then inbred to reveal visible mutants. In two of the stocks it was impossible to inbreed as there was an extremely distorted sex-ratio in favour of males such that in one case out of 77 flies only 3 were females and in the other case all 67 were males. The reasons for this sex-distortion are speculative at the moment, however, if meiotic drive is implicated then such sex-ratio distortion could be a powerful tool for genetic control.

3. Induction of new rearrangements.

3.1. Irradiation of eggs.

Many hundreds of eggs were collected from the large fly cages maintained at I.P.O., Wageningen, within a few minutes of oviposition. The eggs were irradiated with 300 r of X-rays after 15, 45 or 75 minutes at a temperature of 23°C. The adults from the irradiated eggs were screened for rearrangements, none were found.

3.2. Irradiation of fertilized females.

Females ovipositing fertilized eggs were irradiated with 500 rads of X-rays and the F₁ progeny were test-crossed to control insects to screen for the presence of rearrangements. Two F₁'s did show reduced fertility but no rearrangement could be cytologically confirmed. There is evidence from *Drosophila* work that this method of irradiation does lead to a good response in the production of translocations. Only a small number of F₁'s were tested; the experiment will be extended in the following year.

3.3. Irradiation of males with 200 rads X-rays.

This low dose was chosen to try to increase the probability of obtaining a viable homozygote. To the present time 90 F₁'s (both male and female) have been test-crossed to control insects. At least eight of these matings show reduced fertility; cytological observation on these stocks has not yet been completed.

4. New stocks.

Two stocks have been obtained from Canada (Keith Reid, University of Guelph) and both are now being reared at the Association. One stock constitutes the wild-type Canadian stock and crosses will be made with the Dutch strain to see if there is any natural incompatibility. The other stock has a visible mutant as a marker, the eyes are white; in normal flies the eyes are brown. The stock will be used to investigate important aspects of reproductive biology, for example multiple mating and the length of the pre-oviposition period.

Publications-1974.

- ROBINSON, A.S. and CURTIS, C.F. Controlled crosses and cage experiments with a translocation in *Drosophila*. *Genetica* 44: 591-601 (1974).
- ROBINSON, A.S. Gamma radiation and insemination in the codling moth *Laspeyresia pomonella*. *Ent. Exp. and Appl.* 17: 425-432 (1974).
- ROBINSON, A.S. and HEEMERT, C. van. Preliminary radiobiological studies on *Hylemya antiqua* Meigen and data on three radiation induced chromosomal rearrangements. IAEA/FAO. The Sterility Principle for Insect Control. Innsbruck 1973. In Press.
- ROBINSON, A.S. Influence of anoxia during gamma radiation on the fertility and competitiveness of the adult male codling moth *Laspeyresia pomonella*. *Rad.Res.* In Press.
- ROBINSON, A.S. and PROVERBS, M.D. Field cage competition tests with a non-irradiated wild and an irradiated laboratory strain of the codling moth. *Environ. Entomol.* In Press.
- ROBINSON, A.S. A reassessment of the use of chromosomal inversions for insect control. *J. Hered.* In Press.

Table I

Fertility, Segregation Ratio and Homozygous Viability of
Structural Chromosome Rearrangements in Hylemya antiqua.

Rearrangement	Chromosomes Involved	Fertility		Segregation Ratio		Homozygous Viable
		♀	♂	♀ +/+ : Het	♂ +/+ : Het	
T 5	2 ^{s(+)} - 6 ^{s(-)}	81.9 ± 10.9	74.9 ± 5.5	9 : 12	11 : 13	+
T 6	3 ^{l(-)} - 5 ^{s(+)}	62.2 ± 9.2	61.7 ± 7.1	10 : 12	11 : 6	+
T 7	2 ^{l(-)} - 4 ^{l(+)}	60.4 ± 7.8	55.9 ± 6.0	10 : 8	4 : 7	N.T
T 8	2 ^l - 5 ^l	≈ 50	≈ 50	N.T.*		N.T
I 1	6	73.9 ± 6.7	97.4 ± 2.8	30 : 31	49 : 48	-
I 2	3	70.0 ± 6.4	96.2 ± 5.9	(see text)	N.T.	N.T

* N.T = Not yet tested.

Resultaten van het project No. 28

Hoofd van het team en wetenschappelijke medewerkers:

D. Snieder

Titel van het project:

Genetical control of Adoxophyes orana F.v.R.

Beschrijving van de resultaten:

The investigated subjects of radiobiological research for a genetical control of A. orana were:

To test the hypothesis that the difference found between the heridity of chromosomal aberrations induced in irradiated male and female moths, can be explained by the moment of meiosis in relation to the moment of irradiation (Annual Report, 1973). Third and fifth instar larvae have been irradiated with 2 and 5 krad ^{60}Co γ -rays (Fig.1). It appears that the F_1 -progeny from the irradiated females shows a fertility above that of the irradiated P-generation (fig.1a). These data are in close agreement with theory because only premeiotic stages have been irradiated (Suomalainen, Department of Genetics, Helsinki, pers. comm.). However, in the case of the irradiated males data are contradictory; after treatment of premeiotic sperm by irradiation of third instar males with 5 krad γ -rays approx. 50% of the F_1 -progeny has a fertility below that of the P-generation (fig.1b). Irradiation of fifth instar male larvae treats postmeiotic sperm. Then after irradiation with 2 krad γ -rays 71% of the F_1 -progeny has a fertility above that of the P-generation (fig.1c). These data mean that the theory is not valid, or that at least other factors, as threshold values or sensitivity differences, may disturb the model. From Fig. 2 it is concluded that sperm irradiated with a substerilizing dose of 15 krad γ -rays has a rather poor competitiveness as compared to untreated sperm: a mating with an untreated male after a first mating with an irradiated male, diminishes the influence of that first mating to almost zero (determined by % egg hatching), while in the reciprocal order of mating, the influence of the normal first mating is reduced for approx. 67% by the mating with the irradiated male. The preliminary conclusion (Annual Report 1973) that irradiated sperm (25 krad γ -rays) is less competitive than untreated sperm, is therefore confirmed by these experiments. However, for a proper estimate of the degree of loss of competitiveness by both doses, i.e. for a proper comparison of the sterile-male technique, and the delayed sterility method more detailed experiments are necessary. The developmental time of F_1 larvae from irradiated male moths (15 krad, X-rays) was compared with that of normal larvae. From Fig.3 it is clear that there is a big change in developmental time after irradiation of the P-generation.

For A. crana, with two rather distinct flight periods in the field this may mean that the emergence of the sterile F_1 -adults is very poorly synchronized with that of the normal adults i.e. the effect of the F_1 -progeny from semisterile moths may be negligible in that case. As a step between the laboratory results obtained up till now and field trials, cage experiments were started. Many difficulties were encountered to get uniform, well defined populations and to analyze the effects of mating order, competitiveness of moths, sperm and F_1 -larvae, population density, ratio of treated to untreated moths, radiation doses applied etc. The relatively long generation time also hampers a quick feedback of results on new experiments. Using visible markers these experiments might be speeded up. A recessive marker has been isolated, demonstrating a smooth, orange colour of the moths and related experiments are in progress. In the release programme of Dr. Ankersmit (Laboratory of Entomology, Wageningen) again certain aspects are checked:

1. before the releases, larvae from the release-orchard have been checked for population density and examined cytologically. Contrary to the results of 1973 (Annual Report 1973) in not a single larvae visible chromosomal aberrations could be detected. Considering larvae from insecticide or fungicide sprayed orchards, evidence was got that these products do not induce chromosomal aberrations like the ones detected in the 1973-larvae.
2. Checks are performed on the degree of sterility of the released moths and their capability to leave the transport boxes. Twenty-five krad ⁶⁰Co γ -rays sterilize the moths almost completely and unknown factors cause an enormous variation in viability and longevity.

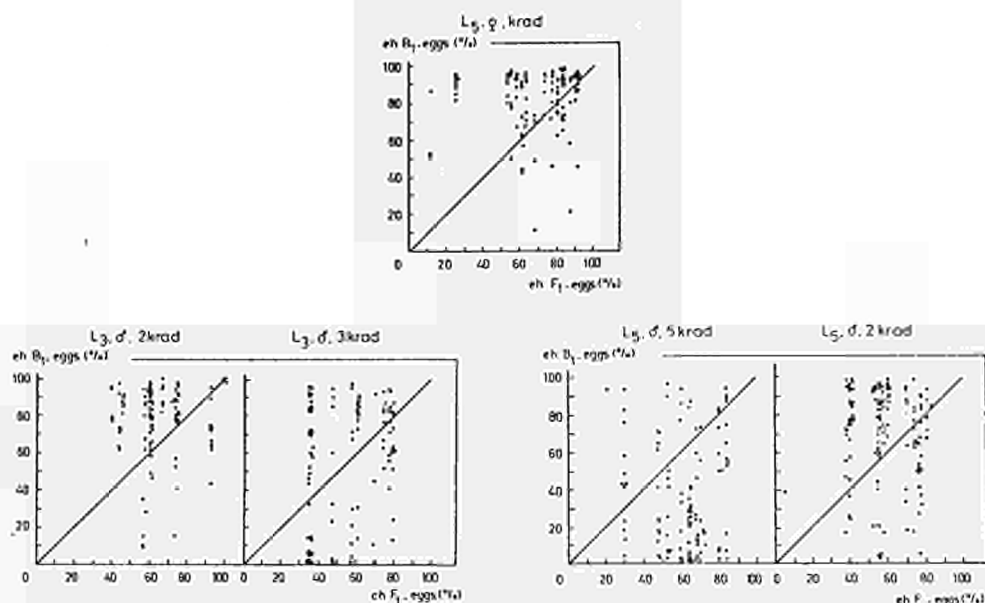


Fig. 1. Relationships between fertility of irradiated P-generations and their F_1 -progeny. Third and fifth instar larvae (q, σ^7) irradiated with 2 and 5 krad ^{60}Co γ -rays. %eh = % egg hatching.

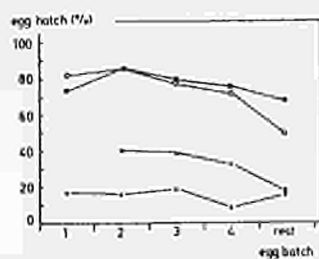


Fig. 2. Influence of mating order on % egg hatching (%eh).
 1. eggs fertilized by a mixture of irradiated and untreated sperm
 2. eggs fertilized by untreated sperm
 3. eggs fertilized by a mixture of untreated and irradiated sperm
 4. eggs fertilized by irradiated sperm.

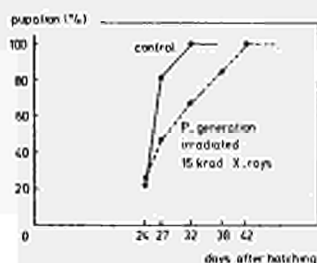


Fig. 3. Developmental time of F_1 -progeny from substerilized male moths.

Resultaten van het project No. 29

Hoofd van het team en wetenschappelijke medewerkers:

A.M. Feldmann

Titel van het project:

Genetical and radiobiological studies on the two-spotted spidermite
Tetranychus urticae Koch.

Beschrijving van de resultaten:

During the year 1974, the research was continued on the following subjects.

- I Dominant- and recessive lethals.
- II Induction of structural chromosome mutations and isolation of these mutations.
- III The influence of ionizing radiation on competitiveness of treated males
- IV Repair of radiation induced genetic effects
- V Temperature sensitive mutations.

Results

i. Dominant- and recessive lethals.

A. Sperm irradiation.

In addition to the radiation doses, presented in the 1973 Annual Report of the Association males were irradiated with resp. 12, 16 and 24 krad X-rays and with 8, 12 and 16 krad fast neutrons. The survival frequencies after 12 and 16 krad X-rays fitted well to the already established regression-line.
($y = ae^{+bx}$) y = survival; x = dose in krad.

The 24 krad survival frequency was below this line. The regression coefficient (b) = -0,136; $Y_{x=0} = 96,4$ and the correlation coefficient is 0,991. With fast neutrons it was found that the survival frequency of the 16 krad irradiation experiment did not agree well with the regression, in contrast to the results of the 8 and 12 krad irradiations $y = ae^{-bD}$ ($b = 0,192$; $y = 99,2$; $r = 0,992$, D = Dose in krad) (r = correlation coefficient).

The R.B.E. for the induction of dominant lethals in spermatids of *T. urticae* is 1.4. In the literature much higher R.B.E. values are given for mature sperm irradiation; for other sperm developmental stages the R.B.E. values are lower, but still higher than observed in the spermatids of *T. urticae*.

B. Oocyte irradiation.

As in the previous work, oocytes were irradiated during the diplotene of M1. Using fast neutrons and X-rays, oocytes were irradiated with a 8 krad dose, in addition to the doses applied in 1973, and fertilized after irradiation with untreated sperm. In other experiments irradiated oocytes were sampled, which were not fertilized; these gave rise to haploid individuals. The survival frequencies of the fertilized eggs (1-dominant lethal frequency) was compared with the survival frequencies of the unfertilized eggs. The frequency of recessive lethals was calculated by the formula

$$r.l. = 1 - \frac{V_u}{V_f}$$

Vu = survival of haploids
Vf = survival of diploids
r.l. = frequency of recessive lethals.

The dose survival curves for F₁-diploids and F₁-haploids after oocyte irradiation with X-rays and fast neutrons, are presented in fig. 1 and 3 respectively. In the same figures are shown the dose response relationships for the induction of recessive lethals.

Survival of F₁-diploids and F₁-haploids fits adequately to a two target dose response curve described by the equation $y = ae^{bd^2}$ (y = survival; b = regression coefficient; D = dose).

The best fit for the neutron irradiation of oocytes was observed when the value at 8 krad was excluded. It has to be considered if a better fit can be found when a linear component is included. The regression (b) and correlation-coefficients (r) for the survival of diploids after neutron and X-ray irradiation of oocytes are $b_{nf} = -0,054$; $r_{nf} = 0,989$; $b_{x-rays} = -0,030$; $r_{x-rays} = 0,999$, respectively. The R.B.E. for the survival of F₁-diploids after irradiation of oocytes with fast neutrons and X-rays is 1,8.

The dose response relationship of recessive lethals are linearly plotted in fig. 2 (X-rays) and 4 (fast neutrons).

A striking fact is the reduction at low doses of neutrons of the percentage of recessive lethals. This reduction is not found to be significant. However, other authors observed the same phenomenon after irradiation of oocytes of *Drosophila* suggesting a repair induction by low doses of radiation. For neutron irradiation the best fit for a linear regression is found when only the results are taken into account from 1 krad and higher. The respective regression and correlation coefficients are: $b_{nf} = 15,56$; $r_{nf} = 0,985$; $b_{x-rays} = 6,60$; $r_{x-rays} = 0,997$. The R.B.E. for the induction of recessive lethals is 2,3.

II. The induction of structural chromosome mutations and isolation of these lines.

A. The induction of structural chromosome mutations (S.C.M.) by ionizing radiation is treated extensively in a publication (Feldmann, 1974). Since then, experiments were set up in order to clarify the irregular dose response relationship for the induction of S.C.M. in sperm of *T. urticae* by fast neutrons. In the first experiments it was observed that the frequency of S.C.M. was the same for 0,5 krad, 1.0 krad and 2 krad irradiation (see fig. 5). Those experiments differed from all other experiments in the time during which the males were kept together with the females (24 h). When the experiments were repeated, (0,5 and 2 x 2,0 krad Nf), the mating time was shortened to only one hour. With 0,5 krad the percentage of S.C.M. was 6.0%. None of the S.C.M. were associated with a recessive lethal. Two krad irradiation induced in each experiment 36,7% and 25,0% S.C.M. of which 12,3% and 0,0% respectively were associated with a recessive lethal. From these results it is concluded that the length of the mating time is of primary importance for the construction of a dose-response curve. Longer mating may lead to heterogeneous sampling of sperm composed of different developmental stages with possibly different radiosensitivities.

B. The isolation of lines homozygous for a S.C.M.

Up till now 3 attempts have been made for isolating S.C.M. in a homozygous condition. The first experiment involved the irradiation of mature sperm with 500 r X-rays. Two hundred and fifty F₁-females were tested and 25 were found to be heterozygous for a S.C.M. After inbreeding of these 25 different

lines, 4 (15%) could be isolated in the homozygous state.

In the second experiment males were irradiated with 500 r fast neutrons. One hundred and ninety F₁-females were tested of which 10 were heterozygous for a S.C.M. and of these 10% could be brought in a viable homozygous condition.

In the third experiment, males were irradiated with 200 rad X-rays.

Of 480 F₁-females, 23 were heterozygous for a S.C.M. Out of these 3 (13%) could be isolated as viable homozygotes.

The isolated lines are kept in the laboratory and are subjected to tests on viability, stability and competitiveness.

III. The influence of ionizing radiation on competitiveness of males of *Tetranychus urticae*.

In table I are presented the data on competitiveness, observed after irradiation of males of *T. urticae* with different doses of radiation. After 4 krad X-ray irradiation, the treated males were as competitive as untreated males, when the F₁-progenies produced in the period of 2-5 days after irradiation, were studied. At higher radiation doses, the males become less competitive. After 16 krad neutrons, inducing the same frequency of dominant lethality as 24 krad X-rays, the males suffered the same impairment of competitiveness as after 24 krad X-ray-irradiation.

IV. Repair of radiation induced genetic effects.

For the application of the delayed sterility method of control, it is of ultimate importance to have data on the repair of damage induced by ionizing radiation in the germ cells of the treated and released males. During a 15 days period after irradiation with 4 krad X-rays, males were mated four times on days 1, 5, 10 and 15 with virgin females for one hour. During the 3 periods of 5 days, between the different mating periods, the males were kept with virgin females. It was observed that at the end of the 15 day-period the males had regained normal fertility within the 10-15 day period after irradiation treatment. The preceding data are, however, only true for survival of F₁-diploids. Considering the fertility of F₁-adult-females, the occurrence of F₁-females with normally fertility was observed already in the F₁-progeny, sampled 5 days after irradiation of the parental males. At the end of the 15 days period the treated males gave rise to F₁-diploids, which were all fully fertile. It is possible that the described repair effects influence the applicability of the delayed sterility method of control. The size of this influence will, however, depend on the course of the competitiveness of ageing males compared to younger ones. This relationship has to be studied in the near future.

V. Temperature sensitive mutations.

In 4 replica experiments, males were treated with 0.05 M-E.M.S., 0.025 M. E.M.S., 0.005 M-E.M.S. and distilled water.

No influence on dominant lethality by E.M.S. in the used concentrations was observed. The F₁-females were tested for heterozygosity of temperature sensitive mutations under 3 different temperatures e.g. 35°C, 28°C and 22°C. Only the F₁-females, derived from males treated with 0.05 M-E.M.S. were tested. It was observed that 3 out of 128 F₁-females, were probably heterozygous for a temperature sensitive mutation. However, in the series of untreated males, 20 F₁-females were also tested for heterozygosity of t.s.-mutations. Among 20 F₁-females, 1 female produced a progeny which acted at the different temperatures as a temperature sensitive mutation.

Publications - 1974

FELDMANN, A.M. The induction of structural chromosome mutations in males and females of *Tetranychus urticae* Koch. (Acarina: Tetranychidae). (in press).

FELDMANN, A.M. Contribution to the ESNA/IOBC Working group on Genetical Methods of Pest control
Competitiveness of irradiated males of *Tetranychus urticae* Koch.

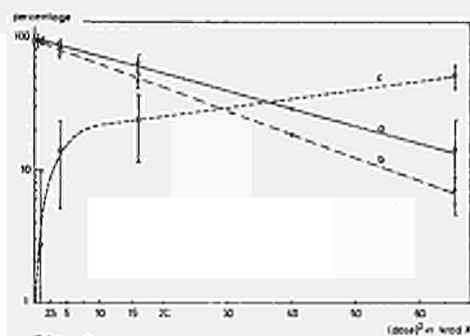


Fig. 1

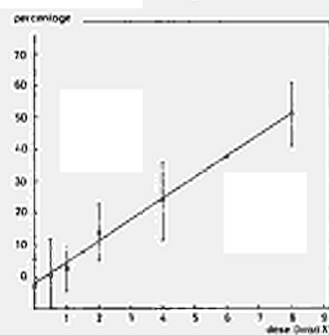


Fig. 2

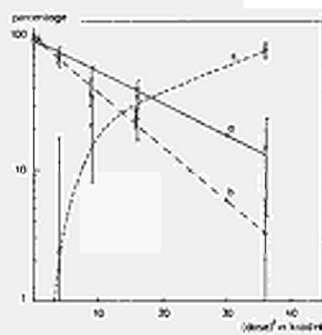


Fig. 3

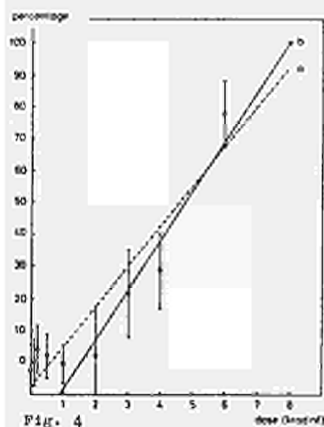


Fig. 4

- Fig. 1 - The dose-response relationship between dose of X-rays applied on oocytes in diplotene of M_1 and the % survival of F_1 -diploids (a), % survival of F_1 -haploids (b) and % of recessive lethals (c).
Horizontally are linearly given the squared radiation doses and vertically are logarithmatically given the percentages. (X-rays, 250 kV, 100 r/min).
- Fig. 2 - The dose response relationship between dose of X-rays (250 kV; dose rate = 100 rad/min; no filter used) and the percentage of recessive lethals induced in oocytes in diplotene of M_1 . Horizontally are linearly given the doses and vertically are linearly given the percentages of recessive lethals.
- Fig. 3 - The dose response relationship between dose of fast neutrons (1,5 MeV mean energy; dose rate = 100 r/min), applied on oocytes in diplotene of M_1 and the percentage survival of F_1 -diploids (a), % survival of F_1 -haploids (b) and % of recessive lethals (c).
Horizontally are linearly given the squared radiation doses and vertically are logarithmatically given the percentages.
- Fig. 4 - The dose response relationship between dose of fast neutrons (1.5 MeV = mean energy; dose rate = 100 rad/min) and the percentage of recessive lethals induced in oocytes in diplotene of M_1 .
Horizontally are linearly given the doses and vertically are linearly given the percentages of recessive lethals.
Line a is constituted of all data.
Line b is constituted of the data from 1.0 - 6.0 krad.

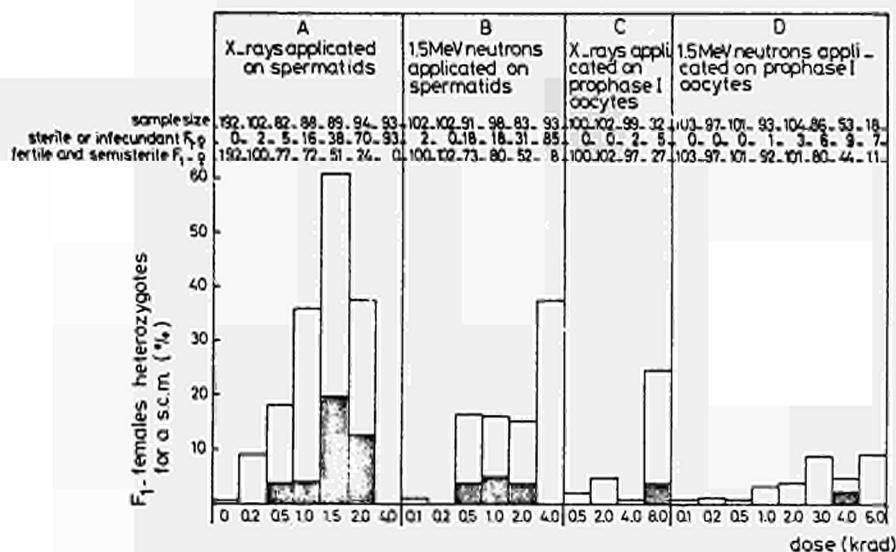


Fig. 5 - Percentages of structural chromosome mutations in the F₁-progeny of *Tetranychus urticae* Koch after irradiation of spermatids and prophase I oocytes with 1.5 MeV neutrons and 250 kV X-rays. On the horizontal axis are the different radiation doses in krad and on the vertical axis is the percentage of the tested F₁-females, heterozygous for at least one structural chromosome mutation. The total height of the column gives the percentage of the F₁-females, heterozygous for s.c.m.. The shaded area gives the percentage of F₁-females, heterozygous for at least one structural chromosome mutation, associated with at least one recessive lethal. At the top of each column is given the number of tested F₁-females, the number of either sterile or infertile F₁-females: and by subtraction the number of normal and semisterile F-females.

Table 1 - The influence of different doses of male irradiation on competitiveness of males of *Tetranychus urticae* Koch

dose	survival	competitiveness	no. days after irradiation
4 krad X-rays	0,558 ± 0,096	1,00	2 - 5
		0,89	5 - 8
		0,89	8 - 11
8 krad X-rays	0,356 ± 0,081	0,89	2 - 5
		0,86	5 - 8
		0,86	8 - 11
24 krad X-rays	0,010 ± 0,019	0,78	2 - 5
		0,74	5 - 8
		0,74	8 - 11
16 krad neutrons	0,008 ± 0,017	0,78	2 - 5
		0,82	5 - 8
		0,82	8 - 11

Resultaten van het project No. 30

Hoofd van het team en wetenschappelijke medewerkers:

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

Titel van het project:

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

Beschrijving van de resultaten:

Preparation of plant material for microlocalization of diffusable substances.

The existing methods for microlocalization considerably differ in complexity of preparation steps and possible disturbance of the tissue during the processing. The aim is to fix the minerals and other substances to be localized inside the tissue, while preserving the structure.

For dehydration and embedding the following points have been considered:

a. Damage to the tissue during freezing and drying.

From literature the following points can be deduced:

(a) To prevent the formation of intracellular ice during freezing, the rate of freezing should be as high as possible. (b) Ice-crystal growth changes rapidly with temperature. Damage will occur in days at -70°C , weeks at -80°C , months at -90°C and years at -100°C .

Small samples (2-3 mm \emptyset) shockfrozen in liquid isopentane (-160°C) and dried at temperatures below -60°C are expected to be nearly undamaged by ice crystals.

b. Investigation of the freeze drying process.

Equipment.

The micro-freezedryer has been modified and provided with four thermocouples to allow measurement and regulation of the temperature at different points (collaboration H. Roelofsen). The temperatures at four positions are shown simultaneously on a digital display. In two channels a temperature can be preset at which the measured point will be stabilized. An automatic liquid nitrogen supply system is controlled by a third thermocouple, acting as a level sensor. The fourth measuring point can be chosen according to the requirements of the actual experiment.

The whole measuring system consists essentially of a four-channel analog multiplexer, automatic cold-junction compensation, amplifiers, linearization network, analog to digital converter, four channel digital multiplexer, comparators between displayed and preset temperatures and output drivers. Its accuracy in the range from -200°C - $+100^{\circ}\text{C}$ is better than $\pm 2^{\circ}\text{C}$.

Results

Figures 1 and 2 show the dependence of the sublimation rate of pure ice on temperature and vacuum. The data in Figure 1 have been obtained at a vacuum of $1-4 \times 10^{-3}$ torr. Comparing the temperature dependence of the saturated partial pressure of H_2O over ice and the sublimation rate it can be concluded that the diffusion is not the limiting factor at this vacuum. As an increase in temperature from $-80^\circ C$ to $-60^\circ C$ causes a twenty times higher sublimation rate, the drying temperature should be taken as high as possible, determined by the ice crystal growth. The drying rate of plant material in different conditions still has to be investigated.

c. Removal of rest water.

To reduce the rest water in the dried tissue, which will influence negatively the polymerization of the embedding resin, the freeze-drying procedure is completed by a period at higher temperatures (e.g. $+30^\circ C$). The samples are subsequently kept over P_2O_5 and the embedding is carried out in a glove box in which the air is circulated over silicagel.

d. Quality of embedding and sectioning.

An épon-araldite mixture has been chosen as basic embedding resin. Very hard and liquified tissues (tomatostem or fruitstem of beans) show a good penetration of the resin and a homogeneous polymerization after a normal roomtemperature dehydration. The final hardness of the polymer is not exactly reproducible. However, the colour of the freshly prepared mixture is a very good indication for the final hardness. Freeze substituted tissue shows some gas bubble formation after penetration of the plastic, mainly in the cells of the bark. This could be a side effect of the CO_2 -saturated acetone during the freeze substitution (cooled with dry ice). Some tests using Vestopal-W as an embedding agent did not show an advantage compared to the épon-araldite mixture. On the contrary, the viscosity is very high and the resin is very hard after polymerization. Dry sectioning (1-2 μm thick) leads to acceptable results with well prepared plant material. If the difference in hardness in the tissue between the different cell layers is too big, the sections tend to break and wet-knife sectioning is preferred.

Application of semiconductor detectors to study the translocation of β -emitting tracer elements.

Lateral movement of ^{115m}Cd .

A series of experiments has been started to determine the lateral movement of Cadmium (e.g. ^{115m}Cd) in the stem of tomato plants (*Lycopersicon esculentum* (Mill) cv Money Maker) in collaboration with project No. 6 of the Radiation Protection programme. (Ch. Petit). The lateral distribution from the transport vessels to other tissue parts, more close to the surface, is measured by determining the maximum energy of the emerging β -rays. ($E_{max} = 1.63$ MeV; without absorption). Figure 3 illustrates the change in maximum energy as a function of time. An energy loss of 100 keV in this instance corresponds with a layer thickness of 600 μm . It can be concluded that the cadmium in the stem of tomato plants is distributed in 30-60 hrs from the xylem towards the epidermal cell layer, dependent on the anatomy of the plant. The location of the initial transport pathway and the xylem was coincident, as confirmed by a microscopic observation.

The lateral translocation seems to be nearly independent on changes in the nutrient medium after uptake of the ^{115m}Cd , whereas very marked changes in the measured count rate are resulting. Experiments aiming at an autoradiographic determination of the internal distribution are actually in progress.

In depth distribution profiles of ^{45}Ca .

The computer programme developed to make a reconstruction of the depth distribution profiles of ^{45}Ca has been reconsidered. The mathematical procedure followed up till now (weighted least squares) didn't allow the introduction of constraints on the parameters, given by physical requirements like non-negativity of the amount of radioisotope at any position. This led to solutions having no physical meaning. The problem has been solved applying an iterative procedure using the Simplex method, which is well known from Linear Programming, to the quadratic problem of minimizing the residual sum of squares, subject to the above mentioned constraints. Consequently the solutions having no meaning are eliminated, and a better interpretation is possible.

Microlocalization by autoradiography; testing a new emulsion.

From the moment we became aware of a new photographic process developed at the Philips Laboratories at Eindhoven, we anticipated possibilities for the application of autoradiography at the electron microscopic level.

In this process benzeendiazosulfonate or benzeendiazosulfide is used as U.V. sensitive compound. These organic salts are uniformly and in molecular form distributed in the emulsion substrate. The photosensitive emulsion does not contain any silver. At the places where the emulsion is exposed to U.V. the photosensitive molecules are activated, the transform is converted into the cis-form. Silver or any other metal is added to the developer and the metal image is built up starting from an atomic basis and not from a crystal basis as it is in the normal photographic procedure. This at first sight, opens wide perspectives for autoradiography at the electronmicroscopic level. The first results were very disappointing. It turned out that the relatively high energies deposited by β particles (in comparison with the U.V. energy quant) desintegrated the radiation sensitive substance instead of activating it. This desintegration resulted in a negative image.

Since theoretically it must be possible to use the negative image by exposing the emulsion afterwards to an overall U.V. illumination, this process has been tried out.

The rather primitive apparatus that has been built for this purpose made it impossible to determine the exposure time very correctly and to have a good reproducibility. Nevertheless we got some acceptable results. A very faint blackening of the emulsion caused by the U.V. exposure permitted the observation of the underlying tissue with the light microscope. Silverless spots showed up where the β rays had affected the emulsion. Experiments with the electronmicroscope will be done in due time.

The next aim was to test the sensitivity of this emulsion. For this purpose we used a β source consisting of tritiated perspex.

A rough calculation yielded as the effective activity of the source about $2 \cdot 10^6$ dpm.

With this source 10 min was a fairly good exposure time and even 5 min was acceptable. The next step will be to compare results with the sensitivity of stripping film and liquid emulsion.

Publications - 1974.

VAN DE GEIJN, S.C. On the energy loss of electrons in absorbers; a study of the ^{137}Cs -spectrum.
Nucl. Instr. and Meth. 114: 135-136 (1974).

VAN DE GEIJN, S.C. In depth localization of beta-emitting isotopes. Dependence of the range of applicability upon the maximum energy and complexity of the spectrum.
Nucl. Instr. and Meth. 117: 109-113 (1974).

VAN DE GEIJN, S.C. An improved method for the determination of the maximum energy of distorted β -spectra and the in-depth localization of spatially distributed β -emitting isotopes.
Nucl. Instr. and Meth. 120: 107-112 (1974).

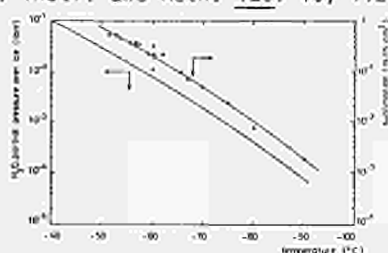


Fig. 1 - Sublimation rate (ml/hxcm^2) and saturated partial pressure of water over ice (torr) as a function of temperature ($^{\circ}\text{C}$).

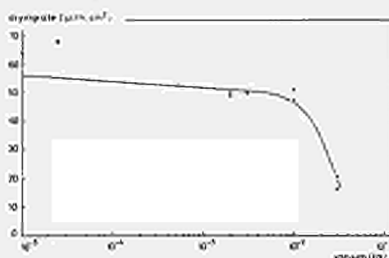


Fig. 2 - Influence of vacuum (torr) on the drying rate ($\mu\text{l/hxcm}^2$) of ice at -70°C ; coldtrap at liquid nitrogen temperature.

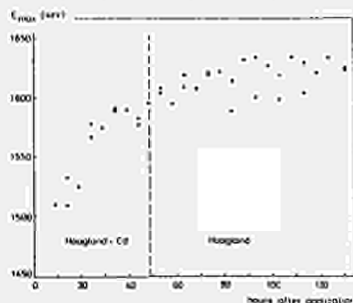


Fig. 3 - Extrapolated maximum energy of the $^{115\text{m}}\text{Cd}$ beta-spectra measured in the stem of tomato plants, as a function of time after application.
o - single measured spectra
 Δ - addition of 2 or 3 successive spectra.

Resultaten van het project No. 31

Hoofd van het team en wetenschappelijke medewerkers:

A.F. Groneman

Titel van het project:

Recycling of liquid waste

Beschrijving van de resultaten:

Introduction

Recycling liquid waste promises to be a good and economical practice. Spectacular results have been obtained in some cases. However, many problems have to be solved in this area. This was one of the conclusions of the International Conference of Industrial Waste Water Treatment and Disposal within the EEC, which was covered by Groneman (1).

Recycling of water born wastes in agriculture is one of the most common, cheapest, and most complete methods of disposal of sludge. However, when used on agricultural land, the contents of toxic compounds have to be at acceptable low levels and the sludge must not be a source for potentially infectious organisms. If disposal of sludge on land would be reduced or forbidden serious financial repercussions would result.

Disinfection of sludge can be done by ionizing radiation or by thermal pasteurization. Thermal pasteurization has many disadvantages. Dewatering properties of sludge deteriorate by pasteurization while the sludge volume increases by the condensed steam used to warm up the sludge in the common pasteurization facilities. Corrosion is increased by the high temperatures which also generate obnoxious smells. With the nuclear method sludge is being disinfected at full scale level in a German pilot plant. This method promises a number of important advantages. Besides its disinfecting power, it was found that ionizing radiation has sludge conditioning properties and is capable to decompose a number of noxious compounds (Groneman (2)).

Sedimentation and dewatering of sludge are time consuming and costly processes. Therefore emphasis was placed on research dealing with the effects of ionizing radiation on sedimentation and dewatering of sewage sludge. Basic research was done in order to detect the governing mechanisms involved in view of the likelihood that they will be of fundamental importance in the optimalization of the dewatering effects. Experiments were done both in the laboratory and at full scale level, which made it possible to correlate the results.

The technical and economic feasibility of disinfection of sewage sludge by ionizing radiation at pilot plant level was investigated in a German-Dutch cooperation and results were reported (Groneman(3)). Aspects of disinfection of effluent to be discharged in open waters with recreational or ecological functions have been described by Groneman (4).

Results and discussion

Sedimentation applies to those operations in which a suspension is separated into a clarified fluid and a more concentrated suspension. Particles can settle out of suspension in four different ways depending upon the flocculating properties of the particles and the concentration of the suspension. The four different types of settling are:

1. Settling of discrete particles,
2. Settling of flocculent particles,
3. Zone settling,
4. Compressive settling.

Experiments confirmed that the sewage sludges available were dominantly flocculent suspensions. For the sedimentation of flocculent particles no satisfactory theories are formulated yet. Therefore the evaluation of the flocculation effect on sedimentation and the way it is affected by ionizing radiation was performed in a settling-column analyser of ca. 250 cm length. Observations and results indicated a positive effect of gamma radiation on the sedimentation. A dose of 300 krad clearly improved the floc formation in both laboratory and full scale experiments. The clarified supernatant solution was cleaner, the interface height of the sludge was approximately twice as low and the suspended solids content in the concentrated sludge was higher than in the control column containing untreated sludge.

Colloidal aqueous dispersions can be distinguished into two major general classes according to their mode of stabilization:

1. hydrophobic colloids that are stabilized by an electrostatic repulsion between particles,
2. hydrophilic colloids that acquire stability by solvation of the interface. Attempts were made to detect which of the modes of stabilization was altered by the irradiation treatments.

Effects of the radiation on the electrostatic repulsions between particles were determined by electrophoretic velocities, which were measured microscopically with a Rank particle electrophoresis apparatus. Zeta potentials were calculated approximately from the electrophoretic mobility. Typical results are presented in Table 1 and are means of 20 replications.

Data of both laboratory experiments and experiments at full scale level showed that gamma radiation at a dose of 300 krad had drastically changed the electrophoretic mobility. Statistical analysis revealed that the differences were highly significant (Probability level $< 1\%$). Gamma radiation decreased the mobility and the zeta potential somewhat more in sludge irradiated at the pilot plant facility than irradiated in laboratory experiments.

Table 1. Effects of irradiation treatments on the electrophoretic mobility and the zeta potential of colloidal particles in sludges from two waste water treatment plants.

Treatment	Sewage treatment plant A		Sewage treatment plant B		Means	
	electrophoretic mobility *	zeta potential **	electrophoretic mobility *	zeta potential **	electrophoretic mobility *	zeta potential **
Not treated (control)	2.4	31	2.3	30	2.35	30.5
Irradiated in laboratory, 300 krad	1.9	24	1.6	21	1.75	22.5
Irradiated in pilot plant, 300 krad	1.4	18	1.3	17	1.35	17.5

* Electrophoretic mobility in μ cm sec $^{-1}$ volt $^{-1}$.

** Zeta potential in mVolt.

The values of the zeta potentials were reduced from values of moderate stability to the threshold of delicate dispersion according to criteria of colloidal stability characteristics established by Riddick et al. (Industrial and Engineering Chemistry, 62 (1970). A detailed interpretation of such results will be presented elsewhere by Groneman (5).

Effects of gamma radiation on the hydration or waterbinding capacity were determined as bound water by a dilatometric method proposed by Heukelekian et al. (Sewage and industrial wastes, 28 (1956). Although irradiation tended to decrease the contents of bound water, the statistical analysis indicated that the results obtained were not conclusive due to high variations in experimental data.

In reality the two modes of stabilization discussed are not mutually exclusive. They nearly always occur together although often one evidently predominates. The absorbed ions that interact with the electric charge concomitantly provide a solvated layer on the surface of the sludge particle.

Gamma radiation at a dose of 300 krad improved the dewatering properties of domestic sludges in laboratory experiments as well as in full scale experiments. The standardized specific resistance of typical domestic sludges decreased by 45 to 60%. This dewatering parameter has a sound theoretical basis and is found to be well correlated with results obtained on drying beds and by mechanical dewatering devices. Detailed discussions of the results will be presented at a conference of the International Atomic Energy Agency by Groneman (5).

Publications - 1974

1. GRONEMAN, A.F. International conference on industrial waste water treatment and disposal within the EEC. Internal Report No. 149 (1974). Association EURATOM-ITAL.
2. GRONEMAN, A.F. Effecten van ioniserende straling op afvalwater en zuiveringsslib. Internal Report No. 148 (1974). Association EURATOM-ITAL.
3. GRONEMAN, A.F. Technical and economic feasibility of the use of gamma radiation for disinfection of sewage sludge. External Report No. 18 (1974). Association EURATOM-ITAL.
4. GRONEMAN, A.F. Behandeling van water, stedelijk afvalwater en slib met hoog-energetische elektronen. Internal Report No. 147 (1974). Association EURATOM-ITAL.
5. GRONEMAN, A.F. Effects of gamma radiation at pilot plant level as compared to effects of pasteurization on the dewatering of sewage sludges. Proceedings of International Symposium on the use of high level radiation in waste treatments—status and prospects. In press.

Resultaten van het project no. 32

Hoofd van het team en wetenschappelijke medewerkers:

J.F. Stoutjesdijk.

Titel van het project:

The propagation of nuclear methods in biology and agriculture.

Beschrijving van de resultaten:

1. Courses

.1 General Radionuclide course.

A general radionuclide course was organized from February 18 till March 8, 1974, with 22 participants, of whom seven students of the Agricultural University of Wageningen and one guestworker from Morokko at ITAL.

.2 Health Physics course.

In cooperation with the Health Physics Department of the Association a Health Physics course was organized from March 18-22 and April 1-5, 1974, with 18 participants. The course was concluded with an official examination under auspices of the Ministry of Public Health and Environmental Hygiene.

Of the participants 16 took part in the examination, of whom 11 with good results. Another candidate who had failed this examination at an other institute, was no more successful this time. According to the Dutch Nuclear Energy Law the responsible leaders of radiochemical laboratories should have a sufficient knowledge of health physics: people who have passed the above mentioned examination are considered sufficiently capable for normal radiochemical laboratories.

.3 Liquid scintillation courses.

Two liquid scintillation courses have been organized from November 25 till December 6 and from December 9 till 20, with 16 and 19 participants respectively. Among them were five members of the personnel of the Association.

2. Evaluation of radiobiological techniques

.1 Effect of quenching on the effect of wavelength shifters in Cherenkov-counting of β -emitters.

By the addition of the wavelength-shifter 4-methylumbelliferone the counting efficiency of the Cherenkov-measurements of ^{32}P could be increased from 44 to 65%. The effect was smaller in plastic vials than in glass vials due to the dispersion of the Cherenkov-light by the plastic (see table 1).

Table 1.

Effect of wavelength shifters on the Cherenkov-counting efficiencies of ^{32}P in glass and plastic vials.

Wavelength shifter (100 mg/l)	Counting efficiency	
	Glass vial	Plastic vial
None	44.6%	51.0
4-Methylumbelliferone	65.4	66.2
β -Naphthylamine	59.9	63.7
Naphtol-2	54.2	
Fluorescein	30.9	

The counting efficiency of ^{36}Cl , with lower maximum energy of the β -particles than ^{32}P (0,71 MeV for ^{36}Cl , 1,71 MeV for ^{32}P), increased from 5% to 20% by the addition of 4-methylumbelliferone. The drawback of these wavelength-shifters is, that the countrate may be influenced by chemical quenchers: lower counting efficiencies were found with several compounds. In fig. 1 the effect of several mineral acids and NaOH and in table 2 the effect of some other quenchers is shown.

Table 2.

Effect of chemical quenching in ^{32}P Cherenkov-counting in the presence of 4-methylumbelliferone.

Quenching agent (20 g/l)	Counting efficiency
none	44.6% (*)
formic acid	38.8
acetic acid	41.0
Na-acetate	42.3
trichloro-acetic acid	33.0
acetone	40.1
2-ethanolamine	38.4
glycerol	43.2
H_2O_2	37.0

*) The countrate was determined in a smaller measuring channel than in the experiments mentioned in table 1.

Quench-corrections could be applied with the channels ratio method (fig. 2).

2.2 Effect of the concentration of H_2SO_4 on the Cherenkov-counting efficiency of ^{32}P .

In H_2SO_4 -solutions a decrease of the counting efficiency was found with increasing acid-concentration. This effect can be explained by the higher density of the H_2SO_4 -solutions in comparison with water. In the presence of 4-methylumbelliferone a small amount of H_2SO_4 already caused a decrease of the counting efficiency from 70% to 46.3%, but in solutions with more than 10% H_2SO_4 the counting efficiency increased to about 74% for which phenomenon no explanation can be given. In 90% H_2SO_4 the counting efficiency again decreased to 71% (fig. 3).

3. Determination of heavy metals and radioactive corrosion products from nuclear reactors in fish and fishery products in behalf of the Ministry of Agriculture and Fisheries

The results of the determinations with atomic absorption spectrometry of heavy metals in fish and fishery products are collected in table 3. No increase in the content of the metals was found. For the low-activity measurements a leadcastle, prepared from old lead has been constructed. However, when the semi-conductor detector arrived (the detector which has been used formerly, did not fulfil all our wishes), it did not fit in the leadcastle due to an unexpected change in the dimensions, so an adaption of the leadcastle was necessary. With the adapted leadcastle one series of samples was investigated for the presence of radioactive elements with the help of a Ge(Li) semi-conductor detector with processing of the measuring data or with a PDP-11/40 computer: only ^{137}Cs was found.

4. Cooperation with other institutes

- .1 Cooperation with ir. P.W.L. Tas and dr.ir. D. Peters of the Department of Virology of the Agricultural University of Wageningen about the use of ^{125}I in investigations about the localization of proteins in viruses.
- .2 Cooperation with dr. Van Broekhoven and Mr. P. Wijkens of the Organical Chemistry Laboratory of the State University of Utrecht in investigations about the juvenile hormone. In het 'hot-lab' of ITA the 2- ^{14}C -labelled juvenile hormone III, starting from 2- ^{14}C -methylbromoacetate was synthesized.
- .3 Cooperation with dr. Forenz of the Department of Entomology of the Agricultural University of Wageningen about the use of ^{125}I in tracerwork.
- .4 Orientating discussion with drs. P. Verijken of the Institute of Phytopathological Research at Wageningen about the use of radio-nuclides in the study of the quantitative relation between the presence of aphid on cereals and the economical damages.
- .5 Orientating discussion with dr.ir. D. Peters of the Department of Virology of the Agricultural University at Wageningen about the behaviour of aphid on pea-leaves.

Table 3.

Contents of metals in fish and fishery products determined by atomic absorption spectrometry

Product	Sampling location	Sampling date	Content in mg/kg fresh material								
			Cr	Mn	Fe	Co	Cu	Zn	As	Cd	Hg
Sole	Northsea near Texel	October 1973	0.05	0.18	3.2	-	0.2	5.3			0.04
		April 1974									0.16
	Northsea near Scheveningen	October 1973	0.11	0.18	3.3	-	0.22	4.8			0.09
		January 1974	0.13	0.15	5.8	-	0.33	4.4			0.10
	Northsea near Breskens	April 1974									0.11
		January 1974	0.13	0.31	11	-	0.16	4.6			0.07
	April 1974									0.21	
Cod-fish	Northsea	January 1974	0.10	0.07	1.1	-	0.08	2.5			0.10
Eel	IJsselake	October 1973	0.13	1.0	26	-	0.41	22			0.21
		April 1974									0.51
Pike-perch	IJsselake	January 1974	0.16	0.09	2.2	-	0.21	5.3			0.77
Shrimps	Dollard	October 1973	0.13	2.9	43	-	13	21			0.17
		January 1974	0.16	1.3	34	-	6.4	26			0.07
		April 1974									0.14
	Northsea near Texel	October 1973	0.22	3.5	59	-	9.6	22			0.06
		January 1974	0.17	1.5	30	-	8.3	25			
	Northsea near Breskens	April 1974									0.08
		October 1973	0.49	3.1	99	-	9.7	19			0.08
		January 1974	0.18	1.9	38	-	8.4	29			0.08
	April 1974									0.09	
Mussels	Shallows near Texel	October 1973	0.44	5.5	89	0.14	1.4	16			0.05
		January 1974	0.62	5.6	93	0.27	1.3	28			0.11
		April 1974									0.06
	Oosterschelde	October 1973	0.40	6.9	105	0.20	1.4	18			0.05
		January 1974	0.62	5.6	93	0.27	1.3	28			0.11
		April 1974									0.06

x) - = not detectable

- .6 Orientating discussion with drs. B.J.R. Scholtens, ir. T. v. Vliet and ir. W. Norde of the Department of Physical and Colloid Chemistry of the Agricultural University at Wageningen about the use of radioactively labelled polymers in the study of liquid-liquid interfaces.

5. Work for committees

.1 Work for C.C.R.A.

For the Coordination Commission Radioactivity Measurements the annual report 1973 was prepared. This commission has now been transformed into the Coordination Commission for the Measurement of Radioactivity and Xenobiotic Compounds (C.C.R.X.), which will consider also other products harmful for man and his environment. As the technical secretary of this C.C.R.A.(X) I was sitting in a commission of the Health Council to prepare safety standards for the biosphere after nuclear accidents. This commission has met five times in 1974.

In the same function I have participated in the preparation of an alarmscheme for nuclear reactors.

6. Publication of a Newsletter

In May a second issue of the Newsletter on the Application of Nuclear Methods in Biology and Agriculture was brought out, in spite of disappointingly few contributions from the scientists who had declared to be interested in this Newsletter. However, for the third issue, published in November 1974, thirteen contributions had been sent in, which is an encouraging development.

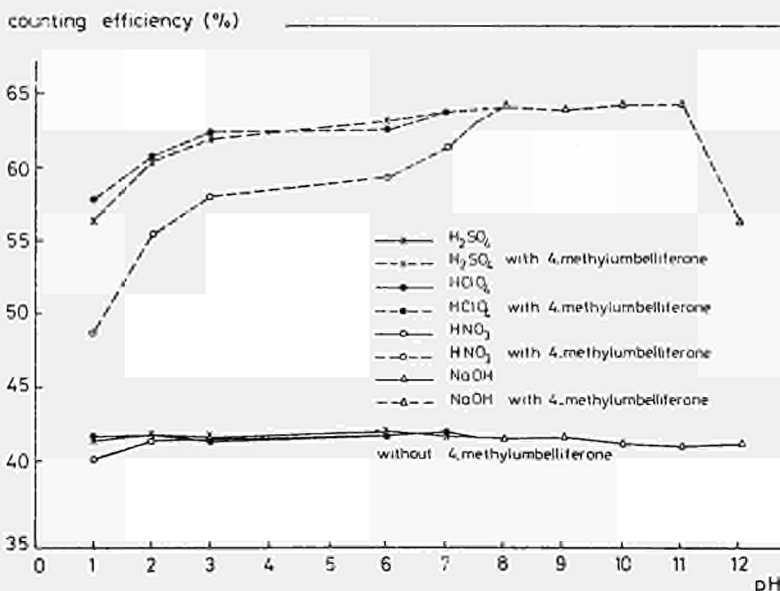


Fig. 1. Influence of pH on Cherenkov-counting of ³²P in the presence of 4-methylumbelliferone. The samples contained 10 ml of a solution of 1 g/l of Na₂HPO₄ + 10 μCi/l of ³²P, pH 7.5.

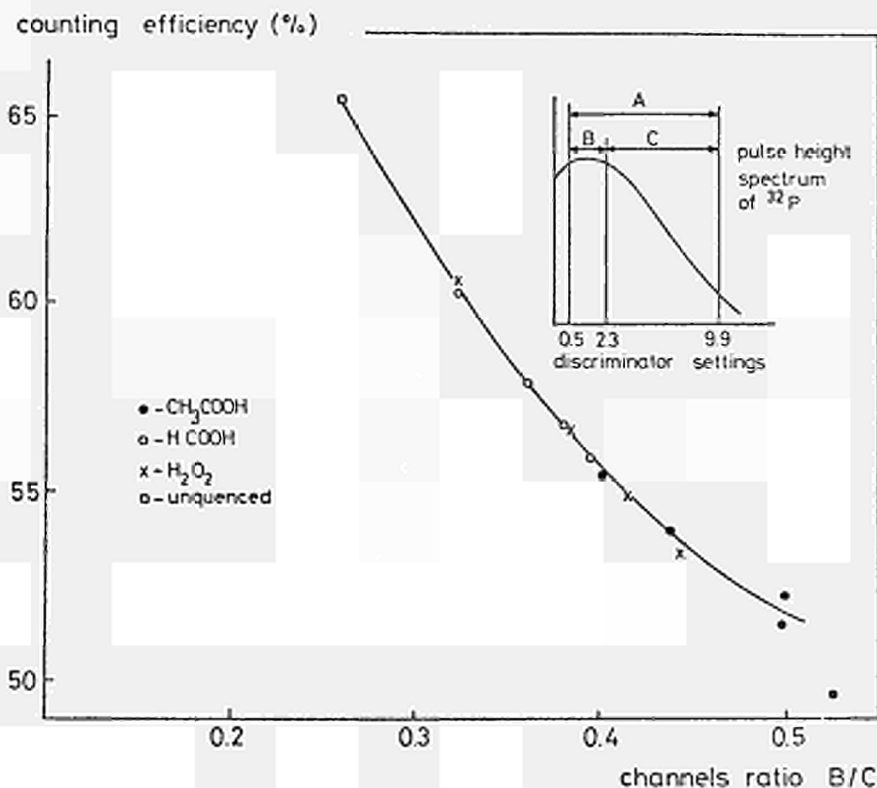


Fig. 2. Channels ratio quench-correction curve for Cherenkov - counting of ³²P in the presence of 100 mg/l of 4-methylumbelliferone.

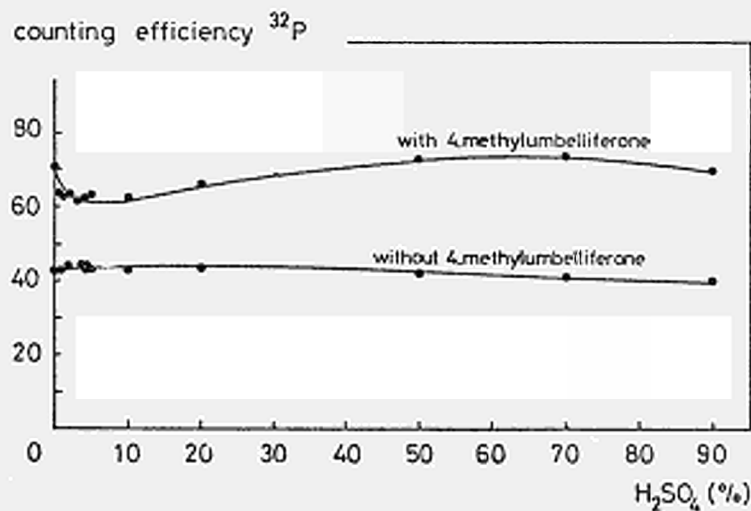


Fig. 3. ³²P-Cherenkov-counting in H₂SO₄-solutions.

Resultaten van het project No. 33

Hoofd van het team en wetenschappelijke medewerkers:

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

Titel van het project:

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

Beschrijving van de resultaten:

The items 1, 2, 3, 4 of this report are dealing with work directly related to the Association's programme. The items 5, 6, 7, 8 and 9 are related to work carried out in cooperation with institutes outside the Association.

1. 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 compounds in fishery products, for the measurement of heavy metals in activated samples for the determinations of such products by means of neutron activation analysis. The detector is shielded in this system with about 400 kg old lead (at least 170 years old). The samples are transported into this leadcastle by means of a pipe-line construction. The system, including computerinterfacing has become operational.

2. 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.

Nevertheless the prototypes work satisfactorily and a number of units have now been built.

3. Tape recording units.

The Racal cassette recorder had to be adapted to the ITAL instrumentation. The unit has been interfaced as tape-recorder to the computer and was built in in the system.

A prototype of a "users module"-recorder has been realized. This unit can also be used to make the registration visible on a Teletype. Several of these units have been produced.

4: pH-Measurements.

For measurement and registration of pH values and ion concentration in complex experimental set-ups, a NIM module with two channels has been realized.

Signal disturbance, due to transport over longer distances through a high impedance system (source impedance may be as high as $10^9 \Omega$) is avoided by separate pre-amplifiers coupled as near as possible to the detectors. (input impedance $10^{13} \Omega$). Each measurement channel has a digital display and output, suitable for connection to our data-collection system.

5. Measurements of density variations of flowing granular material in silo systems.

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

The method and the results have been presented in a paper ("Density behaviour of flowing granular material" to be published in "Powder Technology") presented at the symposium "Storage and Flow of particulate Solids" on September, 12 and 13, 1974 in Germany.

6. Velocity measurements of granular material in an elevator system.

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

Experiments were extended by measurements of the density of the flowing material, especially in the acceleration traject, with the help of a γ -absorption technique.

Because of expected oscillations in this density pattern, a correlation technique will be tried out with the help of direct coupling of pressure and density variations.

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

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

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

Because of the small dimensions (8 à 10 mm diameter and 20 mm long) a high precision detector-source combination is under development for applying low energetic gamma absorption techniques to this problem.

8. Velocity measurements of ensilage in a cattle-food tower silo.

(Cooperation with A.H. Bosma and M.G. Telle, Institute of Agricultural Engineering, Wageningen).

In order to apply a time-mode measurement with a γ -emitting radioactive tracer to this problem, some activation experiments have been carried out, from which was observed that

- a. the amounts of usable short living γ -emitters produced by immediate neutron activation of the ensilage (e.g. ^{24}Na) were insufficient.
- b. no appreciable amount of long living radioactive material is formed by the activation.

A labelling procedure of the silage with a suitable, easily activable element (e.g. Na or Cu) is in preparation.

9. Dataway systems - Coala.

The complexity of modern installations results in an increased demand for direct coupling of computers to such installations. The coupling systems required for this purpose, are in fact two directional data-way systems. For economic reasons, in Wageningen a committee has been formed for standardization and advices concerning these data-way systems, named COALA (Commission Automatization of Laboratory Apparatus).

As a result of the membership in this committee, in a combined action with T.F.D.L., Wageningen (Technical and Physical Engineering Research Service), support has been given to the automation of a stopped-flow spectrometer system and a high precision titration installation, for the Department of Physical and Colloid Chemistry, Agricultural University Wageningen (Drs. A. de Keizer). In this system the spectrometer and the double titration units are controlled by a DEC-PDP11 computer, interfaced via a CAMAC dataway system.

Publications - 1974

- STROOSNIJDER, L. and J.G. DE SWART. Column scanning with simultaneous use of ^{241}Am and ^{137}Cs gamma radiation. Soil Science 118 61-69 (1974).
- ZUILICHEM, D.J. VAN, N.D.VAN EGMOND, J.G. DE SWART. Density behaviour of flowing granular material. Powder Technology 10, 161-169 (1974).

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

The study on the distribution and size of the latent image centers has been continued by determining and interpreting the evolution of the intensification factor as a function of development time. An inversion of the behaviour of Lippmann emulsions is interpreted in terms of sub-germs/germs ratio. The formulation of specific activation treatments for these emulsions, which are particularly important in ionography, has been undertaken.

The autoradiographic studies on the incorporation of ^{14}C -thymidine in nucleoli of adult rat liver cells have been continued. The activated track-autoradiographic method applied on isolated nucleoli has shown an unambiguous labelling of part of these organelles. The proportion of nucleoli having incorporated tracer molecules has been determined by track counting.

An autoradiographic study of the incorporation of ^{14}C -thymidine in adult rat nerve cells has been undertaken.

Publications : JAROS G.G., SENSENBRENNER M., WITTENDORP E., RECHENMANN R.V. and MANDEL P. Etude trace-autoradiographique de la prolifération des cellules nerveuses en culture. C.R. Acad. Sc. Paris, t.279, Série D - 1101 (23 septembre 1974).

SENSENBRENNER M., JAROS G.G., WITTENDORP E., RECHENMANN R.V. and MANDEL P. Prolifération et différenciation des cellules nerveuses en culture - Etude autoradiographique. Table Ronde sur la Culture de Cellules - Strasbourg (1974).

WINTZERITH M., WITTENDORP E., ITTEL M.E., RECHENMANN R.V. and MANDEL P. Track-autoradiographic study of nucleolar DNA synthesis in adult rat liver. Expt. Cell Res. (in press).

RESULTATS du PROJET N°1

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

A. IONOGRAPHY

STUDY ON THE DISTRIBUTION AND ON THE MORPHOLOGY OF THE CORPUSCULAR LATENT IMAGE.

In order to respond to the strong need for improvements which becomes urgent in numerous fields tackling with the photographic detection of radiation, the study on the distribution of the latent image in scientific emulsions has been continued. Experiments on development kinetics have been carried out in order to determine the influence of different photographic parameters on the intensification obtained by means of a given activation procedure. In a first stage of these studies we have used the same standard activation solution in all experiments.

Results and Discussion.

The intensification factor D_a/D_0 (optical density with activation / optical density without activation) as a function of development time has been determined for different combinations emulsion-radiation-developer (Fig. 1,2,3,4). The higher intensification effect in smaller AgBr grain emulsions can be interpreted in terms of energy deposition by an ionizing particle crossing a microcrystal.

Indeed, a smaller amount of energy is released in microcrystals with smaller mean diameters; this results in the formation of a smaller amount of silver in each AgBr grain and hence in a higher sub-germs/germs ratio in the general distribution of the latent image.

In the case of ultrafine grain emulsion (Fig. 3), the activation results in an intensification effect which increases with the development time. We may deduce from this result that the latent image formed in these types of emulsions is mainly constituted by small latent image centers, most of them being undevelopable sub-germs.

It appears on figure 4 that the D_a/D_0 ratio depends on the type of reducing solution, which is one of the parameters considered.

From the results obtained, following practical conclusions can already be drawn : a) the latent image centers created especially by low-ionizing particles in small microcrystals have to be more activated than the germs formed in coarser grains; b) a more powerful activation procedure should also be used in the case of less sensitive emulsions; c) a special study

should be devoted to the so-called Lippmann emulsions, which denote a behaviour deviating strongly from the properties observed of the usual ionographic detectors.

For autoradiographic purposes, density development-time curves and the corresponding signal/noise ratios for an amidol developer and a solvent ferro-oxalate solution at different redox potentials for a given combination emulsion-isotope, i.e. Kodak NTB2 and ^{14}C , had to be determined. The results are given on figure 5a and b.

B. BIOLOGICAL APPLICATIONS.

I. EVIDENCE OF NUCLEOLAR DNA SYNTHESIS BY ACTIVATED TRACK-AUTORADIOGRAPHY IN ADULT RAT LIVERS.

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

In previous papers (1,2), we have given the results obtained by applying a specific track-autoradiographic method to preparations of isolated nuclei of adult rat liver, which had been labelled "in vivo" with ^{14}C -thymidine. It appeared that a significant number of electron tracks are emitted from the nucleoli of the isolated nuclei. Even some of the nuclei were very heavily labelled (Fig. 6).

1) Autoradiography of isolated nucleoli.

In a second phase of our study, the nucleoli of adult rat liver cells have been isolated and submitted to the same specific autoradiographic process which had already been used for the nuclei. Part of the isolated nucleoli have been treated by DNAase.

2) Results and Discussion.

The observation of the autoradiograms confirms that a significant number of tracks are originating from part of the nucleoli (Fig. 7). If the nucleolar preparations were treated by DNAase prior to autoradiography, β -tracks due to electrons could hardly be observed, denoting that the labelling of the DNA had been removed.

Counting of the tracks emitted from isolated nucleoli as well as from nucleoli inside the nuclei lead to fairly equivalent figures, like it can be seen on Table I. It appeared that the preparation techniques used for obtaining the nucleoli yielded a representative population from the nuclei.

It could furthermore be shown that 8.5 % of the nuclei were heavily labelled. This figure is higher than the value which could be expected from the mitotic index for adult rat liver. These results support and precise our previous biochemical findings (3). Indeed, accurate visual loca-

Table I. *Counting of β -tracks originating from nucleoli.*

	nucleoli in nuclei	isolated nucleoli
Labelled nucleoli	59	103
Population	737	2666
<u>Labelled nucleoli</u> Total nucleoli	3.2 %*	3.9 %

* A factor of 2.5 nucleoli per nucleus has been considered for this calculation.

tion of the electron tracks unambiguously establishes that the nucleolus is a site of DNA synthesis in adult hepatocytes. Moreover, it can be seen on the autoradiograms that nucleolar DNA synthesis does not necessarily take place in the same cells as chromosomal extranucleolar DNA synthesis in adult liver. The meaning of isolated nucleolar DNA synthesis in non dividing cells is actually unknown; the biosynthesis of nucleolar DNA in non dividing cells might be involved in the transcription of nucleolar RNA and its regulation (3).

II. AUTORADIOGRAPHY OF ISOLATED NUCLEI OF NERVE CELLS.

In collaboration with the same authors, preliminary experiments have been undertaken on isolated nuclei of adult rat nerve cells.

REFERENCES : 1) WINTZERITH M., WITTENDORP E., ITTEL M.E., RECHENMANN R.V. and MANDEL P. C.R. Acad. Sc. Paris, t. 277, Série D, 1033-1036 (24 septembre 1973); RECHENMANN R.V. and WITTENDORP E. Annual Report 1973, EUR. 5138 d-e-f-i-n, 609; Annual Report Association EURATOM-Ital, EUR. N° 5226. 2) WINTZERITH M., WITTENDORP E., ITTEL M.E., RECHENMANN R.V. and MANDEL P. Expt. Cell Res. (in press). 3) ITTEL M.E., WINTZERITH M., ZAHND J.P. and MANDEL P. Europ. J1. Biochem. 17, 415 (1970). WINTZERITH M., ITTEL M.E. and MANDEL P. Bull. Soc. Chim. Biol. 50, 451 (1968).

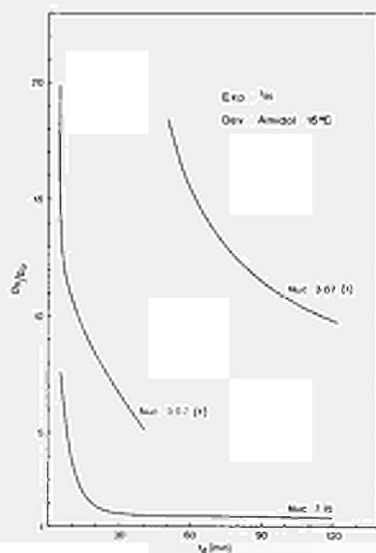
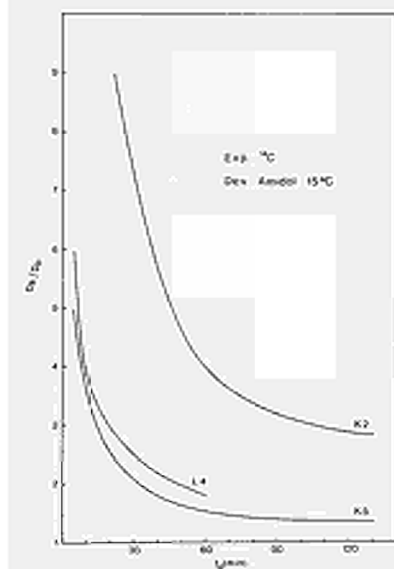


Fig. 1 : Intensification factor D_g/D_0 (optical density with activation / optical density without activation) as a function of development time.

Emulsions : Ilford K2, K5 and L4 - Source : ^{137}Cs .

Developer : Amidol (15°C).

Fig. 2 : Intensification factor D_g/D_0 as a function of development time.

Emulsions : Gevaert Scientia Nuc 7.15, Nuc 3.07 - Source : ^3H .

Developer : Amidol (15°C).

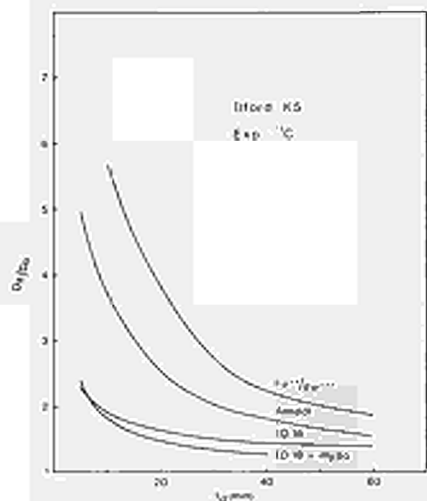
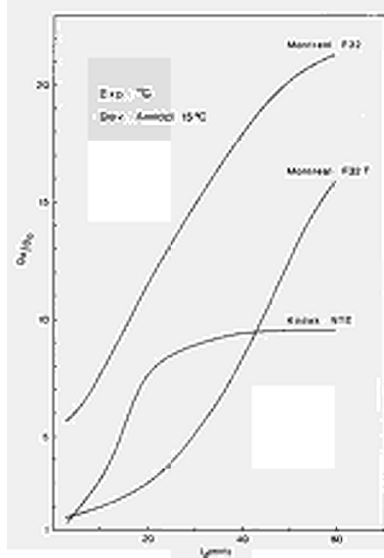


Fig. 3 : D_g/D_0 ratio as a function of development time.

Emulsions : Montreal F32, F32T, Kodak NTE - Source : ^{14}C .
 Developer : Amidol (15°C).

Fig. 4 : Influence of the type of reducing solution on the intensification factor (D_g/D_0).

Emulsion : Ilford K5 - Source : ^{14}C .
 Developer : Fe⁺⁺/Fe⁺⁺⁺, Amidol, ID 19, ID 19 + Hypo (15°C).

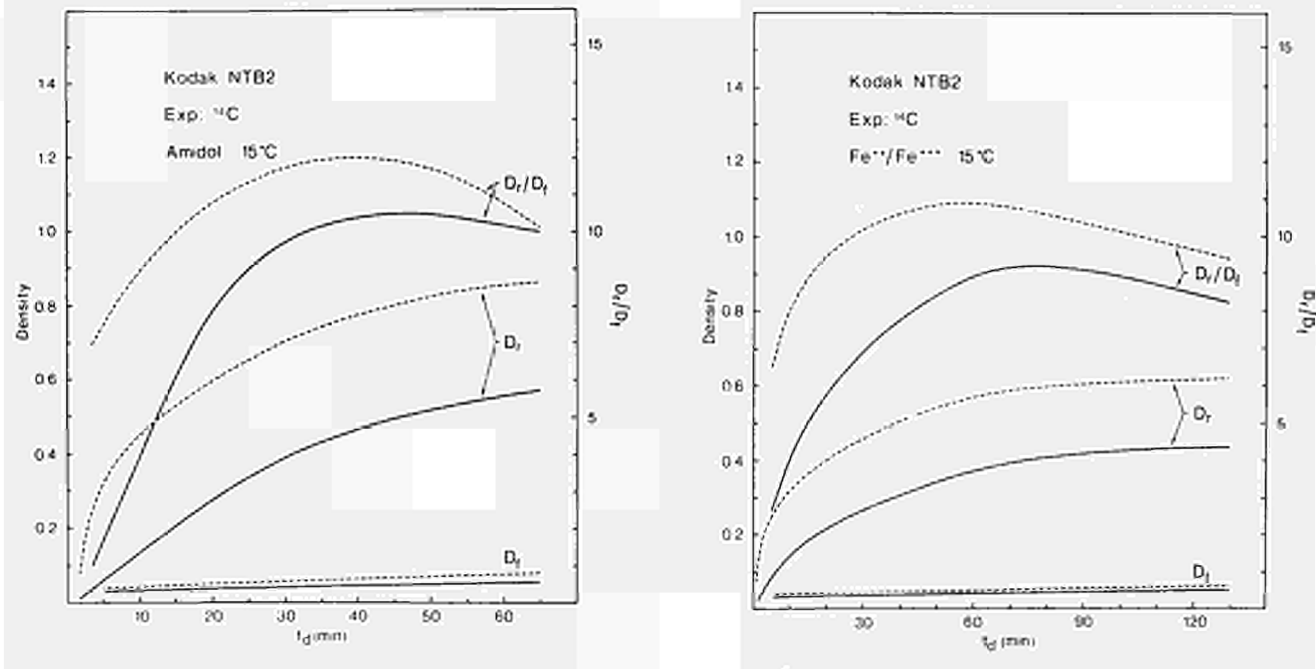


Fig. 5 : Influence of development time on the optical densities measured respectively on exposed (D_r) and unexposed nuclear plates (D_f), as well as on the ratio D_r/D_f . Without (—) and with (----) gold activation.

a) amidol developer

b) Fe^{2+}/Fe^{3+} developer at $E_{redox} \sim -310$ mV.

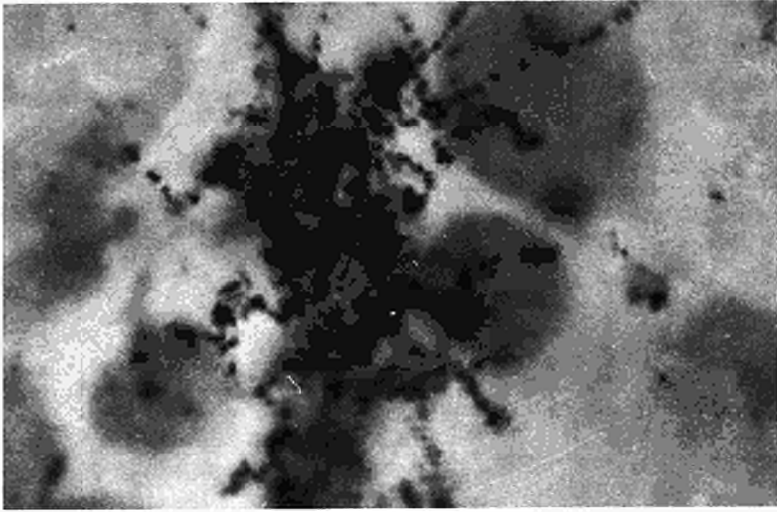


Fig. 6 : Isolated nuclei of adult rat liver after *in vivo* incorporation of ^{14}C -thymidine. Among unlabelled nuclei or others presenting a low nucleolar radioactivity, some heavily labelled nuclei like the one presented in this autoradiogram have been observed.
(x 1,970)

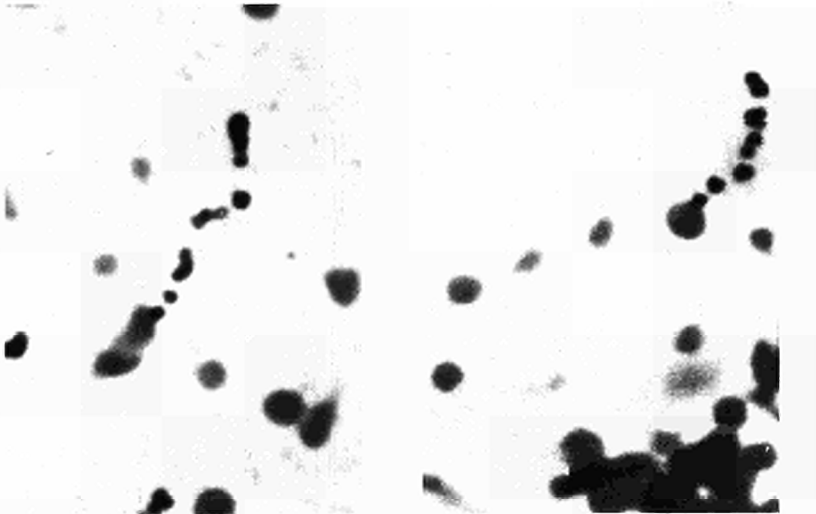


Fig. 7 : Activated track-autoradiograms of nucleoli isolated from rat liver nuclei, after *in vivo* incorporation of ^{14}C -thymidine.
a) (x 5,600)
b) (x 2,100)

Contractant Katholieke Universiteit Nijmegen (The Netherlands)
Dr. C.J.M. Aarts, director of the Faculty of Sciences

Contract Number S.C. 002 - 094 - 72 - 1 BIA.N.

Head of the
Research Team Prof.dr. H.F. Linskens

General subject Radiation biochemistry of pollen and styles of
of contract Incompatible Plants

General description Most of the research, thus far, is directed towards
of Research the unirradiated situation in order to understand
the effect of irradiation

- I. Influence of X-ray-irradiation of pollen and styles on enzyme activity during progamic phase in Petunia hybrida (Drs. L. Gilissen)
- II. Effect of X-ray-irradiation on pollen germination in vitro (Drs. L. Gilissen)
- III. Influence of X-ray-irradiated compatible pollen tubes on incompatible pollen tubes growing in the same style (Drs. L. Gilissen)
- IV. Basic research on the incompatibility reaction
(Prof.dr. H.F. Linskens; Drs. J. v.d. Donk; Drs. J. Schrauwen)
- V. Activation of the ovary upon pollination
(Prof.dr. H.F. Linskens; Drs. J. Deurenberg)
- VI. Electrophysiology of the style in relation to the incompatibility reaction
(Prof.dr. H.F. Linskens and students)
- VII. Translocation and accumulation before, during and after anthesis
(Prof.dr. H.F. Linskens and students)

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

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

Scientists Drs. L. Gilissen
Drs. J. v.d. Donk
Drs. J. Deurenberg
Drs. J. Schrauwen

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

The changes in activity during progamic phase of the following three enzymes have been tested:

1. G.O.T. (=L- aspartate 2-oxoglutarate amino-transferase) because of its intermedial function between carbohydrate and amino-acid metabolism. After pollination a decrease in specific enzyme activity took place. This decrease appeared to begin earlier in cross-pollinated, than in self-pollinated styles.

2. β -glucosidase, because of its function during wilting and the relation which exists between pollination and wilting, and radiation and wilting.

An increase of specific enzyme activity took place, which is greater after cross-pollination than after self-pollination.

3. Acid Phosphatase, because of the differences which could be expected from literature data in styles after cross- and self-pollination.

Contrary to data in literature no changes in enzyme activity of this enzyme has been found in the styles after cross- and self-pollination.

These preliminary experiments will be extended with enzyme assays in pollinated styles after irradiation of either the pollen of the style.

II. Effect of X-ray-irradiation on pollen germination in vitro (Drs. L. Gilissen)

It has been found in in vitro germination experiments, that decay of radicals in pollen of *Petunia hybrida* after X-ray-irradiation in dry air at room temperature does not increase biological damage. Radical concentrations are measured with an electron spin resonance spectrometer (E.S.R.). When pollen is stored during 16 hours at room temperature after irradiation a great part of the formed radicals had decayed, but no change had taken place in germination percentage in vitro. So the damage must have been built up during radiation by action of the radicals then formed.

III. Influence of irradiated compatible pollen tubes on incompatible pollen tubes growing in the same style (Drs. L. Gilissen).

Styles were pollinated with a mixture of irradiated compatible pollen and not irradiated incompatible pollen. Irradiated compatible pollen germinates slowly and pollen tube growth only in the first phase is retarded. In contrary, incompatible pollen germinates fast, but pollen tube growth levels off when reaching the ovary. From some experiments it is be concluded that the incompatibility reaction in the last stage of incompatible pollen tube growth possibly can be broken down by the influence of the irradiated compatible pollen tubes present in the same part of the style as the incompatible ones are.

IV. Basic research on the incompatibility reaction

(Prof.dr. H.F. Linskens; Drs. J. v.d. Donk; Drs. J. Schrauwen).

1. *Petunia hybrida*

The nature of the proteins synthesized in the reaction between pollen and style was studied. At several times after the self- or cross-pollination RNA was extracted from the styles. Protein synthesis was carried out in vitro by injection of this RNA into egg-cells of the tadpole *Xenopus laevis*. After incubation the proteins were extracted and separated electrophoretically in a SDS-urea-polyacrylamide gel. Different patterns were found both between self- and cross-pollinated styles and as a function of time of pollen tube - style interaction.

Proteins synthesized with RNA extracted from styles 3 hours after

pollination and crude protein extract from styles 1 hour after pollination were separated preparatively to their IEP by means of iso-electric focussing technique. Fractions were collected and applied to either pollen or style in order to test their effect on the pollen tube growth.

From protein preparations from both compatibly and incompatibly pollinated styles fractions were isolated that inhibited compatible pollen tubes. These fractions contained 2 or 3 different polypeptides, which are analysed further.

From protein preparations from compatibly pollinated styles a fraction was isolated, which contained at least 4 different polypeptides. This fraction had a slight stimulatory effect on incompatible pollen tube growth, but further purification might be required.

Application of the proteins mentioned above was found to effect the gene expression of the style both quantitatively and qualitatively. However, further research is necessary to explain the function of these proteins.

2. Myxomycetes

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

V. Activation of the ovary upon pollination (Prof.dr. H.F. Linskens; Drs. J. Deurenberg)

The protein synthesis of ovaries from *Petunia hybrida* clone W166K was studied after pollination. At several times after pollination the ovaries of self- and cross-pollinated flowers were collected and their polysomes were extracted. The protein synthesis was determined in an in vitro system with ^{14}C -leucine. It seemed that the ovary of pollinated flowers altered his protein synthesis metabolism from about 12 hours after pollination, compared with ovaries from non-pollinated flowers. Between 12 and 24 hours after pollination the polysomes from cross-pollinated ovaries showed an other protein synthesis rate than polysomes extracted from self-pollinated ovaries. Pollen tubes from cross-pollinated styles had reached the ovary at about 50 hours after pollination, whereas the pollen tubes from self-pollinated styles had reached about $2/3$ of the style length at that time.

So, one can conclude, that a signal has reached the ovary some hours before the pollen tube has grown through the style. This signal has to be different in the case of self-pollination and cross-pollination.

With *Petunia hybrida* clone T₂U essentially the same results have been obtained except for the difference in reaction of the ovary after cross- or self-pollination, which has not been found.

Preliminary experiments are started with ovules from a genetically homogenous line of Paphiopedilum (Orchidaceae). This line was produced by vegetative propagation during the last 6 years. The material which produced a huge number of ovules in one ovary has the advantages of containing less vegetative tissues than other angiosperm plants.

VI. Electrophysiology of the style in relation to the incompatibility reaction (Prof.dr. H.F. Linskens) (with students)

To get better information on the biochemical situation of the

style, where the observed electrical reaction takes place, in investigation of the general growth parameters was started. The measurements were continued with a new type of electrodes. The basic electrical phenomena were studied in collaboration with the Laboratory of Biophysics of the University of Nijmegen. Sources of errors were eliminated.

VII. Translocation and accumulation before and during anthesis (Prof. dr. H.F. Linskens)

As a consequence of penetration of pollen tubes, the style becomes an attraction centre for organic materials, which are translocated from the stamen, the corolla and the green calix. There are significant differences between the flow pattern in cross- and self-pollinated styles. Already 10 hours after pollination the differences become evident, and are outspoken after 18 hours. The flow diagram reflects in a convincing way the altered metabolic state in incompatibly pollinated flowers.

Other activities

All members of the research team participated in the meeting of the Mutation Breeding Group of EURATOM in Wageningen.

Drs. J. Schrauwen, who was absent for a year to the USA, rejoined the group in summer 1974. Most members of the research team were linked with the organization of the International Symposium "Fertilization in Higher Plants", which was organised in August 1974 at the University of Nijmegen and gave presentations of their current "Fertilization" investigations. The research team collaborated with the working group of the Dutch foundation for biological research BION.

Prof. Linskens participated in the Discussion Meeting of the Royal Society, London, on "Incompatibility in Angiosperms" and gave an invited lecture.

Publications in 1974

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- , Some observations on the growth of the style
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- , Translocation phenomena in the *Petunia* flower after cross- and self-pollination.
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116-124 (1974)

Stanley, R.G. and
Linskens, H.F.,

Pollen, Biology, Biochemistry, Management
Springer, New York, Heidelberg, Berlin, 1974, 307 p.

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

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

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

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

General description of the project:

1. Fundamental and applied mutation research in potato (Solanum tuberosum L.)
(several projects). (Van Harten, Bouter).
2. a. The population genetics of the S alleles of a sporophytic incompatibility
system (Bos, Heemstra).
b. Investigations on incompatibility in tuber-bearing Solanum species (Hermsen).
c. Incongruity and sterility in interspecific Solanum crosses (Hermsen, Ramanna,
Mrs. Sawicka, Verdenius): breaking breeding barriers using genetic techniques
and mutagenic treatments.
3. Mutation breeding for disease resistance. Dwarffrust in barley. (Parlevliet,
Van Ommeren).

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

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

Title of the project : Mutation research in potato.

I. Fundamental investigations on irradiated di-haploid potatoes.

Fundamental examinations with di-haploid potatoes ($2n=2x=24$) as to the radiosensitivity, mutability and diplontic selection in the histogenic layers of the shoot apix and their derivatives are continued. Results obtained sofar with testclone 72 F13, which has 6 monofactorial marker genes in heterozygous condition, were disappointing because of high radiosensitivity, very low mutability and high susceptibility to diseases. The production of a better clone to substitute for 72 F13 is in progress.

II. Induction of adventitious sprouts on leaves and leaflets.

For the 4th year in succession, the in vivo induction of adventitious sprouts on leaves and leaflets of plants proved to be unsuccessful. It had been thought that this method would help solve some of the problems mentioned under I and provide chimera-free mutants from L_1 origin. Approximately 100,000 leaves and leaflets were observed in phytotron tests, applying auxins and kinetins and using different daylengths and temperatures. Hardly any adventitious sprouts developed. A new object of observation was the production of callous-like tissues (proliferations on leafsheets). In co-operation with I.T.A.L. preliminary experiments were started to study the possibilities of the in vitro method using explants from aerial parts of potato plants.

III. Screening for induced resistance to diseases and for mutagenically induced yield improvement.

Comperative tests with irradiated Bintje-material for yield improvement and for (horizontal) resistance against leafroll, y-virus and blight have now reached the final stage.

For leafroll resistance e.g. 6 out of 693 sub-clones, obtained from 180 eye-pieces of the c.v. Bintje irradiated in 1963, possessed a significantly better level of resistance. As far as observed 3 of these 6 clones differed from the original c.v. Bintje only in their level of resistance (see table 1).

Table 1. Bintje like sub-clones from irradiated eye-pieces of c.v. Bintje with improved level of resistance to leafroll.

Clonal treatment		percentages of illness in:									
A	kRad	1966	1967	1968	1969	1970	1971	1972	1973	1974	1966 t/m 1974
313	4	0 ^x	7 ^x	67	8 ^x	63	33 ^x	37 ^x	34	14 ^x	29 ^x
315	2½	7 ^x	3 ^x	-	79	75	40 ^x	32 ^x	46	10 ^x	37 ^x
350	3¼	10 ^x	20 ^x	-	66	-	54	39 ^x	26 ^x	10 ^x	32 ^x
x̄ untreated control		35	41	73	83	75	58	47	35	21	55

(^x = significant difference with P < 5%)

Recent results indicate that re-irradiation of this material in 1969 may lead to further improvement with regard to leafroll virus resistance. Already after one year of selection for leafroll resistance there appeared to exist a significant difference with the untreated control, which had P = 1%

IV. Maintenance and multiplication.

About 1,000 sub-clones derived from irradiated plant material of the c.v.'s Bintje, Burmania, Désirée and some di-haploid clones are maintained under favourable conditions in the new polders to serve as basic material for later experiments in Wageningen and for demonstration purposes.

V. Programme for 1975 and later years.

1. Intensification of the experiments to investigate the organisation of the irradiated shoot apex of potato and its histogenic layers.
2. Advanced research at diploid level with di-haploid clones with regard to mutability, radiosensitivity and diplontic selection.
3. The development of an in vitro method for the induction of adventitious sprouts, in co-operation with the I.T.A.L. (Dr. Broertjes and Dr. Roest). Cultivated potato species of different ploidy levels and di-haploids will be used.
4. Preliminary tests with the triploid, cultivated South-American potato species Solanum juzepczukii to investigate the possibility of reducing the high content of glyco-alkaloids via induced mutations.
5. Some additional experiments to complete the studies on the possibilities of improving disease resistance and yield capacity of c.v. Bintje via induced mutations.
6. Within the framework of our mutation research we also commenced studies on Pelargonium zonale. This work is not subsidized, as yet by the Biology Division of Euratom. Investigations into the possibility of breaking undesired linkages via mutagenic treatment will be intensified during 1975.

Publications.

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Results of the project : *Lobularia maritima* (L.) Desv.
Leader and co-worker : I. Bos, G. Heemstra.
Title of the project : The population genetics of the S alleles of a sporophytic
incompatibility system.

In continuation of the preceding analytical studies concerning the dominance or codominance relation between the S-alleles in the stigma and the pollen producing tissues of individual plants, the extensive crossing program needed to reveal this relation, has been adjusted as indicated last year. The application of FABIG & NOWAK's procedure has been completed for the material under investigation. Because disturbances with ^{un}known causes are still present it is hard to draw clearcut conclusions at this stage about the most common of the interaction types for stigma- and pollen-relationship of the S alleles. These disturbances are caused most probably by the imperfect isolation of plant material from insects causing uncontrolled pollinations. During last summer however, a better insect-proof system for the greenhouse compartment has been realized.

Because in nature there is unhampered crosspollination (i.a. by flies) between violet and white flowering varieties of *Lobularia maritima* (L.) Desv. a study about the genetics of habitus and flowercolour has been executed. The erect plant type appears to be dominant over the creeping type (1 locus). Flower colour seems to be determined by 2 independent, cumulative isomeric loci, one of which is tightly coupled with the locus for plant type.

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

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

In 1974 a population of 25 plants, which had been obtained through pseudo-compatibility from G254 (genotype S_1S_3/S_1'') x tester $S_1S_3 \delta$, was investigated for occurrence and frequency of the expected genotypes S_1S_1 , S_1S_1/S_1'' , S_1S_3 , S_1S_3/S_1'' , S_3S_3 and S_3S_3/S_1'' . All genotypes except one (either S_1S_1 or S_1S_1/S_1'') could be detected, but their frequencies showed that fertilization by S_3 -pollen was far more frequent than by S_1 -pollen. This could mean either that S_1 in the style is a stronger allele than S_3 or that the stylar part mutation of S_1'' did not completely inactivate the stylar activity cistron.

Six reciprocal F_1 's involving one self-incompatible (G 609) and two self-compatible dihaploids (G 254 and B16) were retested for segregation into sc:si. In table 1 the results are presented.

Table 1. Number of sc^{and} si plants in six F_1 populations.

F_1	Numbers	
	sc	si
a. G254 x B16	49	17
b. B16 x G254	34	18
c. G254 x G609	45	47
d. G609 x G254	38	42
e. B16 x G609	36	41
f. G609 x B16	47	57

Though not significant, in all F_1 's there is a shortage of sc plants. Therefore it is reasonable to assume a slight certative disadvantage of pollen carrying the duplication with the S_1'' allele (see previous report). In view of the 3:1 ratios in the F_1 's indicated in table 1 as a and b, lethality of duplication-homozygotes has to be assumed.

In 1975 a final and crucial experiment on the supposed lethality of duplication homozygotes will be carried out. For this experiment the test crosses were made in 1974. Investigations on the incompatibility system in S. bulbocastanum will be carried out. Some orientating experiments were already carried out. Though the results suggested a two-loci gametophytic system, they are not ^{at} all conclusive.

Leader: Dr.Ir. J.G.Th. Hermsen. Co-workers: Dr. M.S. Ramanna, Mrs. E. Sawicka M.Sci.
and Ing. J. Verdenius.

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

Interspecific hybridization is becoming more and more important in plant breeding, not only for introducing desirable characters in cultivated crops, but also for a general broadening of the genetic basis for breeding. This is particularly true in potato breeding. Within the framework of this project crossability barriers between Solanum species are being studied together with methods to break or to overcome such barriers.

In the 1973 report two barriers were mentioned, viz. unilateral incongruity in diploid S. tuberosum ♀ x S. verrucosum ♂ and unilateral "eclipse" sterility in S. verrucosum ♀ x diploid S. tuberosum ♂. Investigations on inheritance in 1973 and 1974 enabled the use of genetic techniques to overcome the barriers mentioned. As a consequence the starting material for breeding could be broadened considerably.

The diploid species S. bulbocastanum, like S. verrucosum, is important for resistance breeding in potato. Crosses between S. tuberosum and S. bulbocastanum were not successful in 1974 in spite of extensive attempts in both directions. Crossing S. bulbocastanum with S. verrucosum succeeded only with S. verrucosum as a female. The diploid F₁-plants and also their colchicine-doubled counterparts appeared completely sterile and could not be used in crosses either with diploid or with tetraploid potato. Also this sterility is probably due to the cytoplasm from S. verrucosum: "eclipse" sterile pollen grains were observed. Because of the sterilizing effect of S. verrucosum cytoplasm, extensive crosses were made in 1974 using S. bulbocastanum as female parent, but not one berry was obtained. This implies that in S. bulbocastanum no acceptors of S. verrucosum pollen could be detected, contrary to the results with dihaploid S. tuberosum (see report 1973).

Having found no solution for these problems along traditional genetic lines, it is obvious to resort to mutagenic treatments, aimed at

- a. inducing mutations of genes for non-crossability, which mostly are dominant;
- b. inducing a weakened incongruity reaction in styles;
- c. low-dose stimulation of incongruous pollen tube growth;
- d. mutating sterilizing (S-) cytoplasm of S. verrucosum into normal (F-)cytoplasm.

Preliminary experiments are planned for 1975.

Publication.

Hermesen, J.G.Th. et al. 1974.

Acceptance of self-compatible pollen from Solanum verrucosum in dihaploids from S. tuberosum. Fertilization in Higher Plants, ed. H.F. Linskens, North-Holland Publishing Company, Amsterdam: 37-40.

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

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

To study the possibilities of mutation induction of partial resistance against dwarf(leaf)rust, *Puccinia hordei* Otth, in barley seed of the cultivar Minerva was treated in 1973. The M_2 was grown in a greenhouse in the spring of 1974. Over 5000 M_2 seeds were planted, each seed coming from a different M_1 ear. After emergence seedlings showing chlorophyll mutations or very poor growth were removed and the open space replanted with another M_2 plant of the same treatment kept in reserve. Table 1 shows the number of M_2 seeds planted and the number of chlorophyll mutations observed.

Table 1. Number of M_2 seeds planted and the number of chlorophyll mutations observed. Between brackets percentages.

	No M_2 seeds sown	No M_2 seeds not emerged	No Chlor. mutations
0 K-rad	820	73 (8.9)	1 (0.12)
15 K-rad	2060	349 (16.9)	49 (2.38)
0 m. mol EMS	620	20 (3.2)	1 (0.16)
25 " " "	860	79 (9.2)	39 (4.53)
50 " " "	860	179 (20.8)	121 (14.07)
75 " " "	60	24 (40.0)	13 (21.67)

The percentage not-emerged seed, the proportion of seedlings with stunted growth and the sterility in the adult plants increased in a similar way as the chlorophyll-mutation frequency. Several seedlings displaying a chlorophyll mutation also showed a stunted growth. Apparently chromosome aberrations still occur in the M_2 and it is not unlikely that part of the chlorophyll mutations are in fact due to chromosome aberrations. If on the other hand all chlorophyll mutations would be recessive gene mutations one could expect at least twice as many plants to be heterozygous for such mutations raising the mutation frequency in the 50 and 75 E.M.S. treatments to values of over 40 and 65% resp., which are extremely high frequencies. Within each treatment a number of plants of normal phenotype have been crossed pairwise in order to study the effect of recombination after mutation induction on the variability of quantitative characters (partial resistance is one of these) in comparison with the conventional approach (no recombination).

Table 2 shows, that the number of seeds obtained per emasculated ear decreased in a way similar to the increase in non-emerged seeds and chlorophyll-mutations (table 1). This too suggests the presence of chromosomal aberrations even in normal looking.

Table 2. Number of pairwise crosses and number of seeds obtained. Between brackets the no. of seed per ear.

treatment	No pair crossed	No parent plants included	No seeds
0 K-rad	45	88	512 (11.3)
15 K-rad	47	82	445 (10.5)
0 m. mol EMS	34	64	477 (14.0)
25 " " "	21	42	197 (9.4)
50 " " "	29	38	299 (7.9)
75 " " "	3	6	9 (3.0)

When it is true that chromosomal aberrations are still present to a considerable extent in the M_2 the studies of variation in quantitative characters often done with normal looking plants in the M_2 should be approached with caution.

Associato della Commissione: Comitato Nazionale

per l'Energia Nucleare, Laboratorio Applicazioni Agricoltura

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

Capo del gruppo di ricerca: prof. A.Bozzini

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

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

The researches carried out in 1974 at the Laboratory of CNEN were principally oriented in three directions:

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

Risultato del progetto n. 1: see report of the
Association EURATOM-ITAL

Risultati del progetto n. 2

Capo del progetto e collaboratori scientifici:

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

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

2.1. Analysis of mutation spectra at the S-locus of
N.alata: see report of the Association EURATOM-ITAL.

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

This investigation is the continuation of the stu-
dies mentioned in the annual report of 1973 for which the
details of the objectives and the results obtained till
that period have already been furnished.

In 1974, a total of 56 seeds were available, which have
been obtained after obligate **inbreeding** of S_1S_2 individu-
als that have been previously placed in several different
genetic backgrounds. These seeds gave rise to 29 plants
of which 26 have survived to maturity. All the 26 plants
were crossed reciprocally to the mother clone 006 (S_1S_2)
to test the cross-compatibility. The results of the cros-
ses of the inbred plants (S_1S_2) as pistillate parents with
the mother clone showed that, there are **at least** 9 plants

which show cross-compatibility. All these 9 plants are being tested, using as pistillate parents, by pollinating with the tester-stocks. The work on the crossing programme, of 006 (S_1S_2 - mother clone) as pistillate parent and the S_1S_2 inbred plants as staminate parents, is in progress.

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

Genetic analyses were carried out in the plants originating from in vitro anther cultures of the clone 9 (S_1S_3) and F.13 (S_6S_7), and also in the plants deriving from stem internode cultures of F.9 (S_4S_5).

The results of self-pollinations and backcross-tests with the mother clone showed that there are marked differences between the plants regenerated from anthers and stem internode cultures. Whereas many plants, which derived from anther cultures, showed compatibility, none of the plants regenerated from stem internode cultures showed any compatibility after self-pollinations as well as after reciprocal crosses with the mother clone.

With anther culture-derived plants, interesting results were obtained in respect of S-locus mutations. Eventhough, the results obtained do not permit yet to record any final conclusions, they give valuable data concerning incompatibility and sterility mutations. The results with plants coming from the genotype S_1S_3 show that, there were 4 plants which were compatible (another three were somewhat weakly compatible!), when used as staminate parents, in crosses with the mother clone, but they were found to be incompatible after self-pollinations as well as after crossing as pistillate parents

with the mother clone. There was also one plant that showed compatibility when crossed reciprocally with the mother clone, and incompatibility after self-pollination. Further genetic analyses, by conducting detection tests, and by observations on the morphology of the pollen tube growth in the style, would reveal whether there is any "new specificity" operating in these plants.

In F.13 (S_6S_7), altogether 15 plants were analysed for compatibility relationships. Cytological analyses have revealed that these plants were tetraploids. The results of self-pollinations showed that, of the 15 plants 9 were self-compatible and 6 were self-incompatible. In tetraploids, as would be expected, self-compatibility is induced through the supposed competition between different S-alleles in the diploid pollen. Marked differences were observed among the self-compatible tetraploids in respect of cross-compatibility relationships with the mother clone. In some of the self-compatible tetraploids, it was noted that, after crossing reciprocally to the mother clone, there was fruit-setting, but little or no seed development. These results indicate that, some sterility barriers intervene post-zygotically and prevent the formation or the development of triploid embryos. The backcross tests in the other self-compatible tetraploids show that, the degree to which the sterility barrier affects depend upon the direction of the cross made.

The six tetraploid plants which showed incompatibility after selfing, also gave no fruit-and seed-setting when crossed both as staminate and pistillate parents with the mother clone, implicating the presence of a system restricting selfing, i.e., a system which is controlled by the

incompatibility alleles. Further genetic analysis and observations on the pollen tube growth in the style would reveal, whether any "tetraplex" condition occurs or any "dormant" genes modify at the tetraploid level the intensity and the mode of expression of the self compatibility characters.

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

Analyses have been carried out in the morphological mutants (Spur types) induced by radiation in olives and sweet cherries to find out possible self-compatible types. The procedure adopted was consisted of isolating several flowering branches in each plant, for preventing cross-pollination and screening for plants showing fruit and seed production. This investigation will be continued also in the next year with the forthcoming mutants. Plants coming from the more promising mutants of olives and cherries which have been ascertained to be self-incompatible, have been prepared for the irradiation at PMC stage.

Risultati del progetto n. 3

Capo del progetto e collaboratori scientifici:

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

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

As in 1973, this problem has been tackled at the cytological and genetical levels in collaboration with the Wageningen laboratory of the EURATOM-ITAL Association.

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

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

Cytological analyses were carried out in *N.glauca* with the aim of identifying the S-bearing chromosome. From the first data obtained with the karyotype analyses in the root tip mitoses, it appeared that the S-locus may be located on one of the chromosomes 2, 4, 8 or 9. Recently, the investigations with the self-compatible aneuploid plant C.191 A.18 with 22 chromosomes (Obtained from Wageningen) indicated that among the others, one of the chromosomes 8 or 9 is present three times. Therefore, the presence of S-locus in one of these two chromosomes would exclude the possibility of the involvement of the other chromosome for the presence of the S-locus. In the aneuploid plant C.191 A.3 with 25 chromosomes, it is also possible to ascertain the presence of one of the chromosomes 1 or 2 in three times; if we confirm that the chromosome 2 is present two times than this chromosome will be automatically excluded as S-bearing chromosome.

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

Studies were made to create a collection of trisomics and to find out which trisomic line was self-compatible, and consequently the trisomic for the S-bearing chromosome. The triploid obtained from the cross between self-compatible tetraploid and self-incompatible diploid, was backcrossed to the diploid parent, and also some reciprocal crosses were made between the triploid and other diploid genotypes. Altogether, 217 seeds were obtained from these crosses and they finally gave rise to 167 plants which were tested for self-compatibility. Cytological analyses in root tip squashes were made in 117 plants.

The results of self-compatibility tests performed in these 167 plants have shown that, excepting 16 plants, all the other plants were completely sterile and gave no fruit development. Among the 16 plants that showed fruit-setting to different degree, only 12 were found with seed-setting. However, they differed markedly with regard to the percentage seed-setting. Based on the number of seeds produced per selfed flower or seeded fruit, only five plants can be considered as self-compatible and the other 7 plants as weakly self-compatible.

A detailed cytological analysis of root-tip mitoses in the five self-compatible plants has shown that, three plants (n° 145, 172, 220) were trisomics ($2n=25$), while one (n° 119) had $2n=24$ and the other (n° 125) possessed 48 chromosomes. Karyotype analysis has clearly revealed that, in plant 145, the extra chromosome is the chromosome number 2 whereas in the other plants the extra chromosome is other than the chromosome 2. However, recently

in plant 145, analysis of a large number of mitoses has shown, in some cases, the presence of 50 chromosomes and this is being verified in the meiosis of pollen mother cells in order to see if any periclinal chimeric situation exists in this plant. Further karyotype analyses with other plants, n° 269 (weakly self-compatible) and n° 261 (sterile), have shown that in these plants probably some modification is involved in respect of the satellite chromosome. Meanwhile, all the self-compatible plants were submitted to test-crosses in order to identify the S-allele involved and to obtain information on the mechanisms operating in these plants. Pachytene analyses will be carried out next year to confirm the observations made in root-tip mitoses and to have more detailed information on the cytological aspects in the self-compatible progeny.

Complement to projects n. 2 and n. 3:

Studies on the ultrastructural aspects of pollen tube growth in the styles after self-incompatible and cross-compatible pollinations in the flowers irradiated with gamma rays in *L.peruvianum*.

In collaboration with the group of the laboratory of electron microscopy at the University of Siena (Professor G.Sarfatti, Drs. Cresti and Pacini), we undertook a research programme to study the ultrastructural aspects of the pollen tube growth in the styles after self-incompatible and cross-compatible pollinations in the flowers irradiated with gamma rays in *L.peruvianum*. The motivation for this type of study stemmed from the results

of our previous investigations that, one of the early manifestations of the self-incompatibility reaction seems a general cessation of protein synthesis, which results from an alteration of the endoplasmic reticulum into a whorl of concentric layers and its subsequent degradation throughout the cytoplasm of the incompatible pollen tube. Further, from the previous reports, it is known that radiation treatments could make possible, in self-incompatible species, the pollen tube growth through the style and the fertilization of the ovules.

Experiments were carried out with S_1S_4 and S_6S_7 genotypes. About six hours after pollination, the flowers were excised and transferred to a minimal medium in Petri dishes, and irradiated with gamma rays at 100 kR delivered at an exposure rate of 38.7 kR/hr. Based on a preliminary experiment, this dose was chosen for this study. After irradiation, the flowers were transferred to fresh minimal medium and the styles were fixed the following day to prepare the slides for electron microscopic observations.

The results showed that, in the non-irradiated styles (controls) and in the irradiated styles after cross-compatible pollination ($S_1S_4 \times S_6S_7$) the growth of the pollen tubes was normal. In respect of the irradiated styles after self-incompatible pollination ($S_1S_4 \times S_1S_4$), the pollen tubes grow through the half length of the style, i.e., through that portion where generally incompatibility reaction takes place. Some pollen tubes were also present at the lower portion of the style near the ovules.

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Risultati del progetto n. 4

Capo del progetto e collaboratori scientifici:

L.M. Menti, F. Saccardo, A. Bozzini, L. Tomarchie

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

4.1. Tomato

In tomato the project aims at finding of inducing resistance against two pathogenic agents for which no resistance is so far available, that are Verticillium albo-atrum race 2 and Phytophthora infestans race T1.

So far, more than 12,000 M_2 seedlings (gamete irradiation with gamma rays) were scored against the former fungus by the dip root technique, without finding no resistant plant; also the screening against the latter fungus was started, by spraying the sperangia on the M_2 seedlings at the cotyledonal stage.

Other mutagenic treatments have been performed and more than 5,000 M_1 plants belonging to three varieties were grown in the field and their seeds harvested.

By analysing eight wild species of Tomato, it was found (see Annual Report of ITAL 1973) that Lycopersicum hirsutum showed a partial resistance against race T1 of P. infestans. It has been cleared that such resistance is due to a defense reaction of a physiological type, because of a longer incubation time and a slower propagation rate of the fungus in this wild species.

A leaf disc technique was set up and an analysis of L. hirsutum and L. esculentum, of their hybrids and of F_1BC_1 plants seem to show a polygenic control of such a resistance.

4.2. Wheat

In wheat 166 mutant lines derived from Cappelli and 55 commercial varieties of durum and bread wheat commonly cultivated in Italy were analyzed for field resistance to bunt (Tilletia triticeoides). Analysis of percentage of attack are still under way. Results obtained so far demonstrate that several mutant lines show a percentage of attacked plants both higher and lower than the control lines, confirming data so far obtained. It seems that together with the modified morphology or physiology induced by radiations or chemical mutagens, also the susceptibility to this soil-borne disease has been modified. No clear correlations between particular morphological and/or morphological traits and bunt resistance have been yet found. More detailed analysis on the possible mechanism of resistance or escaping will be performed once the final and pluriennial results are available.

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

N°. del contratto: s/c 005/094-72-1 BIAN

Capo del gruppo di ricerca: Prof. Angelo Bianchi

Tema generale del contratto Qualitative genetic improvement
of plant protein with special refe-
rence to opaque maize.

Induction of variability for protein quantity and quality in opaque-2 and normal maize.

X-ray treatment of dormant seeds of the normal and opaque-2 B 37 inbred versions resulted in the X_2 generation in a recovery of macro- and micromutations. The macromutations were of the seedling (chlorophyll and morphological) and endosperm (defective endosperm) types. The most suitable X-ray dose for practical purposes was 4000 r.

Micromutations for protein quantity were present in the material derived from the X-ray treatments: in fact the coefficients of variation were increased for the irradiated progenies as compared with the non irradiated ones.

The simultaneous evaluation of each progeny for total protein content (micro-Kjeldahl method) and for a quali-quantitative value (DBC method) makes possible the estimation of a quality index. At least on the \underline{o}_2 material the quality index showed greater coefficients of variability in the treated progenies than in the untreated ones. Intercrossing the progenies with higher protein content or higher DBC value should increase the possibility of recovering a B 37 \underline{o}_2 with a better protein endowment.

Risultati del progetto n.s/o 005/094-72-1 BIAN

Capo del progetto e collaboratori scientifici: Angelo Bianchi
Carlo Lorenzoni
Antonio M. Stanca
Francesco Salamini

Titolo del progetto: Qualitative genetic improvement of plant
protein with special reference to opaque maize.

Description of results

Induction of variability for protein quantity and quality in
opaque-2 and normal maize (Zea mays L.)

This experiment started in 1973 with X-ray treatments on the normal (O₂) and opaque-2 (o₂) versions of the inbred line B 37. The irradiated seeds were sown at Fiorenzuola (Piacenza) and, at the flowering time, the plants were self fertilized by hand pollination. The collected ears were shelled and sown in 1974 ear to row. Ten plants were grown for each progeny and 6 - 7 of them were self fertilized and collected at maturity (table 1).

The evaluation of the genetic effect of the treatments has been done either (a) following the appearance of macromutants (defective endosperm and seedling mutations) or (b) searching micromutations by obtaining for each progeny the total N value (micro-Kjeldahl method) and a quanti-qualitative protein value (DBC method).

(a) Induction of macromutations

Two classes of macromutations have been considered: seedling and defective endosperm mutations. The frequencies of seedling mutations have been evaluated in greenhouse-grown X₃ material, with the aim of obtaining useful information about their dose dependance. In table 2 it can be noted that the 8000 r dose gives higher frequencies than the 4000 r dose (0,11 against 0,07 for B 37 o₂ and 0,20 against 0,12 for B 37 o₂).

The defective endosperm mutations have been identified as altered kernel phenotypes segregating with Mendelian ratios on the ears. Again the 8000 r dose seems to be more effective in inducing macromutants than the 4000 r one, at least for B 37 \underline{O}_2 material. However, even if the 8000 r dose is very effective its adoption in radiogenetical experiments with maize is to be avoided on the basis of the extremely reduced X_1 seed germination and plant fertility.

The phenotypes of the defective endosperm mutants varied from medium to extreme reduction of the kernel. In the \underline{O}_2 treated material no macromutations decisely modifying the \underline{O}_2 phenotype without heavy reduction of the endosperm size have been found; similarly in the \underline{O}_2 treated progenies clear Mendelian endosperm mutations only slightly reducing the seed size were absent. In spite of this it can be considered that the defective endosperm mutations with a medium reduction of the kernel size may have some practical or theoretical value in protein studies. This material will be evaluated in 1975, after the obtention of suitable quantity of mutated kernels.

(b) Induction of micromutations for protein quantity and quality

For this type of study one X_2 ear derived from each progeny has been considered. To avoid a possible effect of the number of kernels per ear on the quantity or quality of the seed proteins, well filled ears were chosen. Moreover, when all the ears in a progeny showed poor fertility, the progeny was discarded. In choosing the ear within each progeny special attention has been given to its phenotype: those even slightly deviating from the standard type were usually preferred.

A sample of kernels from the middle part of the selected ears has been milled and the flour passed through a 70 mash sieve. Total protein content were obtained with a micro-Kjeldahl procedure: the flours were also submitted to an evaluation of their capacity of binding to the orange G dye (DBC method). This last method gives a cumulative evaluation of the protein quality and quantity.

In table 3 are reported means and coefficients of variation for the total protein content and the DBC value. As expected the coefficients of variation are greater in progeny derived from treatment with 4000 r or 8000 r

as compared with the values obtained with 0 r progenies. This strongly suggests the possibility of breeding for total protein content by X-ray treatments either in normal or opaque-2 maize. Of practical interest should be the increase of total protein in the opaque-2 version of the inbred B 37, where the mean value (9%) resulted quite lower than the 12% of the normal version. This inbred or its derivatives are widely present in maize hybrid pedigrees.

The correlation coefficient between protein percent and DBC value was high and significant especially in the B 37 α_2 . For this material the regression equation between the two protein estimates has been calculated considering Kjeldahl protein as independent variable. For each sample it was then possible to derive an index of protein quality for any given Kjeldahl protein content as the percent of the observed DBC value on the expected one. The coefficient of variation of this index is given in table 4. Again the treated progenies showed higher values than the control ones, indicating an influence of the X-ray treatment on the modification of the protein quality of the maize kernel.

The effective gain in protein quantity and quality by means of X-ray treatment in our material will be evaluated in the future generations. Particularly we should be able to obtain further increase in the characters under study selecting within the segregating progenies obtained by intercrossing the best plusvariants of this experiment.

Table 1. Number of progenies and ears collected in 1974.

Genotype	Dose	N° of progenies	Ears, progeny
opaque-2	0	40	6,00
	4000 r	748	5,93
	8000 r	140	6,08
normal	0	40	5,50
	4000 r	203	4,72
	8000 r	31	4,48

Table 2. Frequency of seedling mutations and defective endosperm mutations in the X₂ generation.

Genotype	Dose	N° of defective endosperm mutations	Endosperm mutations/progeny	N°.of seedling mutations	Seedling mutations/progeny
opaque-2	4000 r	98	0,13	39	0,07
	8000 r	45	0,32	14	0,11
normal	4000 r	52	0,25	20	0,12
	8000 r	5	0,16	5	0,20

Table 3. Means and coefficients of variation for total protein and dye binding capacity (DBC) and coefficient of correlation between the two characters in X_2 progenies of normal and opaque-2.

Genotype	Dose (r)	N°. of progenies	Protein percent		D B C		r
			m	CV	m	CV	
opaque-2	0	40	9,21	5,59	51,23	7,72	0,430 X
	4000	655	9,15	9,70	53,02	14,98	0,645 X X
	8000	106	8,54	9,46	53,96	16,69	0,609 X X
	0	40	12,00	4,12	59,45	5,59	0,337 ns
normal	4000	138	12,06	7,57	64,76	8,87	0,269 X X
	8000	18	12,21	7,65	63,43	11,92	0,415 ns

X significant ($P < 0,05$)

X X significant ($P < 0,01$)

Table 4. Coefficient of variation of the protein quality index in B 37 α_2 X_2 progenies.

Treatment	N°. of progenies	C.V.
0 r	40	8,69
4000 r	655	13,32
8000 r	106	10,98

Associato alla Commissione:
Università degli Studi di Bari
N. del contratto: 006-I/094-72-1 BIAN

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

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

In the second year of the project, M_2 analyses have been carried out both in field and in greenhouse. 5,288 M_1 -plant-progenies have been grown in field; among them, 738 progenies segregated for suspected mutations. The rate of suspected mutations was higher after chemical treatments than after X-ray irradiation.

Chlorophyll mutations as well as morphological mutations affecting several plant organs have been observed. The frequency of sterile or semisterile M_2 plants was about 2.5%.

All M_2 plants grown in field (about 70,000) have been artificially inoculated with populations of Erysiphe polygoni DC; in the "Sprinter" material treated with 1,5% DES, one progeny segregated for one plant exhibiting a certain degree of resistance.

All segregating progenies have been harvested and seeds collected separately from the suspected mutants as well as from the sister plants. A total of 428,116 seeds are now available for a M_3 analysis.

Furthermore, 2 pods for each normal appearing plant of each M_2 -progeny have been harvested, thus giving rise to a bulk of about 70,000 M_3 seeds to be analyzed in M_3 .

Moreover, using a large-scale screening technique devised in 1973, a total number of 3,258 M_2 progenies, including 64,417 plants, were tested in greenhouse for resistance to powdery mildew. None of them showed any vertical resistance against the disease.

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

Capo del progetto e collaboratori scientifici:

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

Titolo del progetto:

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

In the second year of the project, analyses have been carried out in field and in greenhouse on M_2 plants.

Field experiments

Table 1 shows the number of M_1 plant-progenies and M_2 adult plants grown in field.

In table 2, for each treatment, total number and percentage of M_1 plant-progenies segregating for suspected mutations are shown. The frequencies of mutation induction after chemical treatments appear to be higher than those after X-ray treatment. It was also ascertained that mutation frequencies shown by M_1 progenies from the main stem were higher than those from lateral branches. Moreover, in table 3 the percentage of sterile and semisterile M_2 plants is reported.

The more frequent mutated phenotypes, which obviously have to be confirmed in M_3 , belong to the following classes: chlorophyll mutations, mutations of the leaf shape and size, pod mutations, mutations of seed colour and size; mutations affecting the plant habit (height, waxiness, earliness, length of internodes, absence of stipules, etc.) have also been found.

All 68,782 M_2 plants have been artificially inoculated with populations of Erysiphe polygoni DC; in the treatment SC_2 (DES 1.50%) one progeny (composed by 8 adult plants) segregated for one plant which has exhibited a certain degree of resistance. This plant is 60 cm high, of intermediate type of earliness, of good fertility and with 4 lateral branches.

All segregating progenies have been harvested and seeds collected separately from the suspected mutants as well as from the sister plants. A total of 428,116 seeds (table 1) are now available for a M_3 analysis. Furthermore, 2 pods for each normal appearing plant of each M_2 progeny have been harvested, thus giving rise to a bulk of about 70,000 M_3 seeds to be analyzed in M_3 .

Greenhouse experiments

Greenhouse tests were carried out to ascertain the host range of populations of E. polygoni obtained from pea and other host plants collected from different areas of southern Italy. The preliminary results of cross inoculation tests seem to indicate that populations of E. polygoni isolated from pea plants possess a restricted range of host plant species.

Using a large-scale screening technique devised in 1973, a total number of 3,258 M₂ progenies, including 64,417 plants, were tested in greenhouse for resistance to powdery mildew. None of them showed any vertical resistance against the disease.

TABLE 1

Number of M_1 plant-progenies, number of M_2 adult plants, and number of harvested M_3 seeds of varieties Sprinter (S) and Freezer 69 (F) after treatment with different X-ray (R) and diethylsulfate (C) doses.

Treatment	Number of M_1 plant-progenies	Number of M_2 adult plants	Number of harvested M_3 seeds
FC ₁	637	9,989	98,047
FC ₂	502	9,922	43,758
FR ₁	519	6,737	49,117
FR ₂	972	9,970	50,487
SC ₁	509	6,216	42,641
SC ₂	1,057	11,202	62,978
SR ₁	533	8,668	57,356
SR ₂	559	6,078	23,732
TOTAL	5,288	68,782	428,116

TABLE 2

Number and percentage of M_1 plant-progenies segregating for morphological and physiological mutations.

Treatment	Number of M_1 plant-progenies	M_1 plant-progenies with mutations	Percentage
FC ₁	637	119	18.7
FC ₂	502	73	14.5
FR ₁	519	78	15.0
FR ₂	972	114	11.7
SC ₁	509	78	15.3
SC ₂	1,057	150	14.2
SR ₁	533	77	14.4
SR ₂	559	49	8.8
TOTAL	5,288	738	

TABLE 3

Percentage of semisterile and sterile M_2 plants.

Treatment	Semisterile and sterile plants (%)	Treatment	Semisterile and sterile plants (%)
FC ₁	2.50	SC ₁	1.96
FC ₂	2.57	SC ₂	4.08
FR ₁	1.53	SR ₁	2.26
FR ₂	2.51	SR ₂	3.42
F test	0.47	S test	0.89

Contract partner
of the Commission: Prof. Dr. Werner Gottschalk
No. of the contract: SC 07/94-72-BIAN
Head of the research group: Prof. Dr. Werner Gottschalk

General theme of the contract:

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

General report

During 1974, our former investigations on the yielding capacity and the protein situation of X-ray and neutron induced mutants of *Pisum sativum* were continued. Furthermore, a programme on specific gene-ecological problems was initiated. Some of our useful mutants were improved by means of hybridizations and by stabilizing the unstable penetrance of mutant genes. Furthermore, the reaction of specific mutants to different environmental conditions were studied by cultivating them in a climate chamber and in India. In this way, the adaptability of mutated genes to different climates should be tested. In the field of protein genetics, the protein production during seed development and seed ripening was analysed.

The co-operation with foreign institutes was further intensified. At present, groups of our mutants and recombinants are grown at 3 different universities of India (Kurukshetra, Varanasi, Jabalpur) as well as at the Indian Agricultural Research Institute in Delhi.

Results of project no. 1

Head of the project and scientific co-workers:

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

Title of the project:

The improvement of useful mutants

Most of our fasciated mutants show excellent yielding properties. Unfortunately, they are too tall and too late. Moreover, they show obviously divergent photoperiodic reactions. As a consequence of this, they are not flowering in sub-tropical countries such as India and Egypt. We have tried to reduce the stem height and to shift the flowering and ripening time towards earliness. This has been done by means of hybridizations with other mutants of our collection. In this way, an extraordinarily high number of different fasciated recombinants have been selected in which different characters of breeding value are combined in a different way. So far, about 30 different fasciated recombinants are available, in the form of homozygous strains, 15 of them are considered in fig. 1. This extremely high diversity is due to the complicated mutation genetic situation of our fasciated mutants. At least 10 different genes have mutated in the same embryo during the irradiations. Therefore, a great number of different recombinants are obtained in all the crosses in which one of the fasciated mutants is used as parent.

Fasciated mutants and recombinants of <i>Pisum sativum</i>									
mutants	489C - strongly fasciated - long - late - high yield	123 - strongly fasciated - long - pods shedding - low yield	251A - relaxed fasciated - long - intermediate - high yield	489C } alleles 123 } 251A } 107D } identical 250 } with 489C 2597A }	Ornamenta: - 489C L 5242: non-allelic to 489C				
	recombinants	R 667 - long - not late 489 x 169	R 675A - shorter - not late 489 x cooh	R 656 - very long - intermediate 489 x afile					
relaxed fasciated	R 605 - long - late 489 x 98A 489 x afile	R 657 - long - not late 489 x afile	R 658 D - long - intermediate - increased seed number per pod 489 x afile	R 205 - very long - very early - bifurcated 489 x 46	R 674 - short - late 489 x cooh	R 98a - short - not late 489 x 176a 489 x 1201A	R 163A - short - late - bifurcated - pods shedding 489 x 1201A	R 600 - short - earlier 489 x 445	
	weakly fasciated	R 661 - long - not late 489 x 26	R 701 - shorter - not late 489 x cooh	R 710 - shorter - earlier 489 x cooh	R 161 - short - not late - bifurcated 489 x 1201A				
other combinations	- fasciated/very short/late - fasciated/very short/not late - fasciated/very short/early			} low seed production					

Figure 1: Fasciated mutants and recombinants of *Pisum sativum*. The mutants belong to a series of at least four multiple alleles. All the recombinants have been selected after having crossed the fasciated mutant 489C with other mutants of our collection.

Three mutants showing a dichotomous stem bifurcation in the upper part of the shoot are available in our collection showing good yielding properties (mutants 157, 239, 1201A). Unfortunately, the penetrance of the respective genes is unstable reducing the seed production of the strains. We have crossed this mutant with other useful genotypes in order to select recombinants homozygous for the mutated genes and to study their co-operation. Some of them influence the penetrance of gene 1201A (=a). The presence of gene b (earliness) decreases the penetrance of gene a. The incorporation of gene c (increased ovule number per carpel) into the recombinant aabb decreases the penetrance of gene a even more, but only when gene b is present in the genome. The combination of genes a and c without b does not influence the penetrance of a. The incorporation of gene d deriving from the fasciated mutant 489C into the genome of mutant 1201A stabilizes the penetrance of gene a (figure 2). This stabilization results in the expected increase of the seed production.

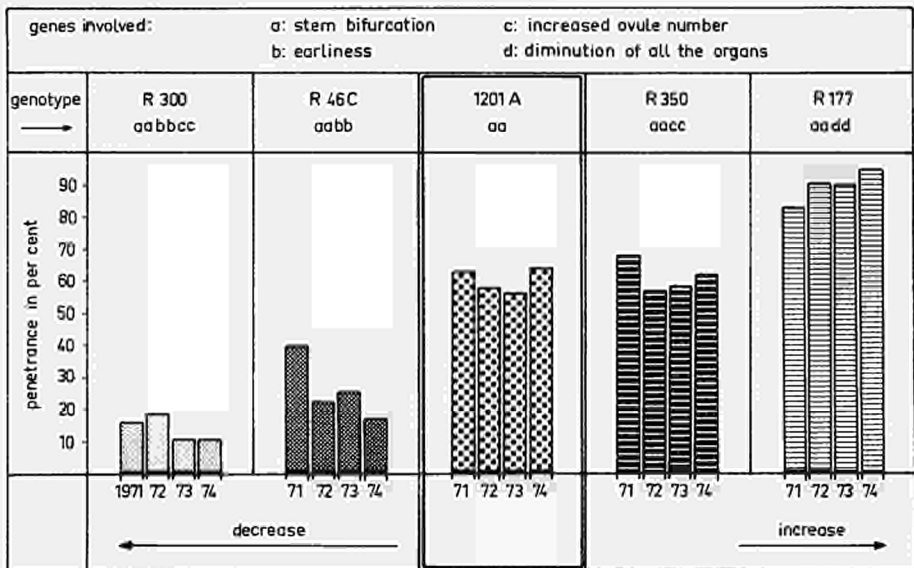


Figure 2: The dependence of the penetrance behaviour of gene a (mutant 1201A) on the genotypic background.

Results of project no. 2

Head of the project and scientific co-workers:

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

Title of the project:

The reaction of mutants to different environmental conditions

Some of our mutants were comparatively grown under 12.000 and 600 Lux in a climate chamber; four different climates were given to them. Mutant 66 having short internodes shows nearly no reactions on the differences of the climatic conditions offered to it. Only with regard to the peroxidase activity, certain differences between the plants grown under the varying climates were found (figure 3). Under 12.000 Lux, 3 isozymes of the peroxidase are present in the stems of the initial line while only two of them are observed in mutant 66. The opposite situation is valid under 600 Lux. These results are of interest for studying problems of the so-called "enzymatic adaptation" which is of direct significance for the adaptability of different genotypes to specific environmental conditions.

The reaction of the initial line and mutant 66 to different climotic conditions

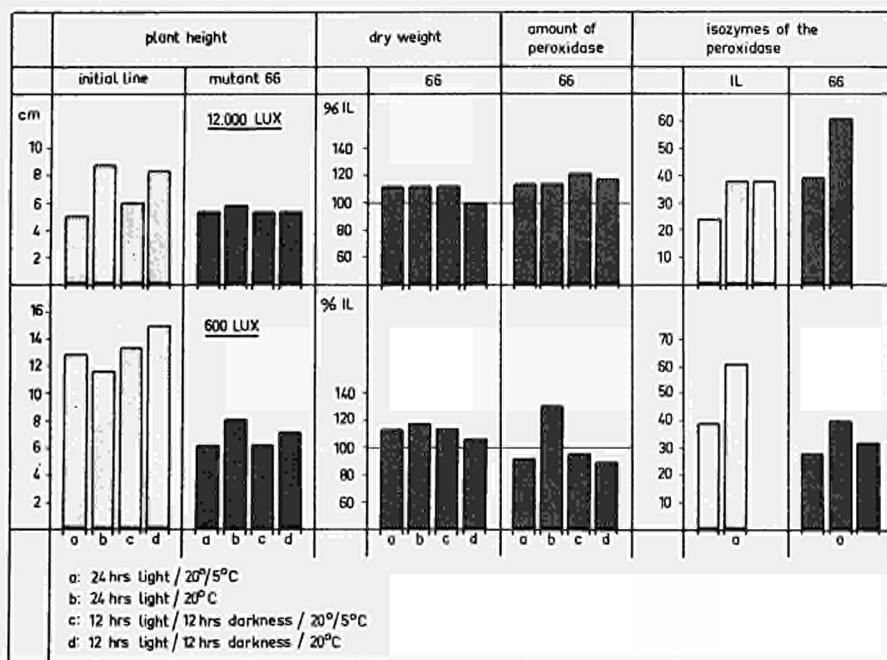


Figure 3: The dry matter production, peroxidase activity and isozyme pattern of the initial line and mutant 66 under different environmental conditions.

Results of project no. 3:

Head of the project and scientific co-workers:

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

Title of the project:

The protein production during seed development and seed ripening

The two main fractions of the proteins - the globulins and albumins - were analysed in three different stages of seed development:

- a: green seeds having nearly reached their definite size;
- b,c: semi- and fully-ripened seeds.

8 different genotypes were analysed; the results of three of them are illustrated in fig. 4. The proportion of the globulins and albumins varies considerably between different genotypes. This is also valid with regard to the globulin-albumin-ratio. This ratio changes during seed development in a manner varying from mutant to mutant. This can be of direct importance for the nutritional value of the seeds of specific mutants.

The qualitative situation was analysed by determining the subfractions of globulins and albumins electrophoretically. The comparison of the protein patterns of the albumins in the three developmental stages just mentioned is graphically presented in figure 5 for 3 genotypes. The albumin fraction becomes more and more heterogenous with continuing seed ripening. From the eight mutants analysed, each shows a specific behaviour in this respect. This could be one of the causes of the diverging amino acid composition of the seed proteins found in our mutants.

List of publications

The following papers were published in 1974:

- Gottschalk, W.: Neighbouring genes of the Pisum genome with high mutation frequency.
Pisum Newsletter 6, 10
- , - Zwei hochpleiotrope, polymere Gene von Pisum sativum.
Decheniana 127 (in press)
- Gottschalk, W. and M. Helmig-Missel: The reaction of different pea mutants to different light intensities.
Pisum Newsletter 6, 11
- Gottschalk, W. and C. Hiemke: Seed protein content of Pisum mutants grown under different climatic conditions.
Pisum Newsletter 6, 12
- Gottschalk, W. and M.M. Imam: The productivity of some useful Pisum mutants in Germany and Ghana.
Pisum Newsletter 6, 13
- Gottschalk, W. and M.L.H. Kaul: The co-operation of the cochleata-gene with other mutated genes of the Pisum genome.
Pisum Newsletter 6, 14-15
- , - The genetic control of microsporogenesis.
The Nucleus (in press)
- Gottschalk, W. and O. Konvicka: Die Meiosis einer partiell asynaptischen Mutante von Pisum sativum.
Cytobiologie (in press)
- Gottschalk, W. and M. Milutinović: Dependence of the penetrance upon genotype and climate.
Pisum Newsletter 6, 17
- Gottschalk, W. and H.P. Müller: Quantitative and qualitative investigations on the seed proteins of mutants and recombinants of Pisum sativum.
Theor. Appl. Genet. 45, 7-20
- , - The protein yield of Pisum mutants and recombinants.
Pisum Newsletter 6, 15-16
- Gottschalk, W., H.P. Müller and G. Wolff: The relations between protein production, protein quality and environmental factors in Pisum mutants.
(in press)

- Gottschalk, W. and G. Wolff: The relation between seed size and protein content in Pisum mutants. Pisum Newsletter 6, 18
- Konvicka, O. und W. Gottschalk: Untersuchungen an der Meiosis steriler Mutanten von Allium cepa. Angew. Bot. 48, 9-19
- Müller, H.P.: Fractionation of the globulins of pea seeds. Pisum Newsletter 6, 39-40
- , - Distribution of the globulin and albumin fractions within the seeds of pea mutants. Pisum Newsletter 6, 41
- Müller, H.P. and S.R. Bacquar: Quantitative evaluation of genetic variations in seed protein content and some other breeding characters of some recombinants of X-ray induced Pisum mutants. Genetika (Beograd) (in press)
- Müller, H.P. and W. Gottschalk: Cooperation of mutated genes in protein production. Pisum Newsletter 6, 39
- Müller, H.P. and G. Isbary: The sugar production of mutants and recombinants and its influence on seed germination. Pisum Newsletter 6, 42-43

During 1974, the following co-workers joint our research programme:

- Dr. Dagmar Müller,
- Dr. Oldrich Konvicka,
- Dipl.Biol. Elisabeth Hasenberg,
- Dipl.Biol. Marianne Helmig-Missel.

They work in the field of gene physiology and cytogenetics. Their results will be included in the next report.

Lectures were given at the Universities of Heidelberg, Kiel, and at EURATOM-Ital in Wageningen by Professor Gottschalk, furthermore at the University of Marburg and Bundesforschungsanstalt für Gartenbauliche Pflanzenzüchtung by Professor Müller.

Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung mbH, München

Nummer des Vertrages: SC O8/94-72-1 BIAN

Leiter der Forschungsgruppe:

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

Allgemeines Thema des Vertrages:

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

In project 1 mutagenic treatment of barley seeds has been continued. In M_2 mildew resistant mutants were selected. They show resistance to the mildew population of Grünbach. The experiments provide information on the frequency of resistant mutants induced by different mutagens and on the efficiency of the mutagenic treatments for the induction of mildew resistant mutants.

In project 2 genetic analyses of mildew resistant mutants are carried out. Both the mode of inheritance and the number of resistance genes are studied. Investigations reveal which resistance genes are included in the mutant collection. The $ml-o$ locus will be studied the most extensively. In some of the mutants mildew resistance is part of a pleiotropic character complex. It is attempted to separate mildew resistance from undesired features being controlled by the same resistance gene.

Ergebnisse des Projekts Nr. 1

Leiter des Projekts und wissenschaftliche Mitarbeiter:
Dipl.-Ing.agr. V. Lind
Prof. Dr. H. Gaul

Titel des Projekts:

Selection of mildew resistant barley mutants following
mutation induction

Darstellung der Ergebnisse:

Several mutants of our collection being resistant against
barley mildew are used in practical breeding projects.
But they all originate from older German varieties.

In order to establish mutants of high yielding new varieties a program for the induction of mutations in "Bido", "Carina" and "Villa" was set up. The X-ray treatment was performed with doses of 15 and 20 kR. The EMS-treatment was conducted with 2 and 3 % for 3 hours at a temperature of 24°C. The mutants were selected after the first EMS- and the third X-ray treatment.

The M_1 generations were grown in isolated plots to avoid outcrossing and admixtures. The M_2 seeds were harvested by hand and sown as a bulk in sterilized soil. Only those seedlings which were not attacked by the mildew population of Grünbach, have been selected. Among a total population size of about 130 000 M_2 plants 12 resistant mutants could be found. It cannot be excluded that some of them might trace back to the same mutation event. But in this case they should have similar characters. In order to prevent an overestimation of the frequency of resistant mutants a comparison of phenotypes has to be done after the selection. Another method for the identification of different mutations is the growing of M_1 -ear progenies. However, in our case it is only practicable after EMS treatment and it is very time consuming.

Ergebnisse des Projekts Nr. 2

Leiter des Projekts und wissenschaftliche Mitarbeiter:

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

Titel des Projekts:

Influence of the genetic background on the expression
of mildew resistance

Darstellung der Ergebnisse:

In 1974 the genetic analyses of 19 mildew resistant mutants have been continued. Crosses were carried out completing the diallel crossing system. The obtained 43 F_1 generations have been growing in the greenhouse. They could be tested for mildew reaction after inoculating with the population of Grünbach. All hybrids proved to be resistant. Consequently the resistance genes of the parents should be in the same locus.

Because of these results the question arised if some of the mutants could come from the same mutation event in one M_1 plant. The mutants used in the experiment were induced in the varieties "Gerda", "Volla" and "Matura" in different years and after various mutagenic treatments. Thus only those mutants coming from the same seed bulk can be identical. Preliminary phenotypic studies showed that this could be true for 4 mutants.

Crosses have been carried out between some mutants and MC 20, a barley mutant obtained from FAVRET. It carries the ml-o gene on chromosome 4 and shows a similar phenotype like the Matura mutants. Studies will give information if the resistance genes of the mutants and the ml-o gene are in the same locus.

Further crossed between MC 20 and Engledow India, carrying a resistance gene at the M1-a locus, will elucidate if interactions between both loci exist. In previous studies such results had been obtained.

Experiments with mutants the mildew resistance of which belongs to a pleiotropic character complex have advanced up to the F_2 generation. However, no resistant plants without the undesired chlorotic flecking could be detected. The selection will be repeated in F_3 when the population size can be increased.

Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung mbH, München

Nummer des Vertrages: SC O8/94-72-1 BIAN

Leiter der Forschungsgruppe:

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

Allgemeines Thema des Vertrages:

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

During the last vegetation period 7000 induced mutants have been tested for improved protein and lysine content. Most of the mutants originate from diploid, a few hundred from tetraploid mother varieties. The diploid mutant strains have been grouped into macro- and micromutants according to morphological and physiological characters.

The selection for protein quantity and quality was accomplished on a percent dry matter basis and on a yield basis, using a two step selection in each generation.

In step 1 all mutants are screened for protein and lysine contents with the DBC-method. In step 2 the 10 % best strains are differentiated into high protein and high lysine mutants by special tests.

During selection two negative relations have to be considered:

(1) protein quality (% lysine in protein) with protein quantity (% protein in dry matter)

(2) protein quantity (% protein i.d.m.) with kernel yield (g/m^2). Including these two relations, a selection scheme was employed, using the field of covariation between both protein characters.

The variability of the mutants in protein and lysine was used for selection of two types of protein-mutants:

(1) mutants, increased in small protein and/or lysine steps. These mutants could be defined as protein-micromutants.

(2) mutants, increased in large protein and/or lysine steps. These mutants could be defined as protein-macromutants.

The protein-micromutants can be used directly as improved strains, the protein-macromutants, however, will be used only as cross-parents.

Ergebnisse des Projekts Nr. 1

Leiter des Projekts und wissenschaftliche Mitarbeiter:

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

Titel des Projekts:

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

Usually selection for high protein and high lysine mutants is performed on a percent dry matter basis (i.d.m.). However several morphological and physiological characters are related with protein and interfering during practical selection work. Therefore relations between protein characters on dry matter basis, protein characters on yield basis and several other characters of economical importance have been tested. The following correlations were found to be of special importance:

% protein i.d.m.	vs % lysine i.d.m.	$r = 0.96^{++} \dots 0.98^{++}$
g protein / m ²	vs g lysine / m ²	$r = 0.98^{++} \dots 0.99^{++}$
kernel yield g/m ²	vs % protein i.d.m.	$r = -0.15^{++} \dots -0.72^{++}$
kernel yield g/m ²	vs g protein /m ²	$r = 0.61^{++} \dots 0.94^{++}$

The relation between kernel yield and protein yield is, according to these results, preferable in selection for protein and lysine. Less pronounced, the same relations are found for thousand kernel weight and protein characters.

Mildew-infection (*Erysiphe graminis*) decreases kernel and protein yield considerably. Lodging, including *Cercospora*-infection, seems to have some negative influence on protein content i.d.m.

Mutants, late in heading and in the degree of maturity at harvest, cause definite losses in kernel and protein yield. Since those kernels are usually immature, the relative amount of protein seems to be higher and therefore also the protein content i.d.m.

Increasing culm and spike length is positively correlated with kernel and protein yield, however shows negative relation to protein content i.d.m.

From all these relations it is obvious that screening for mutants cannot be successful without consideration and inclusion of all interactions from related characters. As far as the results from the last period indicate, selection for improved protein mutants should be carried out also on a yield basis in addition to selection on a dry matter basis. In both cases lysine correlates with protein quite well.

Selection for protein-micromutants is done more easily on a protein and/or lysine yield basis; for protein-macromutants however, the dry matter basis seems to be more useful.

Protein-micromutants at high and constant yield levels occur quite frequently and could be used directly as improved lines. Even if the gain in protein improvement is rather small, these mutants should not be neglected. From protein-macromutants, used as cross-parents, a decrease in protein and lysine content has to be expected in the offspring generations of crosses with high-yielding varieties. Only mutants with supreme lysine and protein values should therefore be used in crosses with high-yielding varieties.

Ergebnisse des Projekts Nr. 2

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Prof. Dr. H. Gaul

Dr. H. Walther

Dipl.-Landwirt W. Friedt

Titel des Projekts:

Utilization of the protein variability of diploidized tetraploid barley

Further investigations on 200 tetraploid barley mutants gave some information on improved protein and lysine content at the tetraploid level. For comparison diploid micro- and macromutants were included in all experiments. All mutants are well-established lines selected from commercial varieties after treatment with EMS and X-rays.

Selection for protein- and lysine-deviating mutants occurred on a dry matter basis as well as on a yield basis. The main results are summarized:

(1) For "% protein i.d.m." and for "% lysine i.d.m." the group mean of tetraploid strains exceeds the group mean of macro- and micromutants considerably. This is true even for the 10 % best lines in each group:

Micromutants: 10.9 % protein i.d.m. 0.42 % lysine i.d.m.

Macromutants: 13.6 % protein i.d.m. 0.52 % lysine i.d.m.

Tetraploids: 16.5 % protein i.d.m. 0.60 % lysine i.d.m.

These means of the 10 % best lines, preselected with the DBC-method, also surpass the mean values of the original mother varieties:

Micromutants: 1.9 % protein i.d.m. 0.08 % lysine i.d.m.

Macromutants: 4.3 % protein i.d.m. 0.15 % lysine i.d.m.

Tetraploids: 2.1 % protein i.d.m. 0.06 % lysine i.d.m.

This positive selection differential indicates some gain of selection in subsequent steps for both protein characters.

(2) The range of variation within the different mutant groups is smallest for the tetraploids and largest for the macromutants. With increasing amounts of protein, the relative content of lysine in the protein decreases. Therefore tetraploids show highest amounts of protein but lowest percent lysine in the protein and therefore lowest biological value of the protein.

(3) If selection for protein and lysine is based on yield (g protein/m^2 , g lysine/m^2), the micromutants show the largest variation in both protein characters and the mean of the group of micromutants is higher than for macromutants. Tetraploids however still surpass both diploid groups. The following mean values for the 10 % best lines were found:

Micromutants:	29 g protein/m ²	1.1 g lysine/m ²
Macromutants:	18 g protein/m ²	0.7 g lysine/m ²
Tetraploids:	44 g protein/m ²	1.6 g lysine/m ²

In macromutants some of the protein increase seems to be achieved at the debit of kernel size and yield. In micromutants and especially in tetraploid strains protein improved lines can be selected at a high yield level.

The primary protein level of the mother varieties has some implications on the derived mutants. Starting with low protein varieties the induced variation seems to be higher than starting with high protein varieties, as with the tetraploid mother varieties. However in each of the mutant groups, a significant range of deviating mutants can be induced by appropriate mutagenic treatments.

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Investigation of selection methods in mutation breeding of barley for protein quantity and quality.

2nd Research Co-ordination Meeting on the Use of Nuclear Techniques for Seed Protein Improvement, Ibadan, Dec. 1973, IAEA, Vienna (in press)

Walther, H., Gaul, H., Ulonska, E., Seibold, K.H.

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, Dec. 1973, IAEA, Vienna (in press)

Walther, H., Friedt, W., Gaul, H.

Variation and selection of protein and lysine in spring barley mutants.

Report, Meeting of the Mutation Breeding Contact Group, Wageningen, Oct. 1974 (in press)

Walther, H.

Variation und Selektion von Protein- und Lysingehalt bei induzierten Sommergerste-Mutanten.

4. Arbeitstagung für Genetik der GSF, Göttingen-Weende, Mai 1974, GSF-Bericht M 199

Lind, V.

Genetische Untersuchungen an mehlttauresistenten Gerstenmutanten.

4. Arbeitstagung für Genetik der GSF, Göttingen-Weende, Mai 1974, GSF-Bericht M 199

Lind, V., Gaul, H.

Investigations of mutagen-induced mildew resistance in spring barley.

Report, Meeting of the Mutation Breeding Contact Group,
Wageningen, Oct. 1974 (in press)

Okamoto, M., Foroughi-Wehr, B.

Uniculate stage of Pollen.

4. Arbeitstagung für Genetik der GSF, Göttingen-Weende,
Mai 1974, GSF-Bericht M 199

Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung mbH, München
8042 Neuherberg, Ingolstädter Landstr. 1

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

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

Allgemeines Thema des Vertrags:

"Strahlenanalyse im Landbau"

Im Berichtszeitraum waren die Arbeiten im wesentlichen auf den Abschluß der eigentlichen Entwicklungsphase konzentriert, so daß im Anschluß daran mit der Prüfung der Meßeinrichtungen und somit den eigentlichen Messungen begonnen werden konnte. Die Apparatur zur Bestimmung der Biomasse an Beständen wurde fertiggestellt; die theoretischen Voraussetzungen haben sich aufgrund von Messungen an einem Getreidephantom als richtig erwiesen und die Messung der Masse an Beständen können somit mit einer Genauigkeit von 1-4% durchgeführt werden. Mit einem Computerprogramm und einer automatischen Steuerung der Anlage werden im weiteren Verlauf der Arbeit Messungen an natürlich gewachsenen Getreidefeldern im Gewächshaus ausgeführt. Mit der Einrichtung zur Simulation des Wasseraustausches zwischen Atmosphäre und Boden mit Hilfe von tritiiertem Wasserdampf sind aufgrund von Verbesserungen an der Apparatur ebenfalls Messungen innerhalb des Bodens vorgenommen worden. Die Bewegung des Wasserdampfes wurde mit und ohne Temperaturgradienten verfolgt, d. h. es konnte auch der Einfluß der Diffusion berücksichtigt werden. Weitere Einflußgrößen werden im Folgenden studiert. Die Entwicklung des Meßverfahrens zur Diagnose von lebenden Bäumen durch Röntgen- und Gammastrahlung wurde durch Einbeziehung der Variation der natürlichen Holzdichte so erweitert, daß das Meßverfahren die wesentlichen Einflußgrößen berücksichtigt.

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- 1) W. Kühn, H. P. Schätzler: "Diurnal Fluctuation of the Mass of a Cabbage Plant". Newsletter on the Application of Nuclear Methods in Biology and Agriculture, 2, 1974
- 2) C. Bunnenberg, W. Kühn: "Movement of Water Vapour in arid Loess Soil under Conditions of Temperature Inversions" Proc. of a Symposium 1973, IAEA, 1974
- 3) W. Kühn, H. P. Schätzler: "Mass Determination on plantations by absorption of gamma-radiation". Atomkernenergie. Bd. 24, 1974.
- 4) H. J. Wiebe, H. P. Schätzler, W. Kühn: "Kontinuierliche Messung der täglichen Schwankung der Masse einer einzelnen Kohlpflanze" Kerntechnik (in print)

Ergebnisse des Projektes Nr. 1

Leiter des Projektes und wissenschaftliche Mitarbeiter:

Dr. W. Kühn, Dipl. Phys. H.P. Schätzler

Titel des Projektes: "Mass determination on Plantations"

Measurements with a prototype were carried out in the laboratory on a phantom of a cornfield consisting of plant equivalent material and of similar shape. The data are recorded by a multichannel analyser working in multiscaling mode. The calculations are performed by data processing. The program evaluates the mass of the field by an Algol-Program on the CDC Cyber 76 computer. The calculation time for the program is about 7 s. The necessary core memory is about 28 000 (decimal). The phantom was constructed to prove the predicted theoretical foundations of mass determination and to optimise the technical construction and the scanning procedure. The single idealized cereal consists of a cylindrical stick out of plexiglass (replacing the culms) on which a thicker one (substituting the spike) of 9 cm length and out of the same material is fixed. The diameter of the "culms" is 3 mm that of the "spikes" 7 mm. By this the natural culm densities of several hundred culms/m² can be reached by the variation of the weight per unit of area. The plexiglass material (polymethylmethacrylate (C₅H₈O₂)_n) presents itself for this purpose as a suitable spare material because of its chemical composition (see Table 1).

Tab. 1: Composition of elements of plexiglass and wheat grains

Element	C	O	H	N	other	μ/g (60keV) theor.
Content in plexiglass	60%	32%	8%	-	-	0.187 cm ² /g
Content in wheat grains	44%	44%	9%	2%	1%	0.187 cm ² /g

Five measurement have been carried out with the phantom. In the first two experiments a field of about (35x200) cm² was built with equal culm- and spikelength but with different culm densities. In the third and the fourth experiment the culmlength were chosen to be normal distributed with different mean values and different standard deviations whereas the spikes had the same length. The culms were arbitrarily distributed in the field of (200x35) cm². In the last experiment the spike length was normal distributed too. In all five experiments the results are in very good agreement with the expected values (Table 2). The computer program contains also a program for least-squares fitting by which a non-linear regression for the mass distribution is resolved assuming a normal distributed culm- and spikelength. By this method the

mass of the culms, and of the spikes as well as the parameters of the Gaussian distribution can be calculated separately. The results show very good accordance to the expected values.

Table 2 Comparison of the measured and expected values of five different field arrangements.

TABLE 2

Experiment Nr.	M_{TE} [g]	M_{TM} [g]	M_{CE} [g]	M_{CM} [g]	M_{SE} [g]	M_{SM} [g]	μ_{CE} [cm]	μ_{CM} [cm]	μ_{SE} [cm]	μ_{SM} [cm]	σ_{CE} [cm]	σ_{CM} [cm]	σ_{SE} [cm]	σ_{SM} [cm]	Error of M_{TM} [%]	Deviation from M_{TE} [%]
1	6086.0	6025.4	2115.4	2148.6	3970.6	3836.9	26.75	27.6	9.05	8.62	0	0.38	0	0.2	+ 0.2	- 0.7
2	4304.7	4172.9	1496.3	1514.2	2808.5	2693.6	26.75	27.34	9.05	8.88	0	0.38	0	0.15	+ 0.2	- 3.06
3	4039.4	3976.8	1100.4	1086.4	2939.0	2923.6	18.8	19.0	9.05	9.54	4	3.76	0	0.7	+ 0.2	- 1.55
4	3869.7	3743.1	926.5	910.2	2943.2	2849.2	15.8	15.9	9.05	8.86	6.0	5.88	0	0	+ 0.2	- 3.27
5	3212.6	3339.9	926.5	980.0	2286.1	2388.1	15.8	16.2	7.0	6.99	6.0	5.72	2.0	3.6	+ 0.2	+ 3.96

- M_T : total mass
- M_C : mass of culms
- M_S : mass of spikes
- μ_C : mean length of culms
- μ_S : mean length of spikes
- σ_C : standard deviation of the distribution of the culms
- σ_S : standard deviation of the distribution of the spikes
- E: Expected value (by whighting)
- M: Measured Value.

Ergebnisse des Projektes Nr. 2

Leiter des Projekts und wissenschaftliche Mitarbeiter:
Dr. J. Handl, Dr. W. Kühn, Dipl. Phys. H. P. Schätzler

Titel des Projekts: "Untersuchungen zur Diagnose und Therapie
von Holzkrankheiten in lebenden Bäumen"

In 1974 investigations about the diagnosis of dry rot in living trees by attenuation of gamma-radiation are continued. Especially the influence of the biological variation of the density in trees are considered, because this is an important factor at the practical application. Systematic measurements of the density on spruce has been carried out on different positions in several trees. On the base of these natural variations calibration-curves are established by absorption of 60 KeV gamma-radiation with ^{241}Am and the results are given for spruce in Fig. 1-4. The most important influence factors are involved in Fig. 4 and the following examples for two trees show the application of the method:

1. Measured Intensity : 2000 cps (tree 3¹)
Weight per unit area : 18,6 g/cm³ (calibration curve)
At a diameter of : 28 cm one gets
a density of : 0,664 g/cm³.

This value lies within the 2 x statistical deviation of the mean density (0,698 ± 0,112 g/cm³); that is to say that the tree is healthy with a probability of 95,5%.

2. Measured Intensity : 1100 cps (tree 1¹)
Weight per unit area : 21,6 g/cm³ (calibration curve)
At a diameter of : 36,8 cm one gets
a density of : 0,585 g/cm³.

This value lies outside the 2x statistical deviation of the mean density (0,698 ± 0,112 g/cm³); that is to say that the tree is healthy only with a probability of 4,5% and this means dry rot with 95,5% probability. In comparison with example 1 (tree 3¹) has tree 1¹ a whole of 6,2 cm diameter; 18% of the diameter is reduced by disease.

The measurements were performed in cooperation with the Institut für Weltforstwirtschaft der Bundesanstalt für Forst- und Holzwirtschaft.

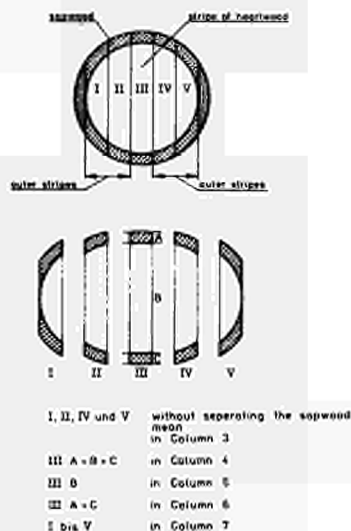


Fig. 1
Position of the density measurements
(by weight)

disk (healthy)	Ø		mean density outer stripes g/cm ³	mean density heartwood g/cm ³	mean density heartwood g/cm ³	mean density sapwood g/cm ³	mean density disk g/cm ³
	min.	max. cm					
1	23.5	25.5	0.667	0.549	0.474	0.972	0.632
2	21.0	22.5	0.609	0.581	0.432	0.944	0.601
3	31.5	33.5	0.760	0.722	0.454	1.030	0.751
4	33.0	35.0	0.728	0.720	0.581	0.956	0.725
5	20.5	22.5	0.735	0.801	0.578	1.000	0.750
6	17.5	18.0	0.711	0.642	0.483	1.018	0.685
7	12.5	13.5	0.668	0.683	0.521	0.963	0.642
8	12.0	12.8	0.696	0.673	0.492	0.939	0.649
9	16.0	16.2	0.779	0.720	0.466	1.019	0.765
10	26.0	26.2	0.703	0.671	0.514	1.017	0.692
11	27.5	29.5	0.678	0.675	0.529	1.000	0.677

disk (rotten)	Ø		mean density outer stripes g/cm ³	mean density heartwood g/cm ³	mean density heartwood rotting g/cm ³	mean density sapwood rotting g/cm ³	mean density disk g/cm ³
	min.	max. cm					
1'	25.2	28.6	0.716	0.565	hohl	0.926	0.717
2'	23.0	21.3	0.727	0.565	0.454	0.916	0.692
3'	27.3	30.2	0.743	0.700	0.487	0.881	0.702

Fresh densities of spruce disks

Fig. 2
Results of the density measurements
(by weight)

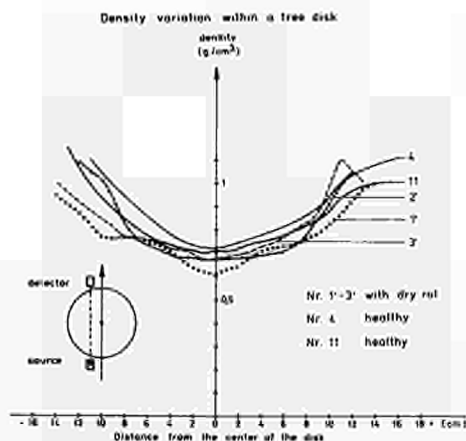


Fig. 3
Diagnose by absorption of 60 KeV
γ - radiation across the diameter

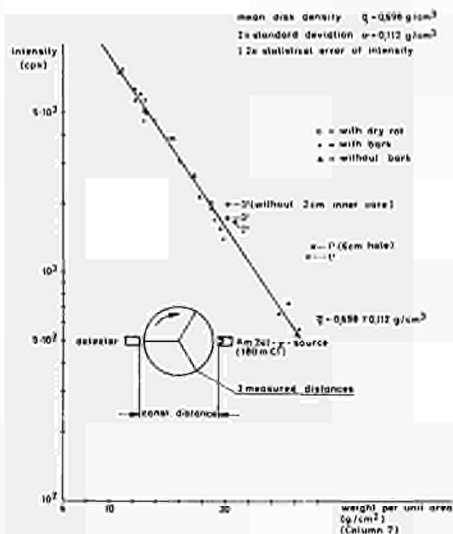


Fig. 4
Diagnose by absorption of 60 KeV
γ - radiation on 3 positions.

Ergebnisse des Projekts Nr. 3

Leiter des Projekts und wissenschaftliche Mitarbeiter:

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

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

With the experimental set-up constructed in 1973 mechanisms of moisture exchange between atmosphere and the upper soil layers and the movement of moisture into deeper soil layers were studied in 1974. The soil column was filled with loess soil of 9,5% moisture content from the Negev Desert (Israel). The atmosphere above the soil surface had a high of 20 cm. According to the temperature curves measured in the Negev at different soil levels the bottom of the column was held at constant temperature (23°C), while the soil surface was heated and cooled in a day-night cycle (41→16°C) by a preset program. The atmosphere was labelled with tritiated vapor for two day-night cycles. The vapor was collected in slotted tubes placed horizontally at different depths of the soil column. After closing the slots it was transported into a flow-type proportional counter by a stream of nitrogen gas. The counter provides high sensitivity, small measuring errors and the possibility of a direct and continuous measurement. A low background is guaranteed by using two coaxial counting tubes and an anticoincidence unit to eliminate pulses from higher energetic radiation. The labelled vapor above the soil column penetrates into the soil due to a vapor pressure gradient directed downwards during the day and moves upwards again during the night phase, when the gradient is inverted. According to the modeled gradients the downward movement has been found stronger than the upward measurement. This results in an accumulation of moisture at a layer of constant temperature. Indications of this accumulation have indeed been detected in the Negev at a depth of about 50 cm below surface. The detection of labelled vapor at a certain depth of the soil column does not necessarily prove a net transport of moisture from the atmosphere down to deeper soil layers as diffusion may play an important part. In order to be able to estimate the contribution of pure diffusion and to obtain more basic relations between net moisture transport and effective forces, further measurements were carried out. In these experiments vapor movement within the soil was investigated using medium sand of 3% moisture content, labelling the middle 10-cm layer of the column and collecting the moisture moving upwards and downwards from the labelled layer. In one case a constant temperature of 35°C was maintained throughout the entire soil column to study diffusion, while in the second experiment the top was heated to 60°C and

the bottom was cooled to 10°C producing a temperature gradient of $1\frac{0^{\circ}\text{C}}{\text{cm}}$ directed downwards. The temperature gradient accelerates the movement considerably. The experiments show that it is more promising to study the mechanisms of vapor transport, when modeling simple conditions displaying the influence of only one parameter. This way the simulation and evaluation of natural conditions can be approached step by step with the developped equipment.

Commission's associate: University of Modena

Contract No.: SC/11-I094-72-1 BIAN

Head of the research group: Ernesto Carafoli

General theme of the contract: Transport of calcium and strontium
in chloroplasts and plant mitochondria

The research has been carried out by Dr. E. Carafoli, Dr. F. Crovetti, Mr. F. Dalgai.

Spinach chloroplasts have been extracted with hypotonic media, and with chaotropic agents (lithium bromide or lithium di-iodosalicylate), or have been fragmented with ultrasonic oscillation. In the supernatants, a protein with a pronounced acid character has been identified by analytical polyacrylamide gel electrophoresis. This protein has been then isolated by preparative polyacrylamide gel electrophoresis, and is currently been studied with respect to its relation to a similar protein isolated from animal mitochondria.

The interaction of a Ca^{2+} -binding glycoprotein isolated from liver mitochondria with Sr^{2+} , and with the inhibitor of the transport of Ca^{2+} in mitochondria, La^{3+} , has been studied. The glycoprotein binds impressive amounts of lanthanides, at the sites where Ca^{2+} (or Sr^{2+}) are normally bound. The interactions of another important inhibitor of mitochondrial Ca^{2+} (and Sr^{2+}) transport, ruthenium red, with mitochondria and with the mitochondrial Ca^{2+} (and Sr^{2+})-binding glycoprotein are under study.

Contractor: Danish Atomic Energy Commission

Contract number: 138-74-1 BIO DK

Head of research team: Jens Sandfær

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

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

Project No.: 1

Research team: Hans Doll, Arna J. Andersen and Bertel Køie.

Title: Genetics and agronomic properties of
high lysine barley mutants.

A repeat in 1974 of a field test of our high lysine mutants confirmed that the 12 mutants have from about 10 to 40% more lysine in the protein than the parent varieties. Further the mutants are characterized by a reduced grain yield, which is mainly the result of a reduced seed size. Only two mutants, nos. 7 and 56, appear to have normal, well-filled seeds.

Studies of the offspring from crosses between mutants and a normal lysine variety showed that mutants nos. 7, 8, 13, 16 and 527 each have a single recessive gene giving the increased lysine content of the protein. Mutant no. 9 has a recessive gene which causes too early seed maturation and consequently a small increase in the lysine content of the protein.

Mutant 1508 is so far the most promising one in our collection. It has a 40% lysine increase in the protein, which is due to the effect of a single gene mutation. A comparison of high and normal lysine lines from the offspring of the cross mutant 1508 x Sultan showed that the mutant 1508 gene causes an about 15% reduction in seed size and grain yield beside the lysine increase. The gene has no significant influence on the total protein production, or on the number of seeds per unit area.

The influence of nitrogen fertilizer on mutant 1508 and its parent variety, Bomi, was studied in outdoor pot experiments. Both the protein content of the seeds and the total protein production increased with increasing supply of nitrogen, but in normal barley this coincided with reduced lysine content of the protein. The lysine decrease was partly due to dominant synthesis of the lysine-poor hordeins, and partly to

decreasing lysine concentration in all the protein fractions. In mutant 1508 the relative amounts of the different protein fractions, as well as their lysine concentrations, were only little affected by the amount of nitrogen fertilizer. Hence this mutant maintains its high lysine content at high nitrogen supply.

Project No.: 2

Research team: Bertel Køie, Hans Doll and Bo Büchmann.

Title: Nutritional value of barley seed protein.

The nutritive quality of the protein in mutant 1508 and in the parent variety Bomi was investigated in feeding tests with rats by the National Institute of Animal Science, Copenhagen. The protein in mutant 1508 had the same digestibility, about 85%, as the protein in Bomi, while the biological value of the mutant protein was increased from about 80 to more than 90%. These studies are now extended to feeding tests with pigs and poultry.

Efforts are being made to develop an in vitro method for determination of the protein digestibility of small barley samples. A ground sample is treated with proteolytic enzymes for 18 hours at pH 7.6 and 43°C. The digestibility is expressed by the amount of dissolved nitrogen after treatment with trichloro acetic acid and centrifugation. The method, which has a good reproducibility, is evaluated by comparing the values obtained in vitro with the results from rat feeding experiments.

Project No.: 3

Research team: Bertel Køie, John Ingversen and Anders Brandt.

Title: Synthesis and composition of endosperm
 storage proteins.

Compared to Bomi, three to four times larger amounts of free amino acids were present in mutant no. 1508 endosperm during the period of intensive seed development. Analysis of the composition of the free amino acids revealed the same relative amount of lysine, a substantial reduction in the relative amount of glutamic acid, and a significant increase in the relative amount of proline in the mutant as compared to Bomi. ^{14}C was incorporated into all endosperm proteins following ^{14}C -lysine injection in the top internode. Supply of lysine at an early stage of endosperm development resulted chiefly in labelling of the albumin fraction followed by globulin and glutelin, whereas at a later stage of endosperm development most label was found in the globulin and glutelin fractions. At this later stage of endosperm development mutant 1508 did not incorporate glutamic acid and proline derived from labelled lysine into the hordein fraction. The Bomi hordein, on the other hand, contained considerable amounts of glutamic acid and proline derived from lysine conversion. That the conversion of lysine to glutamic acid and proline is not generally blocked in the mutant is revealed by the presence of labelled glutamic acid and proline in the glutelin and the residual protein, as is the case in Bomi.

In mature seeds the proteins in the lysine-poor hordein fraction can be divided into three groups, A, B, and C, with decreasing lysine content and different chemical properties. The A hordeins are present in the embryo and the endosperm, while the B and C hordeins are found in protein bodies in the endosperm. Mutant 1508 has very reduced amounts of B and C hordeins, and mutant 56 lacks most of the B hordeins. In normal barley the B hordeins may constitute as much as 1/3 of the glu-

telins, while the remaining glutelins have approximately the same amino acid composition as the albumins and globulins.

Genetic studies of the hordein composition showed that the Bomi and Sultan varieties have different co-dominant alleles at a locus controlling the electrophoretic pattern of the B hordeins. This locus is hypostatic to the recessive gene in mutant 1508, which suppresses the formation of B and C hordeins.

Project No.: 4

Research team: John Ingversen, Diter von Wettstein and
Lars Munck.

Title: Barley endosperm ultrastructure.

Electron microscopy shows that in the endosperm cells the storage proteins, after their synthesis on the endoplasmic reticulum, are deposited in the vacuoles and then concentrated into protein bodies. The formation and morphology of the protein bodies in mutant 1508 are strikingly different compared to the parent variety Bomi.

A procedure was developed to isolate pure preparations of protein bodies from Bomi and mutant 1508. The isolation is based on isopycnic gradient centrifugation of a cell homogenate from immature seeds. The Bomi protein bodies are sedimented at a sucrose density of 1.25 g/ml and the mutant protein bodies at 1.21 g/ml. No sediment was found at 1.25 g/ml in the mutant preparation. Electrophoresis of proteins extracted from Bomi protein bodies showed the band pattern characteristic of the hordein protein fraction, as defined by solubility in 55% isopropanol. These bands were only poorly represented in extracts from mutant 1508 protein bodies, which, however, contained several bands not present in Bomi protein bodies. The alterations in morphology of the protein bodies can thus be related to differences in the composition of the protein bodies.

Publications:

- Andersen, A.J.: Byg med forbedret proteinkvalitet.- "Alt det nyeste 1975", Det kgl. Danske Landhusholdningsselskab, København, 21: 18-23, 1974.
- Andersen, A.J. and B. Køie: Response of a high-lysine and a normal barley genotype to nitrogen fertilization.- (submitted for publication in Agronomy Journal).
- Doll, H.: Forbedring af proteinkvaliteten i byg.- Beretning om AEK's virksomhed 1973/1974: 115-125.
- Doll, H. and B. Køie: Evaluation of high lysine barley mutants.- Proceedings of the FAO/IAEA/GSF meeting on the Use of Nuclear Techniques for Seed Protein Improvement, Ibadan, Nigeria, 10-14 December 1973 (in press).
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- Ingversen, J.: Forbedring af byggenes proteinkvalitet.- Naturens Verden 11/12: 354-358, 1974.
- Køie, B., H. Doll, J. Ingversen and A. Brandt: High-lysine barley mutants.- Barley Newsletter 17: 43-44, 1974.
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- Oram, R.N., H. Doll and B. Køie: Genetics of two storage protein variants in barley.- Hereditas (in press).

Contractor: Danish Atomic Energy Commission

Contract number: 139-74-1 BIO DK

Head of research team: Jens Sandfær

General subject of contract: INDUCED MUTATIONS FOR POWDERY
MILDEW RESISTANCE IN BARLEY

The general aim of the research project is to elucidate the potential of experimental mutagenesis to provide genes conferring resistance to the barley powdery mildew fungus (Erysiphe graminis f. sp. hordei) of value for disease resistance breeding of barley (Hordeum vulgare L.), and to gain knowledge about the nature of the induced mutant genes.

Four independently induced resistant mutants selected at Risø, six induced mutants from other sources, and some spontaneously arisen resistant barleys are being studied with respect to their genetics, resistance characteristics, and agronomic properties. Additional mutants are incorporated in the study from time to time as they appear.

Results of project 139-74-1 BIO DK

Research team: J. Helms Jørgensen, H. P. Jensen.

1. Genetics of the mutants. Ten independently induced mutant genes and one gene of spontaneous origin are non-complementing alleles in a locus designated ml-o, which is located on the long arm of chromosome 4. Some of the ml-o alleles apparently have mutations in different sites within this locus.

A more precise localization of the ml-o locus is under way. F₂ populations involving two powdery mildew resistance genes and five marker genes were grown in 1974 and classified for morphological adult-plant characters; the seedling characters including powdery mildew reaction will be classified on F₃ seedlings early in 1975. With the aim of studying the recombination frequency between six selected ml-o alleles and - if possible - to map the different mutational sites within the ml-o locus, crosses have been made between barley lines each carrying a selected ml-o allele and genetic markers. The genetic markers serve to identify accidental selfings in the crosses and contaminants from admixture or outcrossing.

2. Resistance characteristics of the mutants. Additional data in 1974 confirmed the world wide range of powdery mildew resistance conferred by the ml-o genes. Seedling tests with isolates of the leaf rust and the yellow rust fungus showed that the ten mutants reacted the same as the respective six mother varieties. This indicates that the ml-o genes being very effective against powdery mildew have no effect against other barley pathogens.

A histological study of the infection process for the powdery mildew fungus on the barley mutants and their mother varieties showed that the conidia of the fungus germinated equally well on the mutants and on the mother varieties. However, only 0.5 per cent of the conidia were able to penetrate the mutant leaf surface, and only about 0.25 per cent were able to establish infections, which were only initiated in the subsidiary cells at the stomata. On the mother varieties about 50 per cent of the conidia were able to establish infections, which were initiated in the epidermis cells as well as in the subsidiary cells. Mutual differences were not found between the ten mutants or between the six mother varieties.

3. Agronomic properties of the mutants. The agronomic performance of the resistant mutants and of their mother varieties has been evaluated in previous years in field trials under disease-free and diseased conditions. A necrotic/chlorotic leaf flecking and a reduced grain yield appear to be pleiotropic effects of the ml-o resistance genes; these effects are, however, modified by the background-genotype (the variety).

From 1698 resistant lines derived from crosses, 27 lines with apparently slightly less, and 7 lines with apparently less leaf spotting than the original mutants were selected in 1974. Further, an experiment was initiated in 1974 which utilizes completely homozygous barley lines derived from monploids made on F_1 plants. The experiment aims at elucidating the specific pleiotropic effects of three selected ml-o genes, and the possible influence of specific modifier genes and of the general genetic background.

4. Additional mutants. A number of additional powdery mildew resistant barley lines selected from mutagen-treated material by other research workers was examined in 1974. Some lines resembled their mother varieties in a series of marker genes and in morphology and are thus considered induced mutants. Other lines, however, deviated distinctly from the mother varieties; they are, therefore, considered to be contaminants from other resistant barley material.

Publications in 1974.

- Jørgensen, J. Helms, 1974: Induced mutations for powdery mildew resistance in barley: A progress report. (Abstract). - In: "Induced mutations for disease resistance in crop plants". IAEA, Vienna: 67.
- Jørgensen, J. Helms, 1974: On the mutational and independent origin of mutants induced by seed treatments of selfpollinated plants. - In: "Induced mutations for disease resistance in crop plants". IAEA, Vienna: 57-66.
- Jørgensen, J. Helms, 1974: Location of the ml-o locus on barley chromosome 4. - Barley Genetics Newsletter 4: 43-44.
- Jørgensen, J. Helms, 1974: Inducerede mutanter for resistens mod meldug i byg: Resistens, genetik og udbytte. (Abstract). - Nordisk Jordbrugsforskning 56: 28-29.

Contractor : The Agricultural Institute
Moorepark
Fermoy
Co. Cork / IRELAND

Contract No : 141-74-1 BIOEIR

Head of research team(s) :
F. O'CONNOR

General subject of contract :

The study of the possibility of using radiometric detection of bacteria in milk as a rapid grading index.

- a) We have sought information on a range of liquid scintillation counters, have looked at some models and could make a decision on the model to purchase at short notice.
- b) We are currently running a preliminary trial in conjunction with the Biochemistry Dept. at University College, Cork to check if the method proposed by Buddemeyer (1974), *Appl. Microbiol.*, 28, 177 would be satisfactory for our use. There is no reason to believe that it would not suffice.
- c) We have organised technician help and space to allocate to the project when the funding becomes available.

ZELLKULTUREN

CELL CULTURE

CULTURE DE CELLULES

Commission Associate: Istituto di Genetica della Università, Pisa

Contract Number: 106-72-1 BIOI

Head of research team: Prof. F. D'Amato

Co-workers: Drs. S. Baroncelli, A. Bennici, M. Buiatti, P.G. Cionini, M. Durante, L. Giorgi

Subject of the Contract: Cytology and genetics of plant tissues and cells grown in vitro.

Report on activities 1974

During 1974, the following investigations have been carried out:

1) Genetics of growth and differentiation in plant tissue cultures

Work on the genetic control of differentiation and dedifferentiation in plant tissue cultures was extended to the study of genotype-hormone interactions in cauliflower and to an analysis of differences between ditelocentric lines in hexaploid wheat.

As far as the first problem is concerned, plasticity in the response of six highly inbred lines of cauliflower to varying hormone balances was tested in terms of callus growth, bud and root differentiation in vitro. The results showed significant differences in stability for the tested characters among the six lines. The general conclusion was drawn that the genotype controls in a rather stable way the type of organ differentiation whose amount can be influenced by the environment (hormones in our case). These data, along with earlier ones (see report 1973), suggested a possible use of tissue cultures as a tool for early screening in selection experiments.

With the aim of performing a more refined analysis of the phenomena under study, hexaploid wheat was chosen as a further test material. Particularly, 26 ditelocentric lines of the variety "Chinese spring" were tested in vitro under a constant hormonal level. Striking differences in callus growth were found, suggesting the possibility of localizing in chromosomes genes controlling cell proliferation. Indications were obtained of a possible correlation between callus growth and ribosomal multiplicity of the tested lines. (Baroncelli, Bennici, Buiatti, Sassoli).

2) Cell dedifferentiation in plant tissues grown "in vitro"

Biochemical analyses on the differences between tissue cultures of a tumorous and a non-tumorous strain of the

hybrid Nicotiana glauca x N.langsdorffii were continued. Amplification, revealed by the appearance of a heavy DNA satellite, was found to be limited to the early phases of dedifferentiation of the non-tumorous stock. This fact was confirmed by a selective inhibition of this stock by a BUdR treatment given in the period of DNA amplification. The tumorous strain, on the contrary, was not inhibited by the analogue and seemed to give rise to callus formation without the need of DNA amplification (Buiatti, Durante, Geri, Giorfi, Parenti).

3) Molecular biology of the embryo suspensor of Phaseolus coccineus.

In work on the cytological localization of genes in the polytene chromosomes of Phaseolus coccineus, tritiated 5S and 5.8S RNA was isolated and purified from hypocotyls of Phaseolus coccineus fed with ^3H -uridine. The RNAs, which could be labeled to a high specific activity, was hybridized with DNA of polytene chromosomes of P.coccineus in competition with cold 25S and 18S RNAs to eliminate the risk of erroneous gene localization due to fragments of radioactive 25S and 18S RNAs (Avanzi, Cionini, Cremonini, Durante, D'Amato).

DNA was extracted from roots, shoots, integuments and suspensors (containing polytene chromosome cells). The main DNA has a buoyant density of 1.692 g/ml and in the four tissue there is a DNA satellite with buoyant density 1.700 g/ml. A second satellite with buoyant density of 1.696 g/ml is found in the suspensor only. In the polytene chromosomes the genes for ribosomal RNA are under-replicated (Avanzi, D'Amato, Durante in collaboration with Prof. A. Lima-de-Faria's group, Lund - Sweden).

Publications

- 1) Baroncelli S., Buiatti M., Bennici A. and Pagliai M. (1974). Genetics of growth and differentiation in vitro of Brassica oleracea var. botrytis III. Genetic correlations and ontogenetic unity. Z. Pflanzenzüchtg. 72, 275-282.
- 2) Baroncelli S., Buiatti M., Tesi R. (1974). L'uso delle colture in vitro per la selezione precoce del cavolfiore. Genet. Agraria 28, 170-176.
- 3) Bennici A. (1974). Cytological analysis of roots, shoots and plants regenerated from suspension cultures of haploid Pelargonium. Z. Pflanzenzüchtg. 72, 199-205.

- 4) Bennici A., Baroncelli S., Buiatti M., Pagliai M., Rosellini D. and Sassoli O. (1974). Analisi genetica in Brassica oleracea var. botrytis coltivata in vitro. Atti A.G.I. 9, 53-55.
- 5) Buiatti M., Baroncelli S. and Bennici A. (1975). Genetics of growth and differentiation in vitro of Brassica oleracea var. botrytis. IV. Genotype-hormone interactions. Z. Pflanzenzüchtg. (in the press).
- 6) Buiatti M., Baroncelli S., Bennici A., Pagliai M. and Tesi R. (1974). Genetics of growth and differentiation in vitro of Brassica oleracea var. botrytis. II. An in vitro and in vivo analysis of a diallelic cross. Z. Pflanzenzüchtg. 72, 269-274.
- 7) Buiatti M., Durante M., Geri C., Giorgi L., Martini G., Nuti Ronchi V. and Parenti R. Effetto del BUdR sul DNA satellite di espianti primari di Nicotiana glauca. Atti A.G.I. (in the press).
- 8) Buiatti M., Durante M., Giorgi L., Martini G., Nuti Ronchi V., Parenti R., Grisvard J. and Guillé E. (1974). Dati citologici e biochimici preliminari su colture di tessuto dell'ibrido tumorale Nicotiana glauca x N. langsdorffii e un suo mutante non tumorale. Atti A.G.I. 19, 56-59.
- 9) Cionini P.G. Pattern of binding of tritiated actinomycin D to Phaseolus coccineus polytene chromosomes. II. The entire chromosome complement. Giorn. Bot. Ital. (in the press).
- 10) Durante M., Giorgi L., Parenti R., Buiatti M., Guillé E. and Grisvard J. (1974). Comparsa di un DNA satellite durante la sdifferenziazione di midollo di Nicotiana glauca. Atti A.G.I. 19, 60-62.
- 11) Lima-de-Faria A., Avanzi S., Pero R., Durante M., Ståle U., D'Amato F., Granström H. Relation between ribosomal RNA genes and the DNA satellites in Phaseolus coccineus. Hereditas (in the press).

Vertragspartner der Kommission : Prof. Dr. J. Reinert
Inst. f. Pflanzenphysiologie
u. Zellbiologie, F.U.B.

Nr. des Vertrags : 117-72-1 BIO D

Leiter der Forschungsgruppe(n) : Prof. Dr. J. Reinert

Allgemeines Thema des Vertrags : "Growth and differentiation
of cells and plant tissues
growing in vitro."

During 1974 investigations were conducted on the following aspects:

1. A) Factors influencing the enhancement of haploid tobacco (Nicotiana tabacum cv. Badischer-Burley) plants by anther culture.
- B) Induction of haploid tobacco plants by the culture of isolated pollen.
- C) DNA contents with relation to genetic stability in haploid and diploid cell suspension cultures.
2. A) Isolation, culture and induction of embryogenesis in protoplasts from cell suspensions of carrot (Daucus carota cv. Rote Riesen).
- B) Isolation of protoplasts from pollen tetrad and pollen mother cells of tomato (Lycopersicon peruvianum)
- C) Protoplast fusion and somatic hybridization studies on carrot, tobacco and tomato.

Ergebnisse des Projekts Nr. 1..

Leiter des Projekts und wissenschaftliche Mitarbeiter :

Prof. Dr. J. Reinert, Prof. Dr. H. J. Küster, Dr. Y. P. S. Bajaj, u. E.

Titel des Projekts : "Production of haploid embryos

Heberle

from anthers and cell cultures growing in vitro.

A) Factors influencing the enhancement of haploid tobacco (Nicotiana tabacum cv. Badischer-Burley) plants by anther culture.

In view of the tremendous importance of haploids in plant breeding, it is highly desirable to have a dependable and high efficiency of their production. By studying the effect of various exogenous factors we have successfully increased the efficiency of tobacco anthers undergoing androgenesis as well as the number of plants per anther. Thermal shocks (3°C for 48 hrs) given to the plants encouraged equal division in the microspore nucleus, which eventually increased the production of embryos by about 10%. The anthers taken from flower-buds already sprayed for 3 days with 200 mg/l of ethrel (2-chloroethylphosphonic acid) showed a significant increase in androgenesis. By adding charcoal (0.5, 1 and 2%) to the medium not only there was a tremendous enhancement (control 40 vs charcoal 90%) in the androgenic anthers, but also in the number of plants per anther. By combining the effect of these factors haploid plants were induced from 30% up to 93% of the anthers.

B) Induction of haploid tobacco plants by the culture of isolated tobacco pollen.

The technique of anther culture has proved quite useful for the induction of haploids, however, it suffers from one disadvantage. The plants may not originate from the pollen only but also from various other parts of the anther, with the result a mixed population of plants with various levels of ploidy is obtained. This difficulty can be surmounted by the culture of isolated pollen. In this connection, tobacco pollen obtained from cold-treated anthers (4°C for 72 hrs) cultured for 4 days, have been induced to undergo androgenesis on a synthetic medium enriched with amino acids. This method ensures the production of haploid plants only, and would be an ideal system for the study of direct effects of various mutagens, both chemicals and irradiations.

C) DNA contents with relation to genetic stability in haploid and diploid cell suspension cultures

The maintainance of the genetic stability, especially of the haploid cells in cultures is perhaps as important as the induction of haploidy itself. Tobacco cells (both haploid and diploid) were grown in suspension cultures for various lengths of time, and their DNA contents measured at weekly intervals using micro-spectrophotometric techniques. The haploid cultures appeared to maintain their DNA level for about 4 wks. The effect of stabilizing substances like para-fluorophenylalanine is being studied in order to enhance, and maintain the genetic stability.

Ergebnisse des Projekts Nr. 2..

Leiter des Projekts und wissenschaftliche Mitarbeiter :
Prof.Dr.J.Reinert; G.Gosch; Dr.Y.P.S.Bajaj; A. Gröbler

Titel des Projekts : "Production and fusion of protoplasts from cells growing in vivo and in vitro."

A) Isolation, culture and induction of embryogenesis in protoplasts from cell suspensions of carrot (*Daucus carota*) cv. Rote Riesen).

Protoplasts obtained from cell suspensions of an anthocyanin synthesizing strain of carrot have been induced to divide repeatedly and undergo embryogenesis. An optimal protoplast yield of 80-90% was obtained by treating the cells with 1.5% cellulase (pH 5.0) for 4 hours in a gently shaking water-bath maintained at 33°C. These protoplasts when plated in the agar-solidified modified Murashige and Skoog's medium regenerated cell walls within 3 days, and first division was observed in another 3-4 days. Some protoplasts in addition showed budding, and the formation of sub-protoplasts. The protoplasts continued to divide repeatedly to form cell clumps and colonies, and masses of callus in 4 wks. The calli eventually underwent embryogenesis in 6 wks.

A) Isolation of protoplasts from pollen tetrad and pollen mother cells of tomato (*Lycopersicum peruvianum*).

Techniques have been refined for the isolation of protoplasts from pollen tetrad and pollen mother cells of *Lycopersicum peruvianum*. Nearly 100% of the protoplasts were released from the confines of massive callose walls by treating the tetrad with 0.75-1% enzyme heli-case (in 8% sucrose solution at pH 5.5) at 23-25°C. The protoplasts were released within 30 minutes, and were spherical, highly cytoplasmic with a prominent nucleus. Culture of these protoplasts would be a novel approach for the induction of haploids, and also their fusion for somatic hybridization as it would be close to the conditions of natural fertilization.

C) Protoplast fusion and somatic hybridization studies on carrot, tomato and tobacco.

By combining the polyethylene glycol and high pH techniques, an intergeneric fusion between carrot callus protoplasts and mesophyll

protoplasts from tobacco leaves has been achieved. Aggregates of protoplasts adhered together and at the point of adherence the plasmalemma seemed to dissolve to form dumb-bell shaped bodies, or the contents of one merged with that of the other and the fused protoplasts assumed a spherical form. Taking into consideration the green colour of tobacco and red colour of carrot as the visual marker the fused products could be identified and isolated. Conditions for the culture of these fused protoplasts are being worked out.

Publications

1. Bajaj, Y.P.S.; J. Reinert and E. Heberle, 1975: Induction of haploid tobacco plants from isolated pollen. *Protoplasma* 1975, in press.
2. Gosch, G.; Y.P.S. Bajaj and J. Reinert, 1975: Isolation, culture and fusion studies on protoplasts from different species. *Protoplasma* 1975, in press.
3. Reinert, J., Y.P.S. Bajaj and E. Heberle, 1975: Some factors enhancing the in vitro production of haploid plants. In: G. Morel commemoration volume (ed. R.J. Gautheret), in preparation.
4. Linstedt and J. Reinert, 1975: Occurrence and properties of a cytokinin in tissue cultures of *Daucus Carota*. In preparation.

Associato della Commissione: Comitato Nazionale
per l'Energia Nucleare, Laboratorio Applicazioni Agricoltura
N° del contratto : Euratom-Cnen 107-72-1 B101

Capo del Gruppo di ricerca : Prof. A.Bozzini

Tema generale del contratto: Applications of the in
vitro cultures to radiobiological researches and
mutagenesis of higher plants

The researches carried out in 1974 were
principally oriented in three directions:

- 1) The use of in vitro culture techniques
in relation to self-incompatibility problems.
- 2) The research at the macromolecular level
of the modifications induced by in vitro culture
in different plant tissues.
- 3) Genetical studies on isogenic lines of
tobacco derived from anther cultures and muta-
genic treatments of the microspores from a di-
ploid isogenic line.

Risultati del progetto n. 1

Capo del progetto e collaboratori scientifici:

M.Devreux, U.Laneri, K.S.Ramulu, P.De Martinis*, E.Magnien*

Titolo del progetto: The use of in vitro culture techniques
in relation to self-incompatibility problems.

1.1. Anther cultures of self-incompatible species were performed during all through this year in order to try for the induction of haploid plants. We worked with Lycopersicum peruvianum as well as with the interspecific hybrids between L.esculentum x L. peruvianum. We also tried anther cultures in Oenothera organensis.

After cold pre-treatment by anther cultures, embryoids were obtained in the interspecific hybrids and also some new plantlets in L.peruvianum.

One interesting finding came out during the analysis of the ploidy level of the L.peruvianum plants regenerated from anther and internode cultures: an analysis of the size of the stomata in leaf epidermis and of the pollen grain size, representing respectively the ploidy levels of the first and the second ontogenetic layers (L_1 and the L_2 layers) showed, in some plants, the occurrence of cytochimeric situation ($4n-2n$ or $2n-4n$). Based on this observations, we investigated critically the ploidy pattern of the three ontogenetic layers L_1 , L_2 and L_3 , analysing also the chromosome numbers in pollen mother cells for the L_2 and the chromosome counts in root meristems from the cutting for the L_3 . The results showed that, the distributional pattern of chimerism varied in different branches of the same plant. Further, it was noted

* At no cost for the contract.

that, in some plants, chimerism was lost in the upper branches. Since the plants regenerated from the in vitro grown calluses were believed to derive generally from single cells, the occurrence of cytochimerism in some plants of our materials demonstrated that the plants of L.peruvianum regenerated in vitro from relatively old calluses may also derive from more than one single cell. Cytophotometric measurements performed on calluses of different ages confirmed the occurrence of a large variability in the DNA content per nucleus in these materials.

1.2. The genetical analyses carried out this year in order to find out the origin of the diploid and tetraploid plants coming from anther cultures indicated that, the majority of the plants does not seem to originate from microspore. We thought it desirable to try to eliminate, in our future experiments, the phenomenon of competition between the microspore development and the callus derived from the diploid somatic tissue of the anther walls. For this purpose, we have already performed some preliminary experiments with free microspore cultures in a complete synthetic medium as recommended by Nitsch for Datura and tobacco microspores.

1.3. The in vitro culture of tissues coming from different self-incompatible genotypes of L.peruvianum was pursued in order to constitute a "bank of tissues" characterized by known S-alleles. We are trying to regenerate plantlets from these tissues with the aim to find out if some changes could occur at the S-locus during a prolonged in vitro culture. We do not have yet any good medium to easily regenerate plants from these old calluses, but after having ~~tried different~~ methods, we have

now obtained a good technique for in vitro clonal multiplication of L.peruvianum material. This involves culturing pieces of stem with the epidermal layer on a medium recommended by De Langhe. With this new method, we are culturing periclinal cytochimeric materials in order to verify the ontogenetic origin of the regenerated plantlets.

1.4. With regard to the biological screening method for the mutated pollen grains at the S-locus, the in vitro culture of excised pistil which was pollinated with the pollen treated with a mutagen and carrying the same S-alleles as in the diploid tissue of the style, was pursued this year and this gave us the first positive results with Oenothera organensis.

Gamma ray treatments were applied on whole plants which had several flower buds at different developmental stages from the premeiotic to mature pollen grain stage. Progressively, at the moment of the anthesis of these flower buds, the pollen was collected from them and used for the pollination of several flowers of plants bearing the same S-alleles. Afterwards, the pistil was excised at the proper length and put in vitro in a suitable culture medium. Four hours are sufficient to get the growing of the "mutated" pollen tubes in the medium out of the section at the lower part of the style.

The results showed that many pollen tubes are able to pass through the style without any stop after using an irradiated pollen at maturity. As the number of pollen tubes was very high in this condition, we believe that this may be due to gross structural changes in the vegetative nucleus of these pollen grains. Among the successive developmental stages of the male gametophyte, it appears that the haploid mitotic division separating the

vegetative and the generative nuclei is the most sensitive stage.

We intend to continue the application of this method to test the mutagenicity of different substances.

Risultati del progetto n. 2

Capo del progetto e collaboratori scientifici:

A.Brunori, G.Ancora, K.S.Ramulu, S.Marchetti*, M.Devreux.

Titolo del progetto: The research at the macromolecular level of the modifications induced by in vitro culture in different plant tissues.

2.1. During 1974, we have improved our technique for DNA isolation from different plant tissues (young and senescent leaves, stem, pith and calluses) by isolating the nuclei in a non-aqueous medium (Ethylenglycol) and using the proteinase K as proteolytic agent. This technique seems to work very well and in fact, among the other materials in which it was successful in isolating DNA was the Antirrhinum leaves, a material where all the classical methods have failed because of the very high DNase activity.

Experiments are being carried out to compare the characteristics of the DNA synthesis which takes place during the early stages of stem disk and the pith tissue of tobacco cultured in vitro. We thought it desirable to verify if the presence of meristematic cells in the stem disk can modify the characteristics of the DNA synthesis which seems to be very complex in the pith tissue.

So far the CsCl analysis of the DNA synthesis in stem disk did not show any particularity at any of the time tested. In fact, we treated the explants with H^3 -Thymidine for the last 24 hrs of culture and always the distribution of radiocativity coincided with the optical density profile of cold DNA, isolated from leaves, which was added to the sample in order to mark the O.D. distribution of the tobacco DNA.

* At no cost for the contract.

2.2. In order to obtain informations on the level of nuclear DNA content in tissues cultured in vitro, cytophotometric measurements were performed on nuclei isolated from the calluses grown for various periods of time and obtained from different plant species and tissues (tabacco pith and stem internodes, L.peruvianum anthers and stem internodes).

Tobacco pith and stem internodes were chosen as starting materials because of the highly different relative proportion among the various levels of ploidy. The analyses on both types of proliferating calluses were carried out during the eight months period and the results obtained showed that, in the pith calluses, there was a progressive and surprising reduction in the ploidy levels with most of the nuclei having 4C DNA content at the end of the experimental period. Higher DNA levels (16C and 32C) apparently disappear. On the contrary, in the stem calluses, there was a tendency to maintain the overall distribution of the DNA values present at the time of the explant, with 2C and 4C values being the most frequent. Taken together, the results indicated that under the cultural conditions adopted, there was no occurrence of higher classes of polyploidy than those observed in vivo.

In Lycopersicum peruvianum, calluses deriving from the anthers and internodes and cultivated for one month on the same media that have previously been used in respect of the chimeric plants for the regeneration, were analysed by microdensitometry, in order to observe the variability of the DNA content of the nuclei. In this material, we have not found any haploid level of DNA as it could be expected. The DNA content largely varied from 2C to 32C regardless of the tissues from which the calluses were

derived. We also analysed the calluses coming from anthers which have been maintained for more than two years on L.S. medium. In this case, there was a clear increase in the ploidy levels; the lowest values, 2C and 4C, almost disappeared, while the 8C and 16C were found to be the most common classes. Some nuclei reached a DNA level up to 64C. L.peruvianum seems to be a species in which the in vitro culture promotes high level of polyploidy.

Risultati del progetto n. 3

Capo del progetto e collaboratori scientifici:

M.Devreux, U. Laneri, K.S.Ramulu, M.R.Celestre*, S.Nardi.*

Titolo del progetto: Haploid induction, genetical studies on homozygous lines derived from anther cultures and mutagenic treatments of the microspores.

3.1. We have carried out anther cultures with some new cultivars of tobacco which are important for breeding purposes. Also with Nicotiana sylvestris, new haploids were obtained and the chromosome doubling is being performed by internode cultures on our regenerating medium.

An attempt was made in irradiating the floral buds of N.sylvestris with a series of progressively increasing total exposures of gamma rays and culturing the free microspores in a complete synthetic medium as recommended by Nitsch; however, the experiments gave negative results: no further development was observed and these are being repeated. It is programmed for next year to try with Datura free micropores in order to control the real possibility of this technique.

3.2. Studies were undertaken with anther cultures in strawberry with the aim to produce haploids, since this species is heterozygous and it is impossible to fix the useful characters by selfing considering the severe loss in vigour due to inbreeding effect. Investigations were carried out with some cultivars as well as with some breeding selections using various media. We have also tried some modifications in respect of the cultural conditions, i.e., cold pre-treatment, light and temperature. Calluses

* At no cost for the contract.

have formed with all the media used. More or less 50 plantlets were regenerated from these calluses coming from different cultivars and selections, by maintaining the cultures first in dark and later in light conditions. Cytological analyses of root-tip mitoses have revealed that all the regenerated plants had diploid chromosome numbers ($2n=56$). In order to know the origin of these plantlets, i.e., whether they come from microspores or diploid somatic tissues, we have also regenerated some plantlets from the anther culture of the F_1 hybrid between the short- and long-day flowering plants. Out of 7 plants obtained, 4 started blooming after four months under long-day conditions, while the other 3 did not flower even after 6 months period. This could indicate the gametic origin of these plants.

3.3. Anther cultures were performed this year with Capsicum annuum using the technique recommended by Wang Yu-Ying et coll. (1973). Before culturing the anthers, cold pre-treatment was given to the floral buds in order to increase the yield of plantlet regeneration as was observed by Nitsch and Noreel (1973) in Datura. Also, the environmental conditions in respect of the plants from which the anthers have been collected, were considered. The development of calluses was evident after 10 days of culture. The results on callus formation do not seem different between the cold pretreated anthers and the non treated ones, while the change of the environmental conditions, i.e., the photoperiodism, seems to play an important role in this respect; the yield of callus proliferation reached practically 96% of the anthers.

Also with the same conditions, some plantlets were regenerated (1.72%) after 40-45 days of culture. These plantlets are yet too small for chromosome counting.

3.4. Studies were also undertaken with the in vitro culture of anthers in pearl millet (Pennisetum typhoides), a naturally cross-fertilized agricultural crop. To start with, we have used the various culture media which have been previously adopted for the crops of Gramineae. We have applied some modifications in respect of the media as well as the cultural conditions. Experiments are in progress.

PUBLICATIONS

- D. de Nettancourt, M.Devreux, U.Laneri, E.Pacini, M.Cresti and G.Sarfatti - Genetical and ultrastructural aspects of self- and cross-incompatibility in interspecific hybrids between compatible Lycopersicum esculentum and self-incompatible L.peruvianum. Theor. Appl. Gen., 44: 278-288, 1974.
- M.Devreux and D.de Nettancourt - Screening mutations in haploid plants. International Symposium "Haploids in higher plants", Guelph, june 1974 (in press).
- M.Devreux, U.Laneri and P.De Martinis - Réflexions sur nos cultures d'anthères de plantes cultivées. Giornale Botanico Italiano, 1974 (in press).
- P.Rosati, M.Devreux and U.Laneri - Anther culture of Strawberry. (in press).
- F.Saccardo and M.Devreux - In vitro production of plantlets from anther culture of Capsicum annuum. (in press).
- M.Devreux, U.Laneri, E.Magnien and M.R.Celestre - Biological screening method for mutated pollen at the S-locus by in vitro culture of pollinated pistil. Incompatibility Newsletter (in press).
- K.S.Ramulu, M.Devreux and U.Laneri - Detection and analysis of cytochimerism in Lycopersicum peruvianum plants regenerated from in vitro culture of anthers and stem internodes. Incompatibility Newsletter, (in press).

M.Devreux and U.Laneri - Anther culture, haploid plant, isogenic line and breeding researches in Nicotiana tabacum. Proc. of FAO/IAEA conf. on Mutation and Polyploidy, Vienna, 101-108, 1974.

B.Donini, M.Devreux and G.T.Scarascia Mugnozza - Genetic effects of gametophyte irradiation in durum wheat. Proc. of FAO/IAEA conf. on Mutation and Polyploidy, Vienna, 127-138, 1974.

Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung mbH, München

Nummer des Vertrages: 118-72-1 BIO D

Leiter der Forschungsgruppe:

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

Allgemeines Thema des Vertrages:

Cell-culture genetics in barley, wheat, potato and maize.

For practical plant breeding the production of haploid plants from anthers, as compared to cross breeding, has the following advantages: Mutants can be detected in the M_1 generation; the F_1 hybrids are homozygote immediately after diploidization; new varieties can be obtained in considerably shorter time.

Experiments with barley anthers were carried out with the primary intention of inducing callus growth. The resulting calluses should then be regenerated into whole plants. The experimental series conducted so far varied in their culture conditions, i.e. temperature, light, and composition of the culture medium

Ergebnisse des Projekts Nr. 1

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Prof. Dr. H. Gaul
Dr. B. Foroughi-Wehr

Titel des Projekts:

Development of techniques for the production of haploid plants through the use of anther culture.

Darstellung der Ergebnisse:

Investigations on the detection of optimum growth conditions for barley pollen have been continued. Primarily, the organic additions like auxins, kinetin, vitamins and amino acids have been varied, which is very important for growth and differentiation. Furthermore we have been studying the influence of different lengths of low-temperature-periods, because it is well known, Nitch 1969, that low temperature promotes the organogenesis of Nicotiana.

The investigations are time-consuming and troublesome, because of the great number of possibilities to combine the environmental conditions and the growth factors.

Experiments with anthers of the barley varieties Mutina and Edelmut were conducted with the following components in the indicated concentrations:

Auxin -2,4-D	1-50 ppm	Vitamin-Thiamine-HCl	0,4-2,0 ppm
IAA	1-10 ppm	Ascorbic acid	2-24 ppm
Cytokinin-Kinetin	0,06 ppm	Amino acid-Aminoacetic acid	4-36 ppm
		Asparagic acid	2-12 ppm
		Alanine	100-400 ppm
		myo-Inositol	100-300 ppm
		Gibberellic acid	1-50 ppm

120 nutrient-combinations have been tested. As yet we know from our results that the auxins we used have different influences on callus formation of barley anthers. If we added IAA in quite different concentrations it had no effect on callus formation, while 2.4-D up to 16 ppm promoted callus formation. It seems as if 2.4-D can be replaced by gibberellic acid.

We have got best results when using gibberellic acid, ascorbic acid and asparagic acid together with the nutritive medium of Murashige and Skoog. In this case 2,2 % of the anthers formed callus. But we never could observe any shoot- or root-formation.

In two experiments cut shoots were kept at 0°C and 5°C for 72 and 144 hours in a nutrient solution. Afterwards the cold-treated anthers of those shoots were put on a solid medium. Callus formation happened occasionally without any relation to the pretreatment.

The cytological investigation of treated and untreated anthers have proved that cold treatment delays the development of the pollen grain. Two weeks after treatment 30-40% of the pretreated pollen still were intact, while the untreated pollen were already empty after 5 - 7 days.

The experiments with potatoes were conducted with 5 dihaploid lines (24 chromosomes). The callus formation amounts to 4 - 5 percent.

For improving the used culture-medium, which was worked out for Nicotina, we studied the growth of barley root tips. There we had some difficulties getting roots without bacteria en masse. Therefore we passed over to barley nodes. Some experiments of different anion- and cation-variations were carried out, but they are not yet finished.

RADIOENTOMOLOGIE

RADIOENTOMOLOGY

RADIOENTOMOLOGIE

Vertragspartner der Kommission : Freistaat Bayern, vertreten durch die
Bayerische Landesanstalt für Boden-
kultur und Pflanzenbau, München,
Bundesrepublik Deutschland

Nr. des Vertrags : 114 - 72 - 1 BJO D

Leiter der Forschungsgruppe : Dr. Albert Haisch

Allgemeines Thema des Vertrags : Ökologie, Aufzucht und Sterilisation
der Kirschenfliege (Rhagoletis cerasi L.)

The European Cherry fruit fly serves as object to test the feasibility of the insect sterilization technique under the special fruit growing conditions of Germany. This research project comprises three main fields of investigation. Emphasis is put especially on the artificial rearing of the species because it is the base for every kind of genetic control. This part includes not only feeding experiments with larvae but also the effort to regulate the diapause system of the Cherry fly, for the pupal diapause lasting half a year hinders the rearing experiments heavily. The second topic refers to the radiosterilization of the species. It must be the goal to obtain sterile flies by radiation, however with preserving the vigour of the males as far as possible. The third part of the investigation includes studies of the demecology and populations dynamics. Demecology according to SCHWERTFEGGER is understood as the sum of specific, ecological problems of a population. In particular the factors limiting the living area of the population (= demotop) must be found out because of its significance for the control technique. The overflowing of a population with sterile individuals succeeds only in its control if there exists any kind of isolation. The research contract includes two projects.

Ergebnisse des Projekts Nr. 1

Leiter des Projekts : Dr. Albert Haisch
Titel des Projekts : Laboratoriums- und Massenzucht der
Kirschenfliege (Rhagoletis cerasi L.)

Main attention has been drawn to the feeding of larvae. The standard diet used consisted of brewers yeast (5.5%), sugar (4%), wheat germs (3%) and the vitamin fortification mixture of VANDERZANT (1%). As bulking agents served wheat brans (12%) and paper pulp. Propionic acid (0.45%) at the pH 4.0 - 4.2 had to avoid the growth of microorganisms. The water content was 62%.

At this standard diet 12 131 larvae have been reared. 26.6% of them pupated. But their viability was very low, so that the final yield of pupae amounted to only 7%. This high pupal mortality could slightly be reduced if 0,1% formaldehyd was added to the propionic acid as a second antimicrobial agent. Increased amounts of the vitamin mixture did not significantly improve the survival of the pupae. At present the reasons for the high pupal mortality are unknown. Still it must be checked if perhaps it can be caused by the relative high temperature of 27°C during the larval stage.

Following the systematic study of the protein supply of the larvae the protein - carbohydrate ratio was tested. At earlier experiments yeast contents of more than 6% proved to be detrimental to the larval growth and development. However, if both yeast as well as sugar are enriched to 7% in the diet 45% of the larvae pupated and the pupal mortality was slightly reduced so that the final yield of viable pupae was 23%. This is a significant improvement of the diet compared with the standard food.

Further studies connected with rearing deal with feeding and keeping of adults. Essential differences of the egg production between 80 and 600 eggs per female were observed if the cage size or adult food was changed. The underlying physiological reasons are not yet quite clear.

The heavy impact of the long diapause on the rearing of larvae calls for particular efforts to prevent the initiation of the diapause. It is assumed that the diapause of the Cherry fly is arisen by photo-periodic influences i.e. short day periods. Therefore adults, eggs and larvae have been exposed to 18 h light at 25°C. Also three light intensities and two light qualities have been administered. The eggs laid by the females as well as the individuals of the other stages could complete their development up to pupation under these conditions. But all pupae entered diapause.

The influence of the length of cold exposition and of the temperature during that time on the postdiapause development was studied in detail. Pupae stored at +2°C for 180 days emerged only with 54%. At a storing temperature of 6°C emerged 80.4%. It seemed however, that the hatching rate of more than 85% was reached earlier in the case of a 2°C storing temperature than in the case of a 6°C storing temperature.

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- A. Haisch: Zur Puppendiapause der Kirschenfliege (Rhagoletis cerasi L.)
I. Abhängigkeit der Nachdiapauseentwicklung von den Lagerungsbedingungen während der Diapause. - in preparation

Ergebnisse des Projekts Nr. 2

Leiter des Projekts :

Dr. Albert Haisch

Titel des Projekts :

Ökologie und Sterilisierung der
Kirschenfliege (*Rhagoletis cerasi* L.)

1) Irradiation experiments

The sterilization dose for male Cherry fruit flies lies between 8 and 10 kR depending on the stage at which the flies are treated. After such a treatment the hatchability of the F_1 -eggs is below 1%. There exist many possibilities to check if such a treatment lowers also the vigour and competitiveness of the flies. However, all these studies are carried out under artificial conditions. The significance of its results for the behaviour of the flies in the field must therefore remain unknown. On the base of this idea the following field experiment was conducted.

Pupae have been irradiated with a dose of 9 kR and yellow labelled by fluorescent dyes. They have been put into the field together with the same amount of non-irradiated pupae for emerging. At the same time red labelled flies of an age up to one week have been irradiated together with the same amount of non-irradiated flies at a second separate experimental field. At the experimental fields *Lonicera* bushes as host plants for the Cherry fruit fly have been grown. At six different days the flies on the bushes have been observed, counting males and females and noting also the marked ones. Finally, the mean number of single flies per one copulating fly was computed. This was done for both sexes, for the irradiated and non-irradiated flies at the different experimental fields. 37 males irradiated as adults could be observed before one copulating male of the same treatment was seen. In the case of non-irradiated males only 20 males were established. The irradiation diminished, therefore, the copulation frequency by the half. The same result could be obtained for the females. Also in the case of irradiation of pupae the male copulation frequency was reduced by the half and that of the females by a quarter.

2) Studies on ecology and distribution

During several years it was found that the flight range of the Cherry fruit fly does not essentially exceed 1000 m. They fly in this distance only in the direction of the prevailing wind. The movement against the wind is weak. Forests do not prevent the flies from crossing, even it is unknown if the flies fly through or over the forest. Trials to catch the flies by traps hanging on a balloon failed because traps apart from the crown of a tree are evidently not attractive. According to the observations made up to now, the demotop of the Cherry fly can be limited by wind and large areas free of host plants.

In connection with this topic the flying tendency of the flies depending on age and food was investigated under artificial circumstances. It seemed that well fed flies fly more active than hungry ones and that the inclination to fly increases with time up to a certain age.

A. Haisch: Observations on the flying behaviour of the European Cherry fruit fly (Rhagoletis cerasi L.) in Sterility Principle for Insect Control, Vienna, 1974 - in press

Associato alla Commissione : Istituto di Entomologia agraria della Università di Padova.

N° del contratto: 105-72-1 BIOI

Capo del gruppo di ricerca: Prof. Sergio Zangheri

Tema generale del contratto: Ricerche sulle metodologie e le tecniche per l'allevamento in laboratorio del Dacus oleae Gmel. e di altri insetti di interesse agrario in vista della produzione in massa e ricerche radioentomologiche e biologiche, con particolare riguardo ai ditteri Tripetidi.

General Theme of the contract: Researches on methods and techniques for laboratory rearing of Dacus oleae Gmel. and other insects of agricultural interest for mass-production and radioentomologic and biologic researches with particular regard to fruit flies.

A brief outline of the work carried out in 1974

The program of research on Dacus oleae Gmel. is articulated, so far as regards Project No. 1, on the studies effected on the symbiosis in other Trypetides, to locate the causes of the permanence or otherwise of the bacteria in laboratory rearing, and testing the techniques of culture of symbionts.

For Project No. 2, the investigations have regarded the factors of stimulus or inhibition of oviposition; in particular the activity of oleo-ropine and its derivatives has been studied.

In Project No. 3, the researches have been directed towards the dynamics of population in the area of Garda, accompanied by laboratory investigations into the influence of climatic factors on the survival and on the duration of the various stages of development.

In an olive-growing area near Garda, the investigation into the biological cycle has been nearly completed, bringing into evidence the number of generations and the numerical relations of these. It has further been observed that, in the absence of biotic control factors, the dynamics of po-

pulation show themselves to be influences, in addition to climatic factors, by an auto-regulation of oviposition, connected with the presence and the percentage of olives already attached.

Within the ambit of Project No. 4, researches into the radio-resistance and into the sterilization of D. oleae have been continued, comparing the effects of gamma radiations and rapid neutrons on the various phases in the biological cycle of the insect; the greater competitiveness of males irradiated by rapid neutrons has also been observed.

In Gonocerus acuteangulatus Goeze it has also been possible to observe, with sub-sterilizing doses, an elevated sterility in F_1 and an average one in F_2 .

The researches into the radio-sensitivity have been extended to various species of Coleoptera of stored products.

In Project No. 5 surveys of the population of D. oleae in Liguria have been continued. For an estimate of the results of the introduction of sterilized insects, a mathematical model for the study of the dynamics of population, employable for various types of insects, has been studied.

Risultati del progetto n. 1

Capo del progetto e collaboratori scientifici: Prof. S. Zangheri, Prof. L. Masutti, Dott. V. Girolami.

Titolo del progetto: Influenza dei batteri simbiotici sul ciclo vitale del Dacus oleae Gmel.

The influence of symbiont bacteria on life cycle of Dacus oleae Gmel.

(Researches made in 1974)

While prosecuting the researches into the bacteriosymbiosis of Trypetidae, it has been possible to observe in numerous Tephritinae belonging to the genus Sphenella Rob-Desv., Tephritis Latreille, Trypanea Schrank, Acanthiophilus Bech, Noeeta Rob-Desv., the presence of endocellular symbiotic bacteria.

Since such a type of symbiosis is normal in a vast group of Trypetidae, the existence of an endocellular or celomatic phase in the cycle of transmission of the symbiosis of the Dacinae and Trypetinae may reasonably be admitted. This has been postulated to explain the varying sensitivity to disinfectants of certain fruit flies of economical importance.

In particular, it does not seem possible in any other way to explain the permanence of the symbiosis in C. capitata reared in the laboratory, in spite of the considerable use of disinfectants and chemioterapics.

A more profound knowledge of the mode of transmission of symbionts to the offspring therefore becomes necessary, above all in those species, such as D. oleae which, being sensitive to mould inhibitors, undergo, on account of the rearing technique employed, an interruption of the symbiosis, with negative consequences on the possibility of rearing.

Researches have also been carried out on the possibility of the culture of D. oleae symbionts in vitro, with the aim of providing a suitable bank of bacteria, to the end of restoring the symbiosis once it has been lost.

Among other things we have tried to obtain the multiplication

of the micro-organisms on the interior of the 'vesicles' dissected and placed in the culture liquid for tissues of diptera, or in culture broth containing "membranous agglomerates". The results have not so far been positive and the researches are proceeding.

It has been possible, among other things, to dissolve in basic solutions the afore-mentioned "membranous agglomerates" which, from investigations carried out, do not appear to be constituted of proteinous substances, but probably of polysaccharides.

The following note on this topic has been published:

Girolami V. - Reperti morfo-istologici sulle batteriosimbiosi del Dacus oleae Gmelin e di altri ditteri Tripetidi, in natura e negli allevamenti su substrati artificiali. Redia, vol. LIV, pp. 269-294, 1973.

Risultati del progetto n. 2

Capo del progetto e collaboratori scientifici: Prof. S. Zangheri, Prof. L. Masutti, Dott. V. Girolami, Dott. ssa L. Panizza, Dott. ssa G. Pellizzari.

Titolo del progetto: Ricerche su substrati artificiali per gli stadi larvali del Dacus oleae Gmel. e di lepidotteri defoliatori e sulle tecniche di allevamento.

Researches on artificial media for the larval stages of Dacus oleae Gmel. and of phytophagous Lepidoptera and on rearing techniques.
(Researches made in 1974)

In the course of previous researches, oleoeuropine, a phenolic glucoside characteristic of the olive tree, was shown to have a stimulating action on the oviposition of D. oleae.

Since the active principles are to be considered products of spontaneous degradation of the above-mentioned glucoside, and in a greater measure of its aglucones, various fractions, obtained by chromatographic separation of the products of the enzymatic hydrolysis of oleoeuropine have been tested.

The fractions have not, however, shown any significant capacity of stimulus on oviposition; it is probable that this depends on an excessive dilution or loss of the active principle, possibly present in traces in the starting solution.

With the aim of clarifying the mechanism of formation of the active principle, researches have, therefore, been directed towards the setting up of a method which permits a higher yield in aglucone with the use of a commercial glucoside, and to the research of specific enzymes present in the olive.

Thus an elevated β -glucosidasic activity has been encountered in the olive; researches are in progress to attain the separation and purification of the enzyme.

The following communications on the subject have been presented at the FAO-IAEA Congress at Innsbruck:

Giolami V., Pellizzari G., Ragazzi E., Veronese G. - Prospects of increased egg production in the rearing of Dacus oleae Gmelin by the use of chemical stimuli. Symposium on the Sterility Principle for Insect Control, Innsbruck, July 22-26, 1974, IAEA-SM-186/23.

Risultati del progetto n. 3

Capo del progetto e collaboratori scientifici: Prof. S. Zangheri, Prof. L. Masutti, Dott. V. Girolami, Dott. ssa G. Pellizzari.

Titolo del progetto: Ricerche sulle cause di mortalità del Dacus oleae Gmelin e sulla dinamica delle popolazioni in natura ed in laboratorio.

Researches on mortality causes of Dacus oleae Gmelin and on population dynamic in field and in laboratory.

(Researches made in 1974)

A) The laboratory researches into the influence of climatic factors on the development and survival of D. oleae reached conclusion and the results are the subject of a note in the course of being drafted.

In the past year in particular investigations have been made into the influence of temperature, the duration and survival of the pre-image stages, going so far as to define the liferange of the species.

The development from egg to adult in the laboratory is possible at a temperature ranging from 10°C to 30°C. Above 30°C it is possible to observe the completion of the embryonic development and only a prolonged survival of the larvae. Above 41°C, in all stages, including the adult, death overtakes rapidly.

In relation to less high temperatures, at 7°C one observes the completion of embryonic development, a prolonged survival and partial larval and pupal development.

Below 6°C development is arrested; down to 0°C, however, survival can reach high levels over long periods; death overtakes rapidly, in all stages, around - 10°C.

From a practical point of view it is interesting to note that the temperature which permits a longer preservation of the pupae of D. oleae is comprised between 5°C and 7°C, the pupae being capable of surviving up to 2 months; an alternation with higher temperatures for short periods,

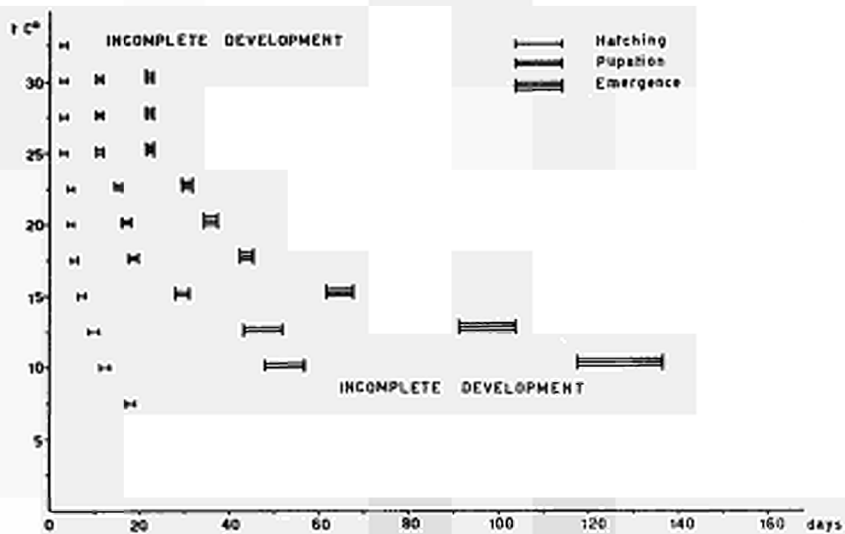


Fig. 1 - Duration of embryonic, larval and pupal development of *Dacus oleae* Gmel at various temperatures. The sections indicate the average intervening period between the beginning and the end of the phase into the successive stage.

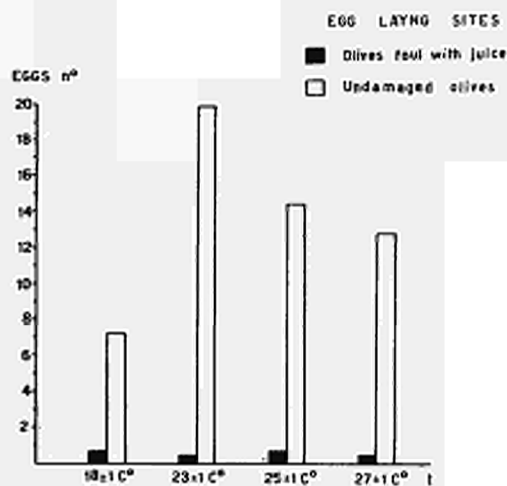


Fig. 2 - Average daily deposition of eggs bred separately at different temperatures in groups of olives renewed daily, respectively sprinkled with olive juice to simulate a previous attack by olive fly, and not treated.

seems to guarantee an even greater survival. All the above-mentioned tests have been carried out in places with U.R. above 55%. Comparative tests with different U.R., which can be encountered effectively in nature, have not brought to light any important U.R. action.

B) Researches have also been carried out on the population dynamics of D. oleae in an olive-growing area near Lake Garda, at the extreme northern limit of distribution of the species. Parallel researches have been arranged with University Institutes in other Italian regions (Apulia and Sardinia) to the end of obtaining comparable data from other places. The investigations arrived at the conclusive stage and the data are in course of elaboration.

The researches made it possible, among other things, to observe that in the above-mentioned places:

- 1) Biotic factors of limitation of the species have no appreciable influence on the population of D. oleae.
- 2) During the winter period, populations are reduced more than 1.000 times, at least as regards the connection between the numerical entity of the immature stages of the last autumnal and the first spring generations.
- 3) In the spring period, during which two or three generations of the insect take place, depending on the host cultivar, on olives still hanging, the populations undergo a notable increase and the adults of the 2nd or 3rd generations await the formation of new olives to give origin to a summer generation.
- 4) The adults of the summer generation give place to the autumnal generation, destined to over-winter; this generation is numerically the highest. (16 million individuals in an area of 4 sq. Km in the year 1973-1974).

The population dynamics of D. oleae is also seen to be influenced by an interesting factor of self-regulation brought to light in the laboratory, on the basis of a hypothesis formulated on the relative field data. The ♀♀ of D. oleae (Fig. 2) reduce drastically the number of eggs deposited in the presence of olives already attached in consequence of the perception of certain repellent stimuli contained in the oxydized olive juice of the attached drupes;

for some time, in fact, it has been known that through such stimuli the ♀♀ avoid depositing more eggs on the same olive.

It is permissible to suppose, on the basis of the quantitative valuation of the phenomenon (that in the laboratory has already shown at infestation of the order of 20% and that at a 50% attack causes fecundity to be reduced to about one-fortieth) that if this also happens in nature, such a mechanism is the most important factor of limitation once a level of population sufficient to produce appreciable infestations has been reached.

A summarized note of the work carried out up to now has been presented to the Italian Entomological Congress of Sassari;

Girolami V. - Indagini demoeologiche sul Dacus oleae Gmel. nell'area Gardesana. X Congresso Nazionale di Entomologia, Sassari, 20-25 maggio, 1974.

Risultati del progetto n. 4

Capo del progetto e collaboratori scientifici: Prof. R. Cavalloro, Dott. G. Delrio.

Titolo del progetto: Effetti biologici comparati delle radiazioni gamma, neutroni e chemiosterilizzanti sui diversi stadi di vita degli insetti.

Comparative biological effects of gamma radiations, neutrons and chemosterilants on different life stages of insects.

(Researches made in 1974)

A. Researches have been conducted on the radio-resistance and on the sterilization of Dacus oleae Gmel., with particular attention to the comparison between gamma radiation and fast neutrons. To this end a Gammacell Apparatus 220 with a source of Co. 60 was employed for the gamma rays and a Van de Graaff linear accelerator Type K.S. 3000 for the rapid neutrons. As regards the radio-sensitivity of Dacus oleae, an increase in resistance to the neutrons was encountered with the advance of the biological cycle of the insect. As regards sterilization, with the highest doses utilized (2979 rad) of fast neutrons, was obtained for adult males, 87,6% of dominant lethal mutations.

The females, also irradiated in the adult stage, proved to be more resistant to the treatment, always as regards the induction of lethal dominant mutations, contrary to what is verified by gamma radiation. In every case, at a dose of 2979 rad fecundity is drastically reduced.

The relative biological efficacy of neutrons in respect to gamma radiation has been calculated: this varies, for sterilizing doses from 2 to 4 in favour of rapid neutrons (Fig. 1). The increase in competitiveness in males sterilized by fast neutrons thus demonstrates the usefulness of the method of irradiation, above all in the application of the technique of the sterile male.

A histological investigation has been made of the mid gut and

the gonads of the irradiated insects. With the doses employed no damage to the mesenteron was encountered under the microscope: as regards the gonads, on the other hand, the dose of 2979 rad of fast neutrons has interrupted the spermatogenesis and the oogenesis.

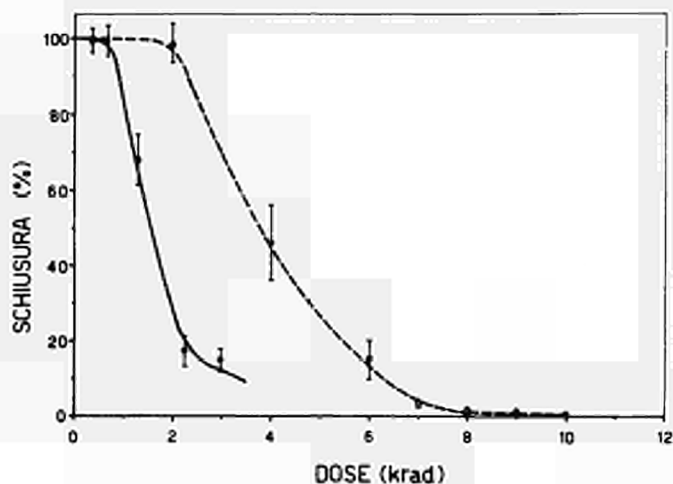


Fig. 1 - Dacus oleae Gmel.: influence on the descendants of the irradiation of males with gamma radiation (hatched line) and with fast neutrons (continuous line) (average normalized relative values of 20 couples for each test; irradiated males in pupa stage 3 days before emerging; 4 replicatons \pm standard error).

The following publications on this subject are in the course of being published:

Cavalloro R., Delrio G. - Irraggiamento di Dacus oleae Gmelin con neutroni veloci (presented at "IV^e Journées de Phyiatrie et Phytopharmacie Circum-Méditerranéennes", Montpellier, Septembre 16-19, 1974).

Cavalloro R., Delrio G. - Sterilizzazione di Dacus oleae Gmel. e Ceratitis capitata Wied. con radiazioni gamma e neutroni veloci (presented

at "X Congresso Nazionale di Entomologia", Sassari, 20-25 maggio 1974).

B. The studies on the induction of sterility of Gonocerus acuteangulatus Goeze have demonstrated the possibility of obtaining a high degree of inherited sterility in F_1 utilizing the sub-sterilizing doses of 4, 5, 6, 7 and 8 krad of gamma radiation.

Sterility is transmitted by either the male or female line to successive generations, but already at F_3 the insects reveal a fertility that approaches more and more nearly to that of non-radiated insects (Tab. 1). Sub-sterilized insects of F_1 have a higher sexual competitiveness than that of the corresponding parental generation.

Tab. 1 - Gonocerus acuteangulatus Goeze: retarded effect of sterility in successive generations, in the male line, following irradiation with gamma radiation (8 krad) of the male.

Generation	Couples studied (n)	Eggs (n)	Hatch(%)
P ♂(8 krad) x N ♀	13	415	20,4
F_1 ♂ x N ♀	10	352	5,3
N ♂ x F_1 ♀	6	175	9,8
F_1 ♂ x F_1 ♀	9	194	4,1
F_2 ♂ x N ♀	4	156	51,2
N ♂ x F_2 ♀	3	113	46,9
F_2 ♂ x F_2 ♀	3	86	25,5
F_3 ♂ x N ♀	6	215	93,9
N ♂ x F_3 ♀	4	198	68,1
F_3 ♂ x F_3 ♀	3	172	83,1

It is interesting to underline that the high radio-resistance presented by certain insects has been co-related to the presence of holokinetic chromosomes of diffused centromere it is to be observed, however, that Rhynchota, while having holokinetic chromosomes like the Lepidoptera, con-

trary to these, manifest an elevated radio-sensitivity, as also appears from the study of Gonocerus.

The following work on this subject is in the course of being printed:

Delrio G., Cavalloro R. - Stérilité héréditaire en Gonocerus acuteangulatus Goeze (Rhynchota, Coreidae) (presented at "FAO/IAEA Symposium on the sterility Principle for Insect Control", Innsbruck, July 22-26, 1974; IAEA-SM-186/24).

C. Having seen the notable discordance, which can be deduced from literature, about sterilizing and lethal doses of radiation for insects dangerous to stored products, tests of radio-sensitivity have been carried out on adults of the following Coleoptera: Sitophilus oryzae L., Sitophilus granarius L., Oryzaephilus surinamensis L., Rhyzopertha dominica F. The studies are proceedings; a growing radioresistance has already been encountered of the species in the order given, evaluated on the basis of their longevity.

Furthermore, studies have been carried out on the radiosterilization of Sitophilus oryzae. Irradiating adults, emerged one day, virgins, a higher resistance was encountered in males in respect to females; the sterilizing dose for males was 11 krad (Tab. 2).

Tab. 2 - Sitophilus oryzae L. lethal dominants in newly emerged males irradiated with gamma radiation (average values relative to 20 couples for every dose; irradiated males coupled with virgin females; 4 repetitions).

Dose (krad)	0	1	2	3	4	5	6	7	8	9	10	11
<u>Lethal Dominant mutation</u> (%)	0	12,85	20,59	65,44	67,65	87,61	92,44	95,80	96,01	97,90	99,62	99,99

Finally; studies have been begun to evaluate the possible effects of an irradiated diet on the longevity and fecundity of normal and irradiated adults

of Sitophilus granarius. To this end wheat irradiated in doses of 10, 50, 100, 150, 250, 500, 1000 krad of gamma radiation was used. The partially obtained results are in course of elaboration.

Risultati del progetto n. 5

Capo del progetto e collaboratori scientifici: Prof. R. Cavalloro, Dott. G. Delrio.

Titolo del progetto: Studi sul comportamento di femmine e maschi normali e sterili.

Studies on the behaviour of normal and sterile females and males.

(Researches made in 1974)

A. A population investigation has been carried out on Dacus oleae Gmel. in certain survey station in an olive-growing area in Liguria. The adults have been surveyed by means of yellow chromotropic traps, from July of last year, and there have been two peaks of captures one in the middle of August, corresponding presumably to the first generation, and one much greater at the end of September (Fig. 2). From the end of December no more adults were caught, but the insect has been surveyed in the pupa stage on ground. A suitable collection of specimens led to the valuation of 31 pupae per square metre in March.

An investigation into drupes has also been carried out so as to study the course of the immature stages of the insect. In this way the presence of entomophagous parasites has also been studied. Thus, at the end of August a parasitisation of about 60% was encountered, operated, in order of importance, by Pnigalfo mediterraneus Ferr. et Del., Eupelmus urozonus Dalm. and Eurytoma martelli Dom. in spite of this elevated parasitisation, the population has developed in such a way that at the end of December the olives attacked amounted to more than 90%.

Studies, which are still in course, have been proceeding to the end of obtaining an economical high-yield diet for the mass rearing of Dacus oleae. In this connection a variation in yield for the same kind of diet has been encountered, depending on the generation of the Dacus with which the breeding began. In the evaluation of the diet, it is necessary, therefore, to take into account other factors hitherto overlooked.

The following work on the field investigation of population, repor-

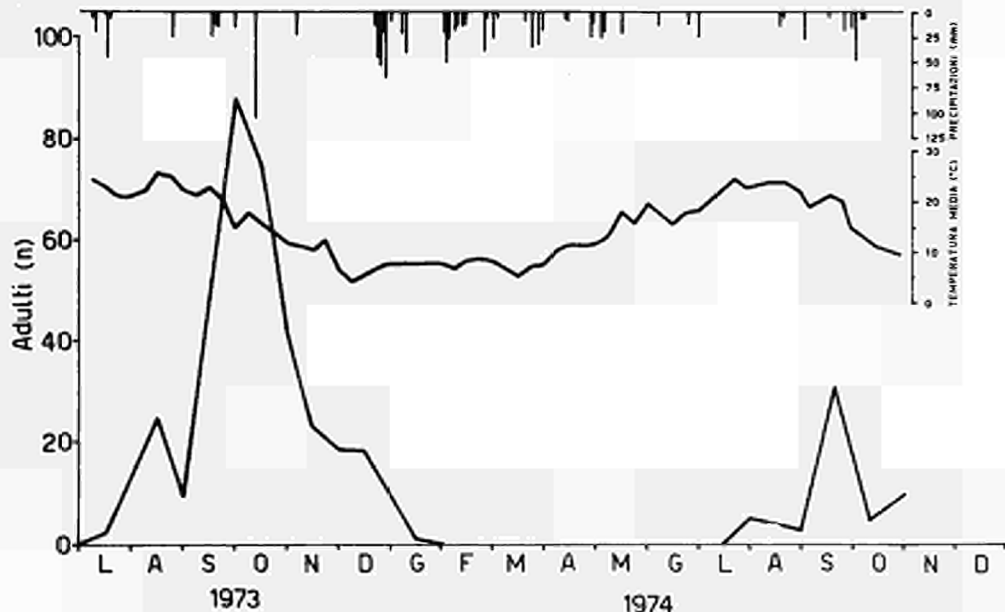


Fig. 2 - Course of the capture of adults of *Dacus oleae* Gmel. by means of chromotropic traps at the experimental station in Liguria.

ting on surveys in various olive-growing districts, is in the course of being printed:

Cavalloro R., Contini C., Delrio G., Girolami V., Prota R., Zangheri S. - Synthèse d'observations eco-ethologiques sur *Dacus oleae* Gmelin dans quelques oliveraies italiennes (presented at 'IV^e Journées de Phytologie et de Phytopharmacie Circum-Méditerranéennes', Montpellier, Septembre 16-19, 1974).

B. A simple mathematical model has been formulated for the study of the dynamics of a population of insects following the introduction of sterilized males, at varying levels of sterility. The model takes into account both the case

of monogamous insects and that of polygamous insects, and takes into consideration, in addition to the sexual competitiveness of the sterilized males, (Fig. 3) the different effect also of the males on the receptiveness of the polygamous females.

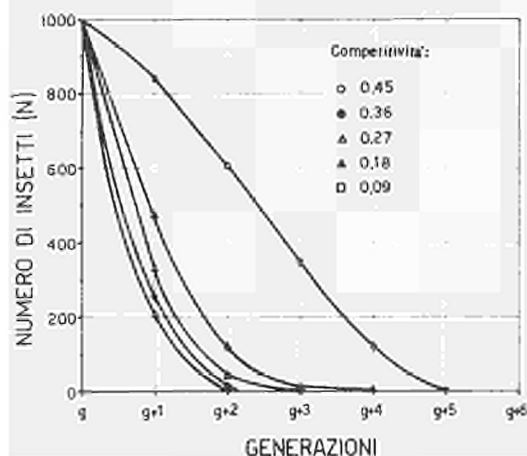


Fig. 3 - Course of successive generations following the distribution of equal numbers of sterile males at different levels of competitiveness.

Finally a formula has been elaborated which is of practical use in tracing back the sexual competitiveness of the insects, once known the percentage of egg-hatch in a mixed population of insects made up of normal females and normal and sterile males, in different and known relations among themselves.

Two publications on the subject are in the course of being printed:

Anselmi L., Cavalloro R., Delrio G. - Modello matematico per lo studio dell'andamento di una popolazione di insetti a seguito dell'induzione di maschi sterili. (presented at "X Congresso Nazionale di Entomologia". Sassari, 20-25 maggio, 1974).

Delrio G., Anselmi L., Cavalloro R. - Valutazione della competitività degli insetti a diversi livelli di sterilità. (Bollettino del Laboratorio di Entomologia Agraria, Portici).

Contractual Partner of the Commission:

Institut für Genetik, Johannes Gutenberg-Universität, Mainz, Germany

Contract No.: 115-72-1 BIO D

Head of the Research Group:

Prof.Dr.H.Laven, Director of the Institut für Genetik

General Topic of the Contract:

Development of genetical control systems in pest insects.

Project No.1 : Mosquitoes

Although the field experiment with an integrated strain in the fall of 1973 (see Report for 1973) was a success, several observations and considerations have lead to the decision to use for any field experiment only genetical systems which are developed from indigenous material. Such a procedure would on the one hand insure that strains for the release are adapted to the local climatic and ecological conditions. On the other hand would the danger be excluded of releasing foreign strains with other and possibly higher capacities of disease transmission. Our former research on genetical systems for control of Culex pipiens was predominantly carried out with European material. In view of the necessity to develop such systems for the use in India, most of the work in 1974 was conducted with strains from that area. No differences have been found between the results with European and Indian strains as regards the feasibility to develop various genetical control systems.

The promising results in this work with Culex has convinced us that other mosquito species can be brought under control with the same genetical systems. Therefore preliminary experiments were initiated with two other mosquito species, i.e. with Anopheles stephensi, the main malaria vector in the Near East and Southeast Asia, and with Aedes vexans, the main mosquito nuisance in Europe.

Project No.1

Title: Genetical systems for control of mosquitoes.

Research workers: Prof.Dr.H.Laven, J.Kutsche-Ohmann, H.Bernd,
M.Ashraf-Choudhary, G.Ackermann

1.1. Screening for viable homozygous translocations (Culex pipiens fatigans)

As reported already in 1972 and 1973, only very few strains with a translocation, originating from X-ray irradiation, can be made homozygous for the translocation. Out of 16 translocations tested so far for viability in the homozygous condition only 3 were viable. But also these homozygous lines have shown serious deficiencies. They did not show the theoretically expected full hatchability. Furthermore, the resulting homozygous offspring were less vigorous in the larval as well in the adult stage. Two of the lines could hardly be maintained under the optimal conditions in the laboratory. These results stand in sharp contrast to observations in *Drosophila* where 46% of the translocations can be made homozygous. In view of these meagre results in homozygotization of translocations in *Culex*, which are corroborated by similar observations with other mosquito species,

this line of research seems to be without prospects. The much propagated model of using two homozygous translocation lines for the production of double heterozygous individuals for release seems not to be practicable. Therefore these attempts have been terminated. However, the screening of translocations, whether they can be made homozygous or not, has been useful for the development of balanced lethal systems with translocations,

1.2. Balanced lethal systems with translocations (Culex pipiens fatigans).

As outlined in the report for 1972, the combination of two or more translocations, which cannot become homozygous, leads to very high lethality percentages up to almost total sterility. The combination of two translocations which can become homozygous does however not enhance the lethality over the level of the heterozygote with a single translocation. Therefore the screening of translocations for their ability to become or not to become homozygous has been useful. There are now available at least three balanced lethal systems with Indian genomes which lead to lethality up to 80-95%.

1.3. Effect of translocation combinations (Culex pipiens fatigans).

The combination of two or more translocations in one line has the disadvantage, that this line has itself a low productivity in mass production. Although a productivity of only 15-20% in such lines compared with a normal line, does not prevent mass production in Culex mosquitoes, another approach to genetical control seems to have equal if not better prospects, i.e. the simultaneous release of individuals with different single translocations. Crossings of two individuals with single translocations which are different results in offspring with double translocations. Numerous such crosses have been made in the laboratory with a series of translocations (Indian material). The resulting double heterozygotes have shown in almost all cases the expected level of higher sterility. If each single translocation had a sterility of 50%, the double translocation individuals had a sterility level around 75%. The few exceptions from this rule are presently under further testing. From these results another policy for genetical control can be suggested, i.e., simultaneous or successive release of single translocation individuals with ensuing combination to double translocations in the wild population. Computer calculations have revealed that the impact on a wild population shows a delay of 1-2 generations but that the end result is the same as if doubly translocated individuals would have been released. The release of single translocations with combinatory effect in later generations has the one advantage that mass production of lines with only 50% sterility makes no difficulty. Such a release program could be extended to releases of three and more different translocation lines with an ever increasing genetical load put upon the wild population.

1.4. Preliminary research on Anopheles stephensi for development of genetical control systems.

Several attempts in different laboratories have been made to develop genetical control systems also for Anopheles species. The results have not been very satisfactory or promising. The main reason for it was the inability of these research workers to get lines with a stable level of sterility. This in turn might be due to the fact that in these attempts old laboratory colonies of Anopheles species were used in which very likely had accumulated a number of recessive lethal factors. With the screening method used for translocation it was not possible to differentiate

between lethality due to recessive lethal factors already present in the laboratory stock and the lethality due to the newly produced chromosomal aberrations. In order to avoid such mistakes and difficulties we selected from wild material of Anopheles stephensi from Lahore (Pakistan) by in-breeding for 4 generations a line with continuous hatchability close to 100%. This line is presently used for the construction of a dose-response curve for dominant lethality after irradiation with different amounts of X-rays. After this curve has been completed the most promising dosage of irradiation for production of chromosomal aberrations can be selected, The first aim of this work will be to screen for a series of lines with a stable level of sterility. The work with Culex will give us the lead for a steady development of an effective genetical control system in Anopheles stephensi.

1.5. Attempts for colonization of Aedes vexans.

The mosquito species Aedes vexans is in many parts of Europe, especially in river valleys, the predominant nuisance species. It is in addition also the main vector of the Tahyna virus, a member of the California virus group. Therefore it appears to be worthwhile to develop a genetical control system against this mosquito species. But such an aim cannot be achieved as long as this mosquito cannot maintained and propagated in the laboratory, Observations on other mosquito species seem now to open the way to successful rearing. If Aedes vexans of both sexes in sufficient numbers are brought together in the same cage with equal numbers of another species which will freely copulate under these conditions, Aedes vexans will be in some way stimulated to copulate also. This has been observed in a combination of A. vexans with A. dorsalis. In this way it seems to be possible to select an Aedes vexans strain which will copulate in laboratory cages, Attempts have begun to get copulation stimulation in combinations with Culex pipiens, Anopheles stephensi and Aedes albopictus,

Project No.2

Title: Production of semisterility in the Mediterranean fruitfly
(Ceratitis capitata).

Research workers: Prof.Dr.H.Laven, N.N.

This project which was started in 1972 and had come to an unexpected interruption at the end of that year, could not be resumed in 1974 due to the failure to find a suitable scientific assistant. This difficulty has now been overcome and work will be resumed as soon as possible.

Other projects:

Title: Production of semisterility in the rice weevil (Sitophilus oryzae),

Research workers; Prof.Dr.H.Laven, R,C,Sharma

Genetic control systems on the basis of chromosomal aberrations have up till now been developed in Diptera (mosquitoes, flies), Lepidoptera, Hemiptera and Acarina. The fact that such developments are possible in such different orders is ample evidence for its general nature. But so far no attempts have been made for such developments in other insect orders than the ones mentioned. Therefore it seems to be appropriate to extend research of this kind to other insect orders, e.g. to Coleoptera, We have chosen the rice weevil, Sitophilus oryzae, as a suitable candidate because it is a serious pest insect and can be easily cultured and studied in the laboratory, Here the preliminary work as mentioned above for Anopheles stephensi has been started and first isolations of chromosomal aberrations have been successful.

Title: Nature of "delayed sterility" in insects with holokinetic chromosomes,

Research workers: Prof.Dr.H.Laven, Dr.R.Klause-Selinger,

In Lepidoptera, Hemiptera and Acarina, which have holokinetic chromosomes, it has been observed that the offspring of irradiated individuals have a higher degree of sterility than the irradiated parents. The cause of this phenomenon, known as delayed sterility, is not yet known, Research is now under way to elucidate the mechanisms for "delayed sterility", It is done with the milkweed bug Oncopeltus fasciatus.

Contractant van de Commissie : Instituut voor Plantenziektenkundig
Onderzoek (I.P.O.), WAGENINGEN, Nederland

Nummer van het contract : 098 - 72 - 1 BLO N

Hoofd van het researchteam : dr. ir. J. Ticheler

Algemeen onderwerp van het contract : Control of the onion fly,
Hylemya antiqua (Meig.), by means of the sterile-male technique.

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The release of sterilized onion flies can reduce the reproduction of this noxious insect to a low level resulting in infestation far below the economic threshold. This has been demonstrated in another two field experiments, one of which carried out for the first time in an onion growing area. The production of the pupae required for these experiments was again rationalized, using less space and labour than before. Prevention of infection in the rearing facilities is very important and progress has been made in this respect.

Stimulating side effects of irradiation of males on the performance of normal females is shown, but is not understood. Use of radioisotopes helped in discovering some of the mortality factors of the juvenile stages of the onion fly under field conditions.

Histological studies helped to understand the phenomena observed in diapause and in storage of pupae. The effects of radiation on the gonads of the fly fit into a much clearer pattern.

We are grateful for the cooperation of university students in the programme. P.M.J. Ramakers (Agric. Univ., Wageningen) completed his study of the induction of diapause. P.V.I.M. Wolff (State Univ., Utrecht) reported on his work on a screening technique for onion fly lures, whereas G.J. Buth (State Univ., Utrecht) cooperated in the ecological studies. Continuing on earlier work, more insight was gained in the phenomena around pupal development.

Publications

- Theunissen, J. 1973 Chromatin transformations in the trophocytes of the onion fly, Hylemya antiqua (Meigen), during egg chamber development. Int. J. Insect Morphol. & Embryol. 2 (4): 267-276.
- Theunissen, J. 1974 Effects of temperature on egg chamber development of the onion fly, Hylemya antiqua (Meigen) (Diptera, Anthomyidae). Ent. exp. appl. 17 (4): 355-366.
- Theunissen, J., Loosjes, M., Noordink, J.Ph.W., Noorlander, J., and Ticheler, J. 1973 Genetic control of the onion fly, Hylemya antiqua, in the Netherlands. Proc. 7th British Insecticide & Fungicide Conf., 3: 981-989.
- Ticheler, J., Loosjes, M., Noordink, J.Ph.W., Noorlander, J., Theunissen, J., 1974 Field experiments with the releases of sterilized onion flies, Hylemya antiqua (Meig.). In : "The sterile-insect technique and its field applications", IAEA, Vienna : 103-107.

Project no. 1

Control of the onion fly, *Hylemya antiqua* (Meig.), by means of the sterile-male technique

General (J. Ticheler)

In 1974 the fourth release experiment with sterile onion flies completed the series at the Schuilenburg site. Moreover, one field experiment was carried out in the onion growing area of Flakkee. Apart from the members of the team, also Miss M. Polhout and Messrs. J. Bos, J.J.M. v.d. Burg, S.B. Meres, F.W. Soerodimedjo, and E. Voorhoeve cooperated in carrying out the experiments. We are again grateful to the SNUiF (Dutch Onion Federation) for the hospitality offered in their headquarters.

Fourth release experiment at the Schuilenburg (J. Ticheler)

By the end of 1973 only a small population of onion flies, 700 pupae, remained at the experimental site of the Schuilenburg, Lienden. This population seemed too small to carry out another meaningful sterile release experiment. Therefore this population has been reinforced in the spring of 1974 with 9 000 fertile flies, dug in as young pupae and hence emerging well distributed in time. In this population, as fourth and last experiment on this scale, weekly around 60 000 sterile insects have been released, again dug in as pupae and provided with a weekly changing dye mark. Protection against birds proved necessary. A web of artificial fibres (Starex) spread over the trays with pupae worked very satisfactory.

The sterility in the field population has been followed during the whole season, on an average it amounted to 94 %. From the scarce fertile females brought to the laboratory only a few egg batches were obtained, too few to draw conclusions on the fertility of the wild females. The observations on the damage, however, show clearly that the fertility has been very low. During the season the infestation accumulated to 0.08 % of the plants. On a check plot, at a distance of 2 km, the infestation was 13 times heavier, from a practical standpoint still hardly noticeable. The reduction of the population, measured at harvest time, was also 13 times bigger than on the check plot. The population remaining on the experimental plot at the end of 1974 was estimated at 200 pupae on 1.3 ha.

The emergence of the onion flies from depots installed during the

autumn of 1973, showed a picture very different from the curves of previous years. The relatively mild weather during the first half of April caused the first flies to emerge on April 15th, again simultaneously with the unfolding of the leaves of the summer oak. Thereafter the flies continued to appear in steady numbers until half June, the last flies appeared even half July. In previous years emergence started two to three weeks later and showed a much more marked peak in a shorter period of time. The decision to release the sterile flies according to a schedule providing for the regular release of constant numbers rather than aiming at following the emergence pattern was thus justified.

Mass rearing (J. Noorlander)

Rearing the pupae, required for the two field experiments, was started on September 10, 1973, putting pupae at 21°C in order to accommodate 34 fly cages. We intended to perform the rearing in the shortest possible time. To enable dealing with the large numbers of trays with larval diet, a climate chamber was installed with 12 racks at each wall. In this way around 2 million pupae could be reared in this cell in three weeks. For the larval diet a cheaper yeast was found. This animal food quality yeast showed, however, strong variation in quality, resulting after initial success in a complete breakdown of the rearing, at the expense of some 3 million larvae. Upon replacement of this yeast by good quality yeast, larval growth remained nevertheless below par. It appeared necessary to sterilize room and trays with formalin before normal production could be resumed. Mixing 0.05 % formalin 40 % through the larval medium gave the same result.

Ultimately 3.5 million pupae have been reared in 4 months. This is an average of 0.9 million per month with a peak production of 2 million per month. The average weight of the pupae was, notwithstanding the setbacks, satisfactory.

This year for the first time, diapauzing pupae were produced, by rearing under conditions of short day (10 hrs. of light) and low temperature (17°C). This method renders the tedious work of separating pupae and larvae daily, superfluous. A disadvantage is the long duration of the larval stage (24 days) when compared with rearing of non diapauzing pupae (at 22°C 14 days).

The industrial mixer (capacity 90 l) used this year produces a satisfactory medium and saves a lot of labour. A start has been made with the development of a new type of fly cage based on a model for rearing Medfly (devised by D.J. Nadel, IAEA, Vienna).

Radiobiology and radioisotopes (J.Ph.W. Noordink)

In the course of the studies with the onion fly, it appeared that in many competition, labeling and irradiation experiments, normal females when crossed with irradiated or isotope-labeled males showed a higher fecundity than when crossed with normal males. In order to test this phenomenon an experiment was set up, irradiating males with increasing doses of X - rays. The results are summarized in table 1.

table 1. Fecundity of females when crossed with irradiated males.

treatment	no. of eggs /female/day
0 R	1.62
500 R	1.68
1 000 R	1.88
1 500 R	2.16
2 000 R	2.69
3 000 R	2.65

In this experiment 1 840 flies and 46 000 eggs were involved. Here again a tendency for higher fecundity, decreasing at higher radiation doses (males are sterilized at 3 kR) appeared. The experiment yielded also data on the life span of untreated females and irradiated males, which are shown in table 2.

table 2. Life span of normal females and of irradiated males when crossed with each other.

treatment of males	life span of males	life span of untreated females
0 R	17 days	29 days
500 R	20 "	28 "
1 000 R	21 "	28 "
1 500 R	23 "	35 "
2 000 R	27 "	39 "
3 000 R	22 "	35 "

Also concerning life span a stimulating effect of sub-sterilizing doses of radiation becomes apparent. An explanation for this effect on the not treated females has not yet been found. Summarizing, in table 3, figures are given for the influence of the treatment of the males on the fecundity of normal females. In this table the results are given of the competition, labeling and irradiation experiments carried out in the course of the years.

In cooperation with M. Loosjes an experiment has been carried out in order to evaluate, using radioisotope labeled onion fly eggs, survival and possibly predation. As we wanted radioactive eggs, and not merely radioactive flies, the initial dosis of ⁶⁵Zn in the larval medium was increased to 1 mCi per 500 g, two times as much as normal. The

table 3. Summary of fecundity data for normal females, crossed with irradiated or labeled males.

mating	treatment	no. of eggs
♀ x ♂ ¹⁾	control	2 348
♀ x ♂ x ♂	3 000 R	4 283
♀ x ♂	control	4 333
♀ x ♂ x ♂	3 000 R	7 862
♀ x ♂	control	4 219
♀ x ♂	1 500 R	7 252
♀ x ♂	2 000 R	8 398
♀ x ♂	3 000 R	7 653
♀ x ♂	control	1 289
♀ x ♂	⁶⁵ Zn	2 306

1) ♂ = treated male.

flies obtained from this had an average radioactivity of 670 c/min. Eggs of these flies have been collected twice a week and sent to Flakkee. In total 10 000 eggs were obtained and put out in the field. During the summer, onion fly larvae and potential predators were sent to the laboratory and autoradiographed. Preliminary results indicate that ⁶⁵Zn is very suitable for survival experiments. The isotope has also been found in several predators. They belong to ; Staphilinidae, Agriotidae, Carabidae, micro spiders and predatory mites. Part of the radioactive eggs have been further bred in the laboratory on artificial

diet. Several specimens in various development stages have been autoradiographed to enable comparison of the blackening of the röntgenfilm with that of animals collected in the field.

Experience elsewhere indicates that mosquitoes treated with substerilizing doses of radiation give rise to offspring with a high level of sterility. By the end of the year an experiment has been started to test whether this holds also for the onion fly. The doses administered to males are 750 and 1 500 R, and normal females, when crossed, show a residual fertility of 20 and 6 % respectively.

The suitability of Timothee grass as food source for the adult onion fly had been demonstrated earlier. This year flowering, ³²P labeled Alopecurus grass was offered to the flies. Both flies and eggs became radioactive, indicating that flowering grasses form an important food source in the field, providing a.o. the proteins necessary for egg laying.

Ecology of the onion fly (M. Loosjes)

This year the effort was concentrated on the application of the sterile male technique under practical conditions on the island of Flakkee, which is an onion growing area. In relation to this experiment study has been made of release methods and of mortality. The release experiment on Flakkee was desirable in order to verify whether the results of the Schuilenburg experiments are applicable in onion growing areas where higher population densities, higher reproduction rates and more immigration of onion flies occur. As experimental area the Pallandtpolder, near Middelharnis, was chosen. This polder borders along one long side on the Haringvliet. Simulation of mark-release experiments carried out on the same site in 1973 made it plausible that this 3 km wide stretch of

water acts as a reflecting barrier for dispersing flies. The fly population, on one of the 1973 experimental sites, was, by means of extensive sampling, estimated at 23 560 pupae. On this spot the new experiment, 1 ha in size, was situated. A not treated checkplot, $\frac{1}{2}$ ha in size, being part of a bigger onion field, was located at a distance of 2 km. Here the population density was twice as big as on the experimental plot.

On the main 1973 onion fields in and around the Pallandtpolder the wild onion fly populations have been mixed with fertile, reared, labeled flies in order to estimate numbers and origin of immigrants on the other onion fields. In the whole area traps, mostly with an attractant, were installed.

On the experimental plot weekly sterile flies have been released, in total 1 400 000, most of them dye-labeled. During the first flight sterile flies, 170 000 in total, have been released near the closest sources of fertile flies in order to restrict the deleterious influence of immigrants.

The percentage sterile females on the experimental plot could be determined rather accurately on the basis of ovary development, since more than 95 % of fertile females caught had clearly developed ovaries, whereas the sterile females have completely undeveloped ovaries. The percentage sterile males generally differed somewhat from that of the females. On the experimental plot, calculated over the whole season, about 90 % of the males was sterile, and on the check plot 0.25 - 4 %. The percentages of pupae which went in diapause during the first and second generation could be estimated very roughly only. The third generation cannot have reproduced in view of the climatological conditions. On the basis of these data a reduction of the population on the experimental plot by a factor 80 could be expected. In reality the population on the experimental plot decreased by a factor 12, whereas on the checkplot the population increased with a factor 10. The actual reduction factor, due to the releases of sterile insects, therefore, was around 120.

With the aid of simulation the deleterious influence of immigration on the results is being studied, this in view of forecasting the results which can be expected from releases on bigger areas.

The damage on the experimental plot, on the basis of germination counts, amounted to 2.4 %, which is far below the economic threshold. Damage on the checkplot was 17.4 %, not taking into account the fact that only 16 % of the second generation of flies reproduced on the checkplot, the remainder laying eggs on the surrounding chemically treated field.

We tried to study the mortality of the juvenile stages by putting into the field over 7 000 eggs labeled with ^{65}Zn via the parents and by sampling these at different times. At the same time we looked for potential predators. The first results indicate the usefulness of this method (see report Noordink).

Histological studies (J. Theunissen)

The research on the normal gametogenesis has been completed with the study of the gonial development in the pupal stage and of the development of the adult testis.

The development of the testis during the pupal stage can be characterized by a.o. the appearance of different cell types at certain stages of the development. On the basis of this type of observations a system of criteria could be formulated that defines the development of the pupal testis at each moment. As the pupal development is a continuous process, a scale has been found for the development of the pupa as a whole. This is important for the study of processes which play a role in the pupal stage, as is the case for the study of diapause. For the development of the ovaries in the pupal stage a similar system has been worked out.

We studied at the same time the stage at which pupal development is arrested when diapause is induced, and how far these pupae differ from pupae in which development is arrested by low temperature (storage). We used gonial development as a standard. Pupae in diapause showed a development similar to non diapausing pupae after 2 - 3 days of normal development. This is precisely the stage at which non diapausing pupae can be stored for a prolonged period at 3°C . Differences between both types of pupae are small at this stage. The cell division activity of the gonial cells is likely to be greater in the non diapausing pupae.

Descriptions have been made of the development of the adult testis at various ages. Some of the conclusions which can be drawn are : that after 10 days already the function of the testis shifts from production to storage, that part of the population of spermatogonia stabilizes, whereas the rest diminishes greatly in number and disappears, and that after 5 days already stagnation in the spermatogenetic processes occurs visible as a decrease of the number of primary spermatocytes.

The study of histopathological reactions of the gonads to various doses of X rays has been continued. The reaction of the testis to sub-sterilizing, sterilizing and higher doses was studied on the level of the individual cell, of the cell population and of the organ. Although

the individual variability is considerable, it could be established that the different cell types react in a characteristic manner to the irradiation. Qualitatively the reaction pattern is rather independent of dose, but the speed at which the pattern proceeds is dependent on dose and on the time between irradiation and fixation. In this respect age of the fly is irrelevant.

Upon irradiation of testes with 0.5 and 1.0 kR, partial repair of the damage done occurs. This repair is visible as a partial repopulation of the testis. The latter process is stopped by a rapid degeneration of the newly formed cells before sperm is produced. Thus the repair does not contribute to the amount of sperm already present (see also Ann. Rept. 1973, p. 750). On the basis of the reactions of the various cell populations to irradiation, criteria could be formulated for their relative radiation sensitivity. These criteria for relatively low and high doses have proven their usefulness as a new approach for comparative radiobiological studies of insects, based upon the histopathological reactions.

Other research topics

Pupal development (J. Ticheler)

In earlier experiments (Houwing, Ann. Rept. 1973, p.751) the emergence of pupae at 12° C did not follow the normal pattern which occurred at temperatures above 12°. Part of the flies appear according to a normal distribution: over a short period an increase in emergence, followed by a maximum, after which a decrease takes place. On a low level then the emergence continues for months. This suggests the induction of diapause in part of the pupae. Another possibility is that the change from the storage temperature, 3°C, to 12°C is too small to start development simultaneously in all pupae. In an experiment, therefore, development of pupae in diapause and of pupae not in diapause has been studied at 14, 12, 10 and 8°C. The diapausing pupae had been subjected, during storage, to enough cold to break diapause. The non diapausing pupae had been stored also for half a year at 3°. Half the number of pupae, moreover, received a "heat treatment" of 6 hours at 14°C. The results for the various temperatures agree very well, albeit that, at the lower temperatures, the effects occur to a stronger degree. At 14°, where no extra heat shock had been applied, the emergence from the non diapausing pupae followed a normal distribution. The last percent of the flies emerged over a period of more than three months. This phenomenon was observed also, but to a higher degree, at the lower temperatures in all pupae developing at 12 and 10°. Non diapausing pupae emerged earlier without than with a heat shock. One might conclude that the heat shock sensibilizes pupae for induction of diapause, which should then be possible also in the early pupal stage. In pupae which were already in diapause, the heat shock accelerates development considerably, it starts the development. Also at 8° a few flies emerged, namely from diapausing pupae with heat shock and from non diapausing pupae without heat shock. It appears contrary to earlier considerations, that the developmental threshold lies below 8°. Extrapolation of the values for the speed of development between 8 and 30° yields a threshold temperature of 6° C.

The data on the speed of development are being used to determine the relation between soil temperature and fly emergence in the field.

This analysis has not yet been completed.

Induction of diapauze under influence of photoperiod and temperature

(P.M.J. Ramakers)

Continuing the work of Kelderman (Ann. Rept. 1972, p. 673) the influence of photoperiod and temperature on the induction of diapauze has been investigated. At 25°C diapauze can hardly be induced by differences in photoperiod. At 14 almost all pupae enter diapauze except under a long day regime of 16 hrs of light or more. At intermediate temperatures interaction between day length and temperature occurs, most clearly observed at 18°. This is shown in table 4.

table 4. Photoperiod induced diapauze at 18° C.

day length	% diapauze
0 hrs.	27 %
8 hrs	52 %
10 hrs	89 %
12 hrs	97 %
13 hrs	83 %
14 hrs	39 %
15 hrs	14 %
18 hrs	3 %
24 hrs	19 %

Depending on day length induction of diapauze can be almost complete or almost totally absent. It is remarkable in a soil inhabiting insect to be so photoperiod sensitive. It is possible, however, to observe day length as night length, which is not in disagreement with the strong ne-

gative phototaxis of the larvae. In connection herewith the observation is important that, both in mass rearing and in infection experiments on onion plants, the larvae were found wandering around on the surface during darkness.

The results of these investigations are being applied in the mass rearing.

Attractive substances (P.V.I.M. Wolff)

The search for lures for the onion fly would be facilitated when a laboratory screening method were available. From a number of types the most suitable olfactometer has been chosen. In this olfactometer attraction by light is combined with a choice between two adjacent sources of scent. The apparatus has been tested by using onion juice as a lure. The age of the test animals appeared to be important: young flies hardly react to onion smell, whereas older flies react to a lesser degree to light. Most suitable are flies of 4 - 5 days old. The attractive substances of onion are found predominantly in the fraction which is extracted with penthane and to a smaller extent in the dichloro methane fraction. Rotting onion juice is more attractive than freshly made juice.

Allium vineale as possible hostplant (P.V.I.M. Wolff)

The wild Allium species A. vineale occurs frequently in the onion growing areas. Therefore, observations have been made on the infestation of this plant by the onion fly in banks of roads and ditches around Wageningen and Lienden. Around 400 plants have been checked regularly but at no time infestation was found. This was confirmed by laboratory experiments. Although the plant is attractive to the fly for oviposition, no larva completed its development on the bulbs. Its skin is probably too firm, as infection succeeded on cut bulbs.

Associate della Commissione: Comitato Nazionale per l'Energia
Nucleare, Laboratorie Applicazioni Agricoltura

N° del contratto: 107-72-1 BIOD

Cape del gruppo di ricerca: prof. A. Bozzini

Tema generale del contratto: Biological control of Ceratitidis capitata
by nuclear technique.

Cape del Progetto e collaboratori: dott. U. Cirio, dott. I. De Murtas,
dott. F. Barbera.

Descrizione dei risultati:

The Precida Medfly pilot experiment

1. Field work

a) One of the main objectives of this experiment is to study the impact of the SIT on the Precida agroecosystem since in this environment many inter-specific competitors of the medfly (larval stage) are present (Tab. 1). The observations are mainly based on the abundance of two key Lepidoptera pests (Grapholitha molesta Brusck and Laspeyresia pomonella L.) during and after the phase of suppression of the medfly in the island. The larvae and the adults of these populations are checked in four systematic sampling areas by fruit (peaches) examination and captures of the adults with pheromone traps. The purpose of these controls was to make a comparison with similar data collected on medfly (Tab. 2). From this figure it appears that suppression of Ceratitidis does not cause an increase of the abundance of the two pidoptera species: It is reasonable to hypothesize that the density of these populations is mainly regulated by other factors, as meteorological conditions and natural enemies, and scarcely influenced by competition for food.

Nevertheless, a longer period of ecological observations are necessary in order to determinate the real impact of this technique in the island of Precida.

b) Behavioral aspects of ecology of Medfly. The field study of the behaviour of this species is of fundamental importance in order to work intelligently with the SIT. These studies could in fact provide:

- parameters for the control of the quality of the sterilized insects;
- criteria for improving the techniques of release;
- information on the movement of the flies.

The experiments on this subject have been carried out using wild and sterile adults in order to have comparative data on the behaviour of the medfly. The studies regarding the long movement of Ceratitidis in the island (Tab. 3) show a clear spatial distribution of the adults which is mainly related to the seasons, the fruit crops, and the food availability. There is a good evidence for a preferential aggregation of flies toward the citrus around all the year. This indicates that in the Precida environment the releases should be initiated only in these crops. The trials concerning the short movement of the Ceratitidis (local flight) show that the sterile ones have a lesser dispersion than the wild ones (the recapture ratios are respectively 10% and 0.5%) (Tab.3). Consequently the utilization of laboratory flies for estimating the natural population density by mark-recapture technique should be used with caution. In Table 4 are reported the data on the movement of the adults observed among the trees and on the trees. The most finding seems to be mainly based on olfactory and visual stimulus. Once arrived on the host, the orientation of the fly toward the fruit is rather visual than olfactory. These experiments also indicate the tendency of Ceratitidis to aggregate on the top and sunny part of the trees. Flight activity, including the reaction to trimedlure, commonly reaches one daily peak in the afternoon (Tab. 3 and 4). For these aspects of the movements no differences were noted between wild and sterile insects. In the

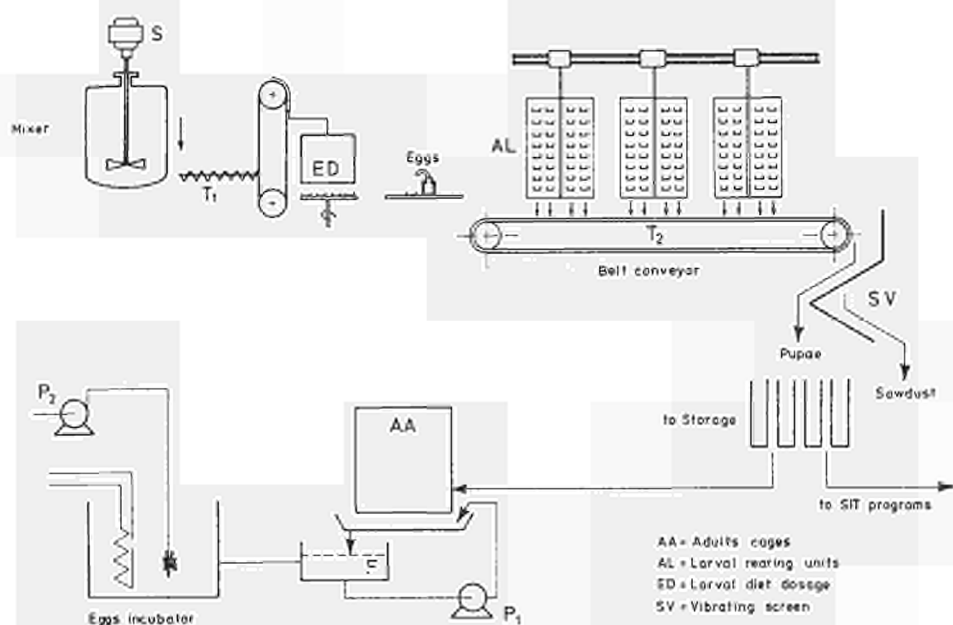
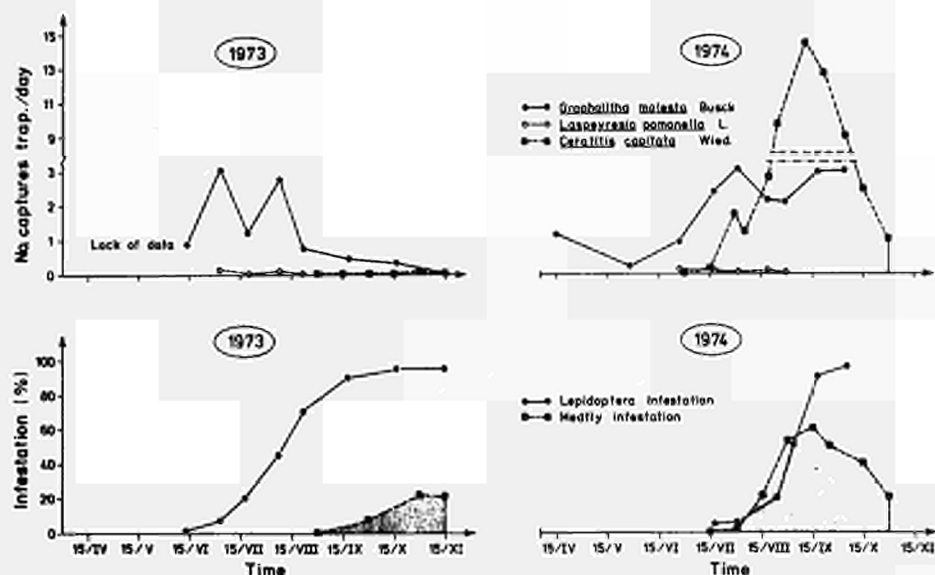


Fig. 1 Pilot plant for mass-rearing of medfly in the Casaccia insectary.

F R U I T S

Drupaceae (Peaches, Apricots)	Rosaceae (Apples, Pears)	Moraceae (Figs)	Rhamnaceae (Jujubes)
<i>Anarsia lineatella</i>	<i>Grapholitha molesta</i>	<i>Ephesia cautella</i>	<i>Carpomya vesuviana</i>
<i>Grapholitha molesta</i>	<i>Laspeyresia pomonella</i>	<i>Lonchea aristella</i>	
<i>Laspeyresia lunibrana</i>	<i>Laspeyresia pyrivora</i>	<i>Caropophilus hemipterus</i>	
<i>Laspeyresia pomonella</i>			
<i>Caropophilus hemipterus</i>			

Tab 1 - Main inter-specific competitors of medfly (larval stage) in Pracida



Tab. 2 - Abundance of medfly and two lepidoptera species in the island of Procida during and after the second year of the suppression of medfly in the island by sterile insect technique. [Data collected only on infested paches]

Subject	General observations	Experiment plans	Data
1. Long movement (Dispersion flight)	<p><u>Basic influences:</u></p> <ul style="list-style-type: none"> - Climate - Seasons - Stress - Fruit crops - Age of the flies - Medfly density 	<ul style="list-style-type: none"> - Use of trimedure traps in different crops, examination of the fruits infestation, visual observation Observation in relation to: <ul style="list-style-type: none"> - Seasons - Fruit crops - Food availability - Sheltering site 	<p>The diagram shows a cross-section of fruit crops: Citrus, Citrus & Stone fruits, Grape, and Grape & Stone fruits. A vertical axis indicates 'Height (m)' from 0 to 1. A horizontal axis indicates 'Adults abundance' with 'X' marks representing captures. The captures are highest in the Grape and Grape & Stone fruits sections during the summer and autumn months.</p>
2. Short movement (Local flight)	<p><u>Basic influences:</u></p> <ul style="list-style-type: none"> - Climate - Shortage of fruit - Stress - Adults age - Adult density 	<ul style="list-style-type: none"> - Use of mark flies and their recapture with traps Observation in relation to: <ul style="list-style-type: none"> - Temperature - Sun position - Quadrants - Hours of the day 	<p>The diagram shows a 50m x 50m square area with a release point 'o' and several capture points marked with dots. A graph shows 'Male captures (%)' over time from 10:00 to 12:00. The captures increase from 0% at 10:00 to a peak of about 80% at 11:00, then decrease to about 20% at 12:00. The legend indicates: Recapture - wild (n=0.5%), sterile (n=10%).</p>

Tab. 3 Dispersive and local movement of medfly in the Procida island

Subject	General observations	Experiment		D a t a																																									
		Plans	Devices	Vertical	Horizontal																																								
3 Host finding (long distance)	<p align="center">Basic influences:</p> <p align="center">Climate → Temperature → Humidity → Wind</p> <p align="center">Flies → Hosts → Noxial</p> <ul style="list-style-type: none"> - Age - Density - Species - Smell - Size - Shape - Topography position - Abundance 	<p>Use of cylinder net coated with slickum around one tree.</p> <p>Observations in relation to:</p> <ul style="list-style-type: none"> - Temperature - Sun position - Quadrants - Hours of the day 		<table border="1"> <thead> <tr> <th rowspan="2">TIME PERIOD</th> <th colspan="2">CAPTURES</th> </tr> <tr> <th>NO.</th> <th>%</th> </tr> </thead> <tbody> <tr><td>8-10</td><td>210</td><td>30.4</td></tr> <tr><td>10-12</td><td>188</td><td>26.5</td></tr> <tr><td>12-14</td><td>102</td><td>14.5</td></tr> <tr><td>14-16</td><td>102</td><td>14.5</td></tr> <tr><td>TOTAL</td><td>502</td><td>100.0</td></tr> </tbody> </table>	TIME PERIOD	CAPTURES		NO.	%	8-10	210	30.4	10-12	188	26.5	12-14	102	14.5	14-16	102	14.5	TOTAL	502	100.0	<table border="1"> <thead> <tr> <th rowspan="2">TIME PERIOD</th> <th colspan="2">CAPTURES</th> </tr> <tr> <th>N</th> <th>%</th> </tr> </thead> <tbody> <tr><td>8-10</td><td>2</td><td>0.3</td></tr> <tr><td>10-12</td><td>3</td><td>0.4</td></tr> <tr><td>12-14</td><td>8</td><td>1.1</td></tr> <tr><td>14-16</td><td>18</td><td>2.5</td></tr> <tr><td>TOTAL</td><td>29</td><td>4.0</td></tr> </tbody> </table>	TIME PERIOD	CAPTURES		N	%	8-10	2	0.3	10-12	3	0.4	12-14	8	1.1	14-16	18	2.5	TOTAL	29	4.0
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4 Fruit localization (short distance)	<p align="center">Basic influences:</p> <ul style="list-style-type: none"> - Climate - Color - Smell - Shape - Size 	<p>Use of artificial oranges coated with slickum on the tree.</p> <p>Observation in relation to:</p> <ul style="list-style-type: none"> - Temperature - Sun position - Quadrants - Hours of the day 		<table border="1"> <thead> <tr> <th rowspan="2">TIME PERIOD</th> <th colspan="2">CAPTURES</th> </tr> <tr> <th>N</th> <th>%</th> </tr> </thead> <tbody> <tr><td>8-10</td><td>102</td><td>11.1</td></tr> <tr><td>10-12</td><td>171</td><td>18.9</td></tr> <tr><td>TOTAL</td><td>273</td><td>30.0</td></tr> </tbody> </table>	TIME PERIOD	CAPTURES		N	%	8-10	102	11.1	10-12	171	18.9	TOTAL	273	30.0	<table border="1"> <thead> <tr> <th rowspan="2">TIME PERIOD</th> <th colspan="2">CAPTURES</th> </tr> <tr> <th>N</th> <th>%</th> </tr> </thead> <tbody> <tr><td>8-10</td><td>2</td><td>0.7</td></tr> <tr><td>10-12</td><td>3</td><td>1.1</td></tr> <tr><td>12-14</td><td>8</td><td>2.9</td></tr> <tr><td>14-16</td><td>16</td><td>5.9</td></tr> <tr><td>TOTAL</td><td>29</td><td>10.6</td></tr> </tbody> </table>	TIME PERIOD	CAPTURES		N	%	8-10	2	0.7	10-12	3	1.1	12-14	8	2.9	14-16	16	5.9	TOTAL	29	10.6						
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Tab.4 Movement of medfly observed among the trees and on the tree in the Procida island

Ingredients	Cost / kg (Lire)	Quantity	Diet type						
			A	B	C	D	E	F	G
Tap water	—	l	38.400	38.400	38.400	38.400	38.400	38.400	38.400
Ground wheat straw	30	kg	8.000	8.000	8.000	8.000	8.000	8.000	8.000
Bear yeast (debufferized)	1300	kg	6.400	3.200	1.600	—	—	—	—
Dried torula yeast	250	kg	—	3.200	4.800	6.400	6.400	6.400	6.400
Sugar	260	kg	9.600	9.600	9.600	9.600	4.800	2.400	—
Sugar beet molasses	50	kg	—	—	—	—	4.800	7.200	9.600
Nipagin	5000	kg	0.096	0.096	0.096	0.096	0.096	0.096	0.096
Sodium benzoate	500	kg	0.096	0.096	0.096	0.096	0.096	0.096	0.096
HCl 37 %	500	l	0.544	0.544	0.544	0.544	0.544	0.544	0.544
Yield % (eggs—pupae)			68.33 (1.333)	63.96 (1.338)	61.05 (1.182)	60.00 (1.085)	58.96 (1.071)	55.55 (1.085)	58.33 (1.107)
No pupae / kg diet			15.600	14.850	14.850	13.940	13.700	12.900	13.950
Weight of 100 pupae (2 days before emergence)			0.755 (1.000)	—	—	0.709 (1.0107)	—	—	0.738 (1.000)
Emergence % (25 °C)			82 (1.81)	—	—	92 (1.81)	—	—	90 (1.81)
Mortality % (after 40 days)			22 (1.31)	—	—	21 (1.31)	—	—	22 (1.31)
Cost in Lire for one million of pupae (may 1974)			11.860	—	—	6420	—	—	3900

Weight to produced 1 million of pupae

Tab.5-Larval rearing diets for medfly in the Casaccia insectary.

preliminary study discussed here, it is shown that the movement of the flies in the Procida environment is a complex function of a large number of interacting factors which need to be studied in greater detail.

2. Laboratory work.

To meet the programme production of 10-15 millions flies per week, the development of efficient and economical rearing methods became of immediate concern. For these purposes the system of mass-rearing of the medfly was modified making changes in regard to egg collection, egg and larval handling and pupae recovery (Fig. 1). For the larval rearing a less costly diet was formulated replacing the sugar and the debitterized beer yeast with sugar beet molasses and dried torula yeast (Tab. 5). The diet type "G" seems the most advantageous comparing the biological parameters here considered. Using this new diet the production costs per million pupae average about \$ 6.

PUBLICATIONS

- U. Cirio, M. Capparella - Conferma dell'efficacia della tecnica di lotta dell'insetto sterile nel controllo della Ceratitis capitata Wied (Diptera, Trypetidae) nell'isola di Procida. X Congresso Nazionale di Entomologia, Sassari, 20-25 maggio 1974 (in press).
- U. Cirio, S. Saleme - Contributo al miglioramento massivo della Ceratitis capitata Wied (Diptera, Trypetidae). X Congresso Nazionale di Entomologia, Sassari, 20-25 maggio 1974. (in press).
- U. Cirio - Basi ecologiche per un programma di lotta contro la Ceratitis capitata Wied nell'isola di Procida. Redia. (in press).

V.

FORSCHUNGSTÄTIGKEIT ANWENDUNGEN MEDIZIN

RESEARCH ON APPLICATIONS IN MEDICINE

RECHERCHES RELATIVES AUX APPLICATIONS MEDICALES

NUKLEARMEDIZIN

NUCLEAR MEDICINE

MEDECINE NUCLEAIRE

Vertragspartner der Kommission:

Universität Ulm, Abteilung Nuklearmedizin (Radiologie III)

Nr. des Vertrages 116 - 72 - 1 bio D

Leiter der Forschungsgruppe: Prof.Dr.W.E.Adam

Wissenschaftliche Mitarbeiter: Dr. F. Bitter, Dr. H. Geffers,
Dr. Ch. Horn, Doz.Dr. Kampmann, Dr.G. Meyer und Ing.grad.R.Weller

Allgemeines Thema des Vertrages:

Entwicklung eines nicht traumatisierenden Verfahrens zur Analyse der Herzkinetik mit Hilfe der quantitativen Funktionsszintigraphie und Anwendung simulierender Verfahren.

Im Jahre 1974 setzte die physikalische Arbeitsgruppe ihre Programmierungsarbeiten fort. Unter den Softwarearbeiten zur automatischen Analyse und Bestimmung von Herzbereichen und zur Datenfilterung soll die Entwicklung eines Programms "Kinetics" hervorgehoben werden, das die Grundlage eines neuen Verfahrens zur morphologischen Beurteilung der Herzkinetik bildet.

Die medizinische Arbeitsgruppe hat im Jahre 1974 folgende Arbeiten durchgeführt:

- 1) Entwicklung des Verfahrens "Kamera-Kinematographie" als klinisches Verfahren zur morphologischen Beurteilung der Herzkinetik.
- 2) Korrelation der Zykluskurven mit EKG, Karotis-Pulskurve und Phonocardiogramm.
- 3) Erste Anwendung des Verfahrens auf herzkranken Patienten und zur Beurteilung pharmakologischer Wirkungen.

Bericht der physikalischen Arbeitsgruppe

Die Softwareentwicklung erbrachte ein Programm "Select heart area", mit dessen Hilfe es möglich ist, Bereiche der Ventrikel einerseits und der Vorhöfe bzw. großen Gefäße andererseits auf dem Display zu differenzieren. Das Verfahren beruht

auf der Auswahl systolisch-diastolisch positiver bzw. negativer Differenzen. Systolisch-diastolisch positive Impulsdifferenzen entsprechen dabei den großen Gefäßen bzw. Vorhöfen, negative Differenzen den Ventrikeln. Die Entwicklung von Filterverfahren, die nicht nur der Verbesserung der Bildqualität, sondern auch der Gewinnung von Kriterien zur Differenzierung verschiedener Herzanteile ^{dienen} wurde 1974 begonnen. Als klinisch bedeutsames Programm wurde "Kinetics" entwickelt, das es ermöglicht, eine beliebige Zeile oder Spalte unserer Matrix auf dem Sichtschirm als Radioaktivitätsprofil darzustellen. Auf Startbefehl wird die Änderung des Radioaktivitätsprofiles als Funktion der Zeit in Echtzeit dargestellt, so daß ein kinematographischer Effekt entsteht.

Medizinische Arbeitsgruppe:

Nach den im Vorjahr erbrachten Nachweisen der Reproduzierbarkeit der Ergebnisse konzentrierte sich im Jahre 1974 die Arbeit der medizinischen Gruppe auf die klinische Ausbeute des Verfahrens.

Folgende Arbeiten wurden durchgeführt:

- 1) Auf der Grundlage des Programmes "Kinetics" wurde eine "Kamera-Kinematographie des Herzens" entwickelt. Die schnelle Repräsentation der 15 Phasenbilder auf dem Sichtschirm ergibt ein grobes Bild der Mechanik des Herzens. Einzelheiten sind dabei allerdings nicht zu erkennen, da die Konturen unscharf und die betrachteten Intensitätsunterschiede zu gering sind. Mit Hilfe des Programms "Kinetics" kann aber Zeile um Zeile und Spalte um Spalte der Matrix dargestellt werden. Die rasch aufeinander folgende Repräsentation der betreffenden Spalte als Funktion der Zeit ergibt einen unmittelbaren Eindruck der Kontraktion und der Erschlaffung des Myocards in dem beschriebenen Herzabschnitt. Ein Sagittalschnitt durch die linke Herzhälfte ermöglicht so eine unmittelbare Differenzierung von Vorhof und Ventrikel an Hand der asynchronischen Bewegung beider Bereiche. Ein Horizontalschnitt durch beide Ventrikel im Herzspitzenbereich ergibt eine unmittelbare Vorstellung des Ausmaßes der Kontraktion und der Kontraktionscharakteristik etwa im Bereich des linken Ventrikels. Das Verfahren ist an-

schaulich und von großer Plastizität. Der Beobachter am Sichtschirm kann jeden beliebigen Bereich des Herzens und seine Mechanik analysieren. Das Programm ermöglicht außerdem die Indizierung eines jeden Punktes, der einer Oberfläche des Herzens von etwa $3/4 \text{ cm}^2$ entspricht. Dabei wird die dem Punkt zugeordnete einen Herzzyklus beschreibende Zeit- Aktivitätskurve simultan dargeboten. Das Verfahren erlaubt selbstverständlich daneben die Darstellung von weiteren Zeit- Aktivitätskurven, etwa des linken Ventrikels. Da aber aufgrund der Bewegungscharakteristik der linke Ventrikel besser als früher abgegrenzt werden kann, dürften die gewonnenen Werte zuverlässiger sein, als die bei den üblichen Bildverfahren gewonnenen.

- 2) Vorgehende im Bericht für 1973 dargestellte Untersuchungen hatten ergeben, daß das Verfahren reproduzierbar ist und bei Wiederholung am selben Patienten Zeit- Aktivitätskurven erbringt, deren Vergleich eine gute Korrelation ergibt. Wir haben 1974 eine Serie mit freiwilligen Versuchspersonen begonnen zur Korrelierung der von uns erhaltenen volumenabhängigen Kurven mit dem EKG, der Karotispulskurve, und dem Phonocardiogramm. Eine statistische Analyse ist bei der zur Zeit laufenden Versuchsreihe noch nicht möglich.

- 3) Erste Untersuchungen an herzkranken Patienten, sowie vor und nach pharmazeutischer Behandlung: Bei den bisherigen Untersuchungen hat sich dabei als besonders wertvoll die Kamera-Kinematographie erwiesen. Obwohl bis jetzt statistische Aussagen noch nicht möglich sind, zeichnet sich doch folgendes ab: Das Radioaktivitätsprofil eines Horizontalschnittes durch beide Ventrikel im linken schrägen Durchmesser läßt einen Einschnitt im Bereich des Herzprofils erkennen, der im wesentlichen dem Septum entspricht, soweit nicht überlagernde Lungenpartien das Bild verzerren. Bei einer Linksdilatation zeigt das Radioaktivitätsprofil links des Septums ein größeres Maximum als rechts. Die Kinematographie erbringt bei einer Herzinsuffizienz eine geringere Kontraktion des linken Ventrikels, darstellbar als sog. "Bewegungsfläche" (Einzelheiten siehe angefügte Literatur!).

Vor und nach Gabe von Digitalis ist die Verkürzungsgeschwindigkeit der Herzmuskulatur verändert. Die an unserem Sichtschirm erzielten Bilder wurden in einem Film festgehalten, der von Herrn Adam im Verlauf des ersten Weltkongresses für Nuklearmedizin, Tokyo 1974, und von Herrn Bitter im "5. Symposium on Sharing of Computer Programs and Technology in Nuclear Medicine", Salt Lake City, 15./16. Januar 1975, vorgeführt wurde. Das Verfahren wurde weiterhin im Verlaufe eines Seminars erörtert, das Herr Bitter am 22. 1. 1975 in Baltimore (John Hopkins Universität) hielt.

Die quantitative Auswertung der Daten zur Bestimmung der Auswurfsfraktion ist nicht ohne weiteres möglich über die diastolisch-systolischen Impulsraten, da eine erhebliche Untergrundbelastung vorhanden ist. In Untersuchungen an einem Herzmodell sowie in Tierexperimenten am Mikroschwein führte Herr Meyer eine Subtraktion des Lungenuntergrundes durch, erzielte jedoch keine befriedigenden Resultate. Die Bestimmung der Austreibungsfraktion ist aber möglich über die Größenbestimmung des linken Ventrikels in Systole und Diastole und Umrechnung mit Hilfe des für die Angiocardiographie bereits aufgebauten Formelmechanismus.

- 4) Beginn der Arbeiten mit einem Ultraschallgerät, zur Standardisierung der Herzgröße und Bestimmung der Auswurfsfraktion.

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Centro di Medicina Nucleare Università Pisa

Contract No. 110-72-1 BIOI

Director: Prof. Luigi DONATO

Subject of Contract: Research and development of diagnostic technique and pathophysiological methods using in vivo and in vitro radioactive tracers.

In 1974 investigations were continued aimed at the development of new methods for early detection of disease state and quantification of function in pathological conditions.

In the field of radiochemistry (Projects 1 & 2) significant progress has been made in the understanding of problems and development of methods for the radioimmunoassay of non antigenic molecules, and in the definition of antibody sites properties in various physicochemical states. Particularly important appear the results on the behaviour of antibody sites in solid phase.

In vivo studies have been reach of positive results. Insulin metabolism definition in physiological conditions, uremia and diabetes, and setting up of a protocol for unsteady state conditions represent significant achievements. In the lung field, a comprehensive review of 4 years work on the control of pulmonary blood flow distribution has been published, and some important methodological innovations have been investigated and are now being tested, ranging from the use of ^{99m}Tc -microspheres for the study of lung ventilation, to the use of antipyrine labelled with cyclotron produced ^{123}I for in vivo study of pulmonary edema distribution.

In the coronary field, the method for the study of regional myocardial blood flow has been further improved and tested in various conditions, leading to new knowledge in the understanding of angina pectoris. These study was awarded the Von Hevesy Prize for Nuclear Medicine in Tokyo, September 1974.

The renal studies proceeded with the development of a scintigraphic methods for renovascular hypertension and an interesting statistical review of 760 renal patients for hematologic disorders which opens important guidelines for better understanding of anemia in renal disease.

The Director of the Group has been awarded the Feltrinelli Prize for Medicine, 1974, by the Scientific Academy of Italy (Accademia dei Lincei) which is assigned every four years.

Results of project number 1

Head of the project and scientific coworkers:

Prof. Umberto Rosa, Dr. Renzo Malvano, Dr. Sergio Albera, Dr. Paolo Cozzani, Dr. C Gandolfi

Title of the project: Development of radioimmunoassay methods of proteic and non-proteic plasma components.

The experimental program developed during 1974 was centered on the following lines:

a) Relationship between chemical structure and specificity in the case of synthetic antigens (e. g. non-antigenic small molecules coupled to proteins)

Using as a model estradiol, progesterone and aldosterone the effects of the structure of immunogens on specificity of antibody sites has been investigated. High specificity to antisera was found to be conferred by coupling at carbon-6, -11 and -3 for estrogens, progesterone and aldosterone, respectively. This findings have been exploited to set up radioimmunoassay methods for direct measurements in plasma extracts without chromatography (Malvano, Rolleri, Rosa, 1974; Malvano, Trojsi, Gandolfi, Attanasio, Crosignani, 1974).

b) Preparation of specific supports for affinity chromatography

A system testosterone-antitestosterone was used as a model to investigate the possibility of results obtained in a) for the preparation of specific supports for affinity chromatography. Preliminary data showed that the nature of the matrix strongly affects the performances of the chromatographic support. The results of a comparative study on the properties of hydrophilic and hydrophobic matrices, as well as on relative merits of different coupling procedures, has been published (Comoglio, Massaglia, Rolleri, Rosa, in press). On this basis, fractionation of an antiserum to testosterone was undertaken. The results showed that among the several fractions eluted after adsorption on a testosterone-Sepharose matrix, two families of homogeneous antibody sites characterized by different binding parameters, were present (Massaglia, Rolleri, Barbieri, Rosa, 1974). Those experiments led to the development of an elution scheme based on the use of mixtures of acidic buffer and organic solvents such as dioxane. Such a scheme proved susceptible of generalization to other steroid systems (Rosa, Malvano, 1974).

c) Synthesis of ^{125}I -labelled steroids.

The coupling procedures developed a) and b) were applied to the problems of ^{125}I -labelling of steroids. Estrogens were used as a model, since their labelling must be carried out in such a way as to avoid ente-

ring of iodine into the aromatic A ring. This was overcome through the synthesis of the reactive ^{125}I -derivative of thyramine which was then coupled to steroid derivatives with a single-step procedure (Comoglio, Saracco, Rosa, in press).

Results of project number 2

Head of the project and scientific coworkers:
Prof. Umberto Rosa, Dr. Renzo Malvano, Dr. Anilla
Massaglia, Dr. Ermanno Rolleri, Dr. S. Comoglio

Title of the project: Study of the properties of antibody
sites in homogeneous and heterogenous phase.

The work which has been developed during 1974 was centered on
two lines:

a) Properties of antibody sites.

Here again antibody to steroids were used as a model to investigate the properties of the sites. The rationale of the study was that the characteristics of antibody sites should reflect the properties of the immunogen used for their preparation. In particular, the interactions with steroids should obey to a polarity rule similar to that found for the sites of carrier proteins. Previous investigations on the effect of pH and organic solvent on soluble immunocomplexes formed by relatively polar and non-polar steroids with their specific antibodies have shown that stability of bonds is more affected by H^+ concentration in the case of estrogens than for testosterone and progesterone; moreover an apposite behaviour was found using organic solvents (Malvano, Rolleri, Rosa, 1974). The stability of immunocomplexes in D_2O medium was compared to that observed in H_2O ; the results showed that the contribution of hydrophobic components in stabilizing the bond decreases when passing to less polar to more polar steroids (Massaglia, Rolleri, Rovano, Rosa, in press).

b) Factors affecting the properties of insolubilized antibodies.

An estradiol-antiestradiol system was used as a model to investigate the relative importance of factors influencing the features of insolubilized antibodies. The study was carried out by coupling under various experimental conditions specific IgG to a Sepharose matrix, and comparing the binding parameters with those of the same IgG in solution. The following factors were taken into consideration: coupling conditions, including pH and composition of the reaction medium; coupling reagents including the use or not of hydrocarbon extensions to keep the protein far from the matrix; and the IgG/matrix mass ratio. The results have shown that preservation of the properties of antibody sites requires the proteins to be kept distant from the matrix microenvironment; antiestradiol IgG were transferred from solution to solid phase with negligible loss of sites and affinity, being the overall recovery of immunoreactivity in the order of 90% or more (Camoglio, Massaglia, Rolleri, Rosa, in press; Malvano, Rolleri, Gandolfi, Rosa, in press).

Results of project number 3

Head of the project and scientific coworkers:

Prof. Renzo Navalesi, Dr. Alessandro Pilo, Dr. Eleuterio Ferranini, Dr. Lorenzo Citti, Dr. Ottavio Giampietro, Dr. Roberto Palla.

Title of the project: Investigation of insulin metabolism with radioactive tracers.

1) Insulin metabolism in uremic patients in chronic maintenance hemodialysis.

These studies have been completed. It has been concluded that "both the lack of functioning renal tissue and toxic factors inhibiting insulin secretion and degradation combine to determine the pattern of insulin metabolism in uremic patients" (1).

2) Insulin kinetics at various plasma insulin levels

Four normal subjects have been studied basally and during glucose infusion (three-to tenfold increase of plasma insulin concentration with respect to pre-infusion levels). The kinetic parameters: metabolic clearance rate (MCR) and fractional catabolic rate (FCR) did not show any statistically significant modification. More studies are needed to definitely establish the invariance of hormone kinetics within the physiologic range of plasma insulin concentrations.

3) Estimation of insulin secretion (IDR) and degradation (IL) in non-steady state conditions.

Five normal subjects, 8 insulin-independent diabetics and 5 chronic uremic patients at various time-intervals after dialysis have been studied so far.

The experimental protocol includes: injection of adequately purified ^{125}I -insulin, plasma sampling up to two hours (22 samples), i. v. glucose load (0.33 g/kg b. w.) and then very frequent blood collections for 90 minutes. Both immunoprecipitable radioactivity and endogenous insulin concentrations are then measured on each plasma sample. Such experimental data yield information about insulin kinetics under basal conditions and under glucose load.

4) The effect of biguanides on insulin metabolism in normal and diabetic subject.

Two insulin-independent diabetics have been studied before and after acute metformin treatment.

Marked variations of kinetic parameters have been observed after the administration of the drug.

The study will be continued.

(1) Insulin Metabolism in Chronic Uremia and in the Anephric State: Effect of the dialytic treatment, J. Clin. Endocrin. Metab. : 40, 70, 1975.

5) Insulin metabolism in obese patients: correlations with "adipose cell size"

Techniques for isolation of adipose cells from biopsy fragments and size measurement have been set up.

The study will be continued.

6) Acute changes in insulin metabolism induced by dialytic treatment

Studies on labelled insulin kinetics in uremic patients before and after submission to regular dialysis program and in anephric patients (see 1) do not tell whether these are acute changes, occurring after each dialysis, or very gradual ones, consequent to the regular dialysis program, cannot be settled from this study.

To investigate this problem we have repeated the study in the same patients at various intervals from the preceding dialytic treatment, using the protocol described in 2).

The disappearance curves of labeled insulin in plasma have been analyzed to obtain the parameters of insulin kinetics as previously reported.

Using the plasma concentration of endogenous insulin after i. v. glucose administration the amount of insulin released in the circulation after stimulation can also be estimated, since plasma IRI after i. v. GTT can be written as the convolution product of secretion function time the disappearance curve of labelled insulin normalized by the injected dose.

The results are currently being analyzed.

Results of project number 4

Head of the project and scientific coworkers:

Prof. Carlo Giuntini, Prof. Giuseppe Pistelli, Dr. Carlo Marini, Dr. Anna Maria Santolicandro, Dr. Massimo Pistolesi, Prof. Mario Mariani, Dr. Antonio Barsotti, Dr. Alberto Balbarini.

Title of the project: Short lived radioisotopes in morpho-functional pulmonary studies in man.

A. Study of the regional distribution of pulmonary blood flow and volume

With respect to the 1974 program, this study was not entirely carried out since the Whole Body Scanner and the computer were not often simultaneously available. On the other hand, the use of a planiscanner allows to study the distribution of pulmonary blood volume only in the supine position.

The regional distribution of pulmonary blood flow was extensively investigated both from the diagnostic point of view (lung tumors, see study C, and other non neoplastic pulmonary lesions) and with respect to the factors that control the local lung perfusion (left heart valvular disease). This second topic was the object of a paper published by the American Journal of Medicine on the issue of September 1974 (see enclosed copy). The technic employed was based on the intravenous injection of macroaggregates of human serum albumin labelled with ^{131}I and, sometimes, of albumin microspheres labelled with $^{99\text{m}}\text{Tc}$. The use of the latter was devised to perform the proper checks in order to introduce a short lived isotope in these studies that appear very fruitful.

Concerning the regional pulmonary blood volume, a simple method of expressing the planiscanner counting rate per unit of lung volume allowed us to investigate the behaviour of the local capacity of the pulmonary vascular bed under various condition of altered cardio-pulmonary hemodynamic. The technic of these investigations entailed the intravenous injection of $^{113\text{m}}\text{In}$ that binds practically at once with the plasma transferrin thus becoming a tracer of the blood pools. Over the second part of the year a few studies were performed using another short lived radioisotope, ^{123}I , bound to antipyrine in order to compare the distribution of the blood pools ($^{113\text{m}}\text{In}$ -Transferrin) with that of the water pools (^{123}I -Antipyrine).

B. Study of regional ventilation and of the muco-ciliary function

The regional lung ventilation was investigated comparing the ^{133}Xe in gaseous form with the $^{99\text{m}}\text{Tc}$ albumin microspheres given as aerosol. A large field gamma-camera was employed to visualize simultaneously both lungs in normal subjects and in patients with different types of bronchitis and emphysema.

Furthermore, since the microspheres, over a few hours following the aerosol inhalation, are cleared from the bronchi mainly through the muco-

ciliary transport function, the ^{99m}Tc -microspheres make it possible to study the efficiency of the bronchial clearance of inhaled particles under various physiologic and pathologic conditions.

The results obtained indicate that the aerosol of ^{99m}Tc -microspheres is quite suitable to assess the regional lung ventilation when compared with the better known and more used ^{133}Xe method. Indeed, the inhalation of microspheres with a diameter of about 0.8 micron made it possible to visualize very peripheral lung regions, in patients with chronic bronchitis, which were very poorly ventilated. The advantages of this technic with respect to that employing ^{133}Xe are twofold: a) the persistence of the tracer for some time where the ventilation carried it, thus allowing its proper visualization in various projections, and b) the absence of distribution outside the lung, thus eliminating the very disturbing effects of the extrapulmonary tissues background that are currently encountered with the ^{133}Xe .

The results of this study were presented, in part, in Padua on December 9th at the meeting of the Societas Europea Physiologiae Clinicae Respiratoriae devoted to the application of radioisotopes in clinical medicine and physiology.

The identification of the indexes of muco-ciliary clearance is in progress.

C. The application of short lived isotopes to the diagnosis of primary lung tumours.

The use of radioisotopes has proven so far of relevant interest in the diagnosis of primary pulmonary neoplasms. The injection into a peripheral vein of ^{131}I -macroaggregates of human serum albumin and their detection by a planiscanner has made it possible to differentiate a different pattern in the alterations of the regional pulmonary blood flow depending on the location of the tumour. Namely, when the neoplasm is located in the periphery of the lung parenchima, the perfusion defect is just local, whereas it extends to the entire lung when the bronchiogenic carcinoma develops at the level of the hilum or near it. Often, especially in the early stage of the neoplasm the extension of the perfusion defect contrasts sharply with the paucity of signs at the röntgenologic examination. Sometimes, even the angiographic investigation fails to reveal lesions of the large branches of the pulmonary artery supplying zones that lung scintigraphy shows markedly underperfused.

Several studies were performed to develop a method, based on the injection of microspheres labelled with ^{99m}Tc and on their detection with a large field gamma-camera, suitable for more extensive application. The technic, now in an advanced state of development, entails the record of the lung scintigraphic images, life size, on an ordinary X-ray film to make easy the comparison with the X-ray pictures themselves.

Results of project number 5

Head of the project and scientific coworkers:

Prof. Attilio Maseri, Dr. Antonio Pesola, Dr. Antonio L'Abbate, Dr. Mario Marzilli, Dr. Paolo Mancini, Dr. Remo Bedini.

Title of the project: Development of the use of short lived radioisotopes in morphofunctional quantitative myocardial studies.

Measurements of total or average myocardial blood flow obtained in man by different methods are, in general, conflicting and inconclusive because, perfusion alterations have been sought using methods that measure average or total myocardial flow, so that changes in opposite directions in adjacent zones could not be detected.

Therefore, after having developed methods for the study of average myocardial flow (1, 2), we have developed a method in our laboratory for the study of regional myocardial perfusion in coronary artery disease at rest and during appropriate hemodynamic or pharmacological stimulations intended to test the coronary reserve capability and its response to acute ischaemia.

This method is based on the detection of the spatial distribution within the myocardium of the initial distribution of $^{133}\text{Xenon}$ injected into a coronary artery and of its regional washout rates, by a gamma camera (Nuclear Chicago Phogamma III) computer system (HP2116B). In order to have a continuous check of the constancy of the geometrical counting conditions we have thought of injecting $^{99\text{m}}\text{Tc}$ labelled human albumin microspheres which remained fixed in the myocardium. The fixed activity of $^{99\text{m}}\text{Tc}$ and the dynamic changes of $^{133}\text{Xenon}$ can be followed simultaneously with the dual counting accessory of the gamma camera which separates the two isotopes by pulse height analysis. We have developed a computer program which subdivides the scintigram into 9 areas and for each provides the representation of the time activity of the two isotopes. When the activity of $^{99\text{m}}\text{Tc}$ remains constant, regional differences in the Xenon washout can be attributed only to a different rate of clearance from the tissue included in the solid angle of each area (Figure 1). Accordingly in successive Xenon injections, performed during different hemodynamic conditions, differences in its fractional distribution to a given zone of the myocardium, outlined on the reference $^{99\text{m}}\text{Tc}$ scintigram, indicate proportional variations of the flow distribution (when the $^{133}\text{Xenon}$ can be assumed to be uniformly mixed with blood at the coronary inlet) (Figure 2).

The whole procedure of analysis has been automatized. Furthermore a system for the acquisition of the "R" wave signal of the ecg simultaneously with the activity of the two isotopes has been developed in order to obtain a stroboscopic analysis of the heart movement within the heart

(1, 2) See 17 and 18 in the list of published papers.

cycle. The system was tested and will be now introduced in routine.

Results

The method gave reproducible results in 4 patients in whom duplicate measurements were performed both for the initial distribution and for the regional washout.

Results at rest. While the standard error of the slopes of the washout curves ranged between 0.02 and 0.07, the coefficient of variation of the values obtained in the 9 areas of the resting scintigram, were significantly larger, ranging from 0.10 to 0.30, indicating that variations in slope in the different areas were in part related to the diffusion-hold up of the indicator in epicardial fat, but in some instances the could be interpreted as related to actual regional differences in flow although. Generally no relation could be established between the site of the coronary obstructions and the regional differences in washout and hence in flow myocardial perfusion. Only in two cases we observed significantly slower washout distally to an obstructed vessel which disappeared following nitroglycerin administration.

Results during induced angina. Pacing in the absence of ecg changes or of typical pain resulted in an increased slope of the washout curves without appreciable regional differences and without changes in the initial scintigram (4 patients). By contrast, when pacing caused typical angina (7 patients) evident alterations of myocardial perfusion were detectable. The alterations appeared in the initial scintigram when the injection was performed after the appearance of angina (indicating that during angina the indicator cannot reach the ischaemic areas) and in the washout slope, when angina was induced during the course of the washout (indicating that the indicator cannot be washed away from the myocardium which becomes ischaemic).

The results obtained show that this approach, from the diagnostic point of view, provides valuable information for the individual patient and, from the pathophysiological point of view, has allowed us to detect the presence of a significant functional regional reduction of perfusion during angina which introduces a new concept in our present understanding of coronary artery disease. From the technical point of view this approach, is susceptible of further improvements when nuclides with more suitable physical characteristics and detecting devices with greater resolution will become available.

The theoretical developments and the results obtained by this method were presented at several Symposia and published in the proceedings or in books and Journals.

This work has been awarded the Von Hevesy prize at the 1st World Congress of Nuclear Medicine, Tokyo, September, 1974.

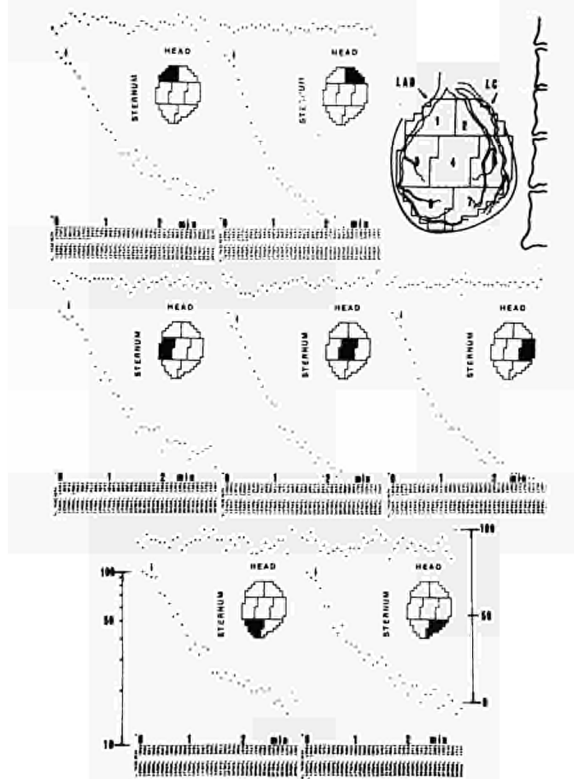


Figure 1 - Computer plot of the simultaneous time-activity curves of ^{99m}Tc microspheres and ^{133}Xe injected into the left coronary artery obtained in each area of the scintigram. Each point is a 5 sec interval. The patients had an isolated subocclusion of the LAD and was studied in the LAO projection. While the ^{99m}Tc activity remains constant in each area (top of each panel) the ^{133}Xe activity decreases as the indicator is washed out from the myocardium.

As the heart rate (hence the myocardial metabolic demand) is suddenly increased from 70 to 150/min, at the arrow, the washout from the myocardial area distal to the critically obstructed LAD coronary branch becomes progressively slower in respect to that from the areas perfused the left circumflex.

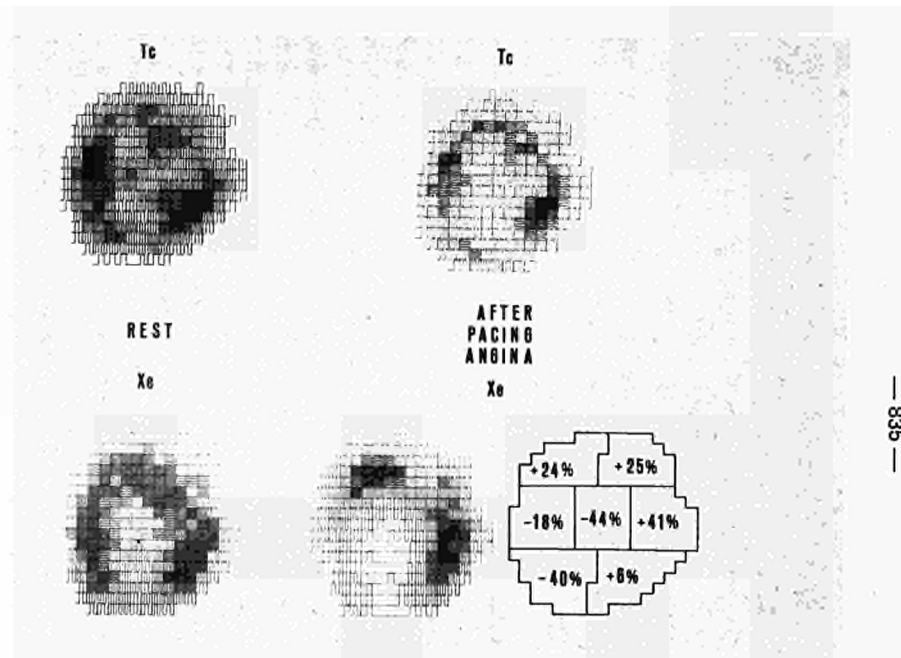


Figure 2 - Myocardial scintigram in LAD position in a patient with proximal subocclusion of the LAD as the only coronarography abnormality, typical angina but no previous infarction. The ^{133}Xe left coronary scintigram obtained during angina is clearly different from that obtained at rest because of the appearance of a large cold area in the territory of the LAD. The scintigram of ^{99m}Tc microspheres (injected into the left coronary at the beginning of the study) recorded simultaneously with the two ^{133}Xe scintigrams are practically unchanged. The percent changes of the fraction of ^{133}Xe distributed to each area outlined on the reference ^{99m}Tc scintigram in respect to control are indicated in the lower right panel.

Results of project number 6

Head of the project and scientific coworkers:

Prof. Claudio Bianchi, Dr. Mario Bonadio, Dr. Gianfranco Tramonti, Dr. Carlo Donadio, Dr. Silvio Figus, Dr. Antonio Coli, Dr. Roberto Palla, Dr. Alessandro Dalle Luche.

Title of the project: Radioisotopes techniques in the morphological and functional study on the renal diseases and in the etiological evaluation of hypertension.

Quantitative sequential renal scans to screen renovascular hypertension

On 45 hypertensive subjects with unilateral renal disease quantitative sequential renal scans were performed with ^{131}I -hippuran using a linear scanner provided with an electronic facility to quantify the relative renal uptake of the tracer substance in each kidney. Scanning began immediately after administering the radioiodinated hippuran and thereafter again, at intervals of: 15, 30, 45 and 60 minutes. In 9 out of 12 cases with renovascular hypertension (as established by aortography) a characteristic pattern developed. The affected side took up the ^{131}I -hippuran slowly; this persisted for a relatively long time, and was eliminated very slowly. The healthy side took up the tracer substance very rapidly and eliminated it very soon thereafter. In only 7 of these 12 cases the I. V. pyelogram was positive. In the remaining 33 hypertensive patients without ischemia affected by unilateral disease, mostly pyelonephritis, the pattern was practically the same on both sides; the images and count rates vanished within 30 minutes after the injection. The aforementioned observations tend to indicate that the described procedure is useful and effective to single out cases of renovascular disease amongst hypertensive patients.

Haematological disorders as a consequence of renal diseases.

The aim of this study is to determine the pathogenesis of blood disorders in renal diseases. For this purpose a statistical analysis was carried out on 760 renal patients to establish the relationship between glomerular filtration rate (GFR) and serum haemoglobin (Hb). Out of these patients, 370 suffered from chronic glomerulonephritis (GN), 371 had pyelonephritis or urinary tract infections (PN) and 19 had polycystic kidneys (PK). In each patient Hb determination (by autoanalyzer) and GFR (by diatrizoate- ^{131}I and external counting over the bladder) were carried out within a 10-day interval. Considering as a whole the 760 patients examined, a progressive linear decrease of Hb was observed in cases where the GFR was less than 70 ml/min. The blood abnormalities are not the same being more marked in GN, less marked in PN and absent in PK. In fact, the decrease of Hb corresponding to a lowering of GFR of 20 ml/min was 0.68 g% for GN and 0.50 g% for PN (difference 36%, $P < 0.02$).

Conclusions: 1. Blood disorders are evident in cases where serum

concentration of urea, creatinine, methylguanidine and other catabolites is still normal; 2. Significant differences between the abovementioned renal diseases were found.

The relationships of these changes to those of the renin and erythropoietin systems remain to be investigated.

Renal haemodynamic effects of a single I. V. administration of prazosin in hypertensive patients. Preliminary results.

Prazosin, 2- [4-(2-furoyl)-piperazine-1-yl] -4-amino-6, 7-dimethoxy-quinazoline hydrochloride is a hypotensive compound active in hypertensive animals and humans. The mechanism of action has been ascribed to vasodilatation which depends on direct relaxation of vascular smooth muscle and peripheral sympatholytic activity.

This paper deals with preliminary results obtained in six hypertensive patients (five females and one male; 37-56 yrs, median 41.5) with mean arterial pressure (MAP) 135-149 mmHg (mean 142), glomerular filtration rate (GFR) 44-105 ml/min (mean 81) and effective renal plasma flow (ERPF) 221-562 ml/min (mean 363).

Arterial pressure was taken by an automatic blood pressure monitor (Arteriosonde 1217); GFR and ERPF were measured by the conventional method of continuous I. V. administration of tracer substances and by collection of urines by vesical catheterization. Diatrizoate-¹³¹I and hippuran-¹²⁵I were used for the measurement of GFR and ERPF respectively.

Patients received a single I. V. continuous administration of Prazosin 7.6-19.5 mcg/kg b. w. (mean 11.0) over a period of time which goes from 28-91 minutes (median 62).

Results (mean + S. E.) obtained before, during and/or after treatment are shown in the following table.

	MAP	GFR	ERPF
Before	141.4 \pm 1.2	88.4 \pm 5.1	391.6 \pm 43.8
During and/or after	115.7 \pm 5.6	52.5 \pm 7.7	228.0 \pm 31.8
% decrease	18.0 \pm 3.8	41.0 \pm 8.2	39.2 \pm 8.8

The results given by one patient did not agree with the above. In fact a decrease of MAP (15%), an increase of GFR (27%) and ERPF (28%) were found. These results remain unexplained.

In conclusion, the I. V. administration of Prazosin was always followed by a decrease of arterial pressure and by an impairment of renal function in five out of six hypertensive patients.

List of Publications

1. S. Comoglio, A. Massaglia, E. Rolleri, U. Rosa: "Preparation of bio-specific supports for affinity chromatography and immunoabsorption" in "Plasma Protein Turnover" Academic Press, in press.
2. S. Comoglio, M. Saracco, U. Rosa: "Preparation of estriol-6-(o-carboxy-methyl)-oxime ^{125}I -tyramine for estradiol radioimmunoassay". J. Nucl. Biol. Med., in press.
3. R. Malvano, E. Rolleri, U. Rosa: "Standardization and control of steroid radioimmunoassay" in "Radioimmunoassay and Related Procedures in Medicine" IAEA, Vienna, 1974, vol. II, p. 97.
4. R. Malvano, L. Trojsi, C. Gandolfi, A. Attanasio, P.G. Crosignani: "Radioimmunoassay of some gonadal steroids: validity and limitation of measurements in plasma extracts" in "Recent Progress in Reproductive Endocrinology" Academic Press, Londo, 1974, p. 229.
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6. U. Rosa, R. Malvano: "Fractionation of antisera to steroids". Proc. of VI Tenovus Workshop "Steroid Immunoassay" April 3-5, 1974, in press.
7. S. Comoglio, A. Massaglia, E. Rolleri, U. Rosa: "Factors affecting the properties of insolubilized antibodies". Biophys. Biochim. Acta, in press.
8. R. Malvano, E. Rolleri, C. Gandolfi, R. Rosa: "Properties of estradiol-antibody complexes formed in homogeneous and heterogeneous phase". Horm. Metab. Res., in press.
9. A. Massaglia, E. Rolleri, S. Rovano, U. Rosa: "Effects of D_2O on the hydrophobic components of steroid-antibody interactions". J. Biol. Chem., in press.
10. R. Navalesi, A. Pilo, S. Lenzi, L. Donato: Insulin metabolism in chronic uremia and in the anephric state: Effect of the dialytic treatment. J. Clin. Endocrin. Metab. 40, 70, 1975.
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Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung,
Institut für Hämatologie

Nr. des Vertrages: 089-72-1 BIAD

Leiter der Forschungsgruppe: Priv.-Doz.Dr.S.Thierfelder

Allgemeines Thema des Vertrages:

Nuklearmedizinische Hämatologie (Proj. 4-7)

(Proj. 1-3 über Strahlenbiologische Hämatologie und Immunologie sind unter Kapitel III "Forschungstätigkeit Strahlenschutz" aufgeführt).

Allgemeine Darstellung der durchgeführten Arbeiten:

The projects of the research group in Munich continued their studies on the analysis and treatment of the consequences of radiation exposure. The principal effort was concerned with the treatment of bone marrow failure following total body irradiation or high doses of cytostatic drugs used in anti-cancer therapy. Experiments in rodents as well as preclinical and/or in vivo studies in dogs and men were undertaken to overcome the immunological complications of bone marrow transplantation in radiation or drug-induced hemopoietic failure. They were concentrated on two research areas: immune-intervention with specific anti-T-cell sera and histocompatibility typing to prevent secondary disease. While the first approach is being studied extensively in our group, collaborative research in histocompatibility typing became particularly fruitful on the European level. The 3rd meeting of the Cooperative Group on Bone Marrow Transplantation in Dogs was held in TNO Rijswijk. A joint project was set up by the participating groups from Holland, Ulm, Paris and Munich to study the long-

term histologic, immunologic and hematologic consequences of marrow transplanted dogs with the member laboratories taking advantage of the animal housing facilities for dogs in our GSF.

In rodents successful immune-intervention led to the complete suppression of acute secondary disease. But also chronic secondary disease responded to this treatment in several H₂ incompatible donor-recipient combinations. An anti-T-cell serum developed against human thymocytes was likewise found to kill T-cells and spare colony forming cells. In dogs collaborative histocompatibility typing for LD in Munich and TNO Rijswijk led to the definition of the MLC-specificities DL 50-DL 57. In men 44% of HL-A-Haplotypes 3-7 were found to carry the MLC-specificity Pi and 46% of the HL-A-Haplotypes 3-W5 had the MLC-specificity Pf. Our development of a technique to store lymphocytes for MLC at low temperatures will facilitate the definition of the MLC-system in dogs and men. The transplantation of SD-LD-heterozygous canine marrow into SD-LD-homozygous recipients conditioned with cyclophosphamide permitted the study of host-versus-graft activity. Histologic studies on lymphnodes from dog chimaeras were performed in the Abteilung für allgemeine und experimentelle Pathologie.

Successful treatment of bone marrow failure depends also on further advances in the diagnosis of the underlying disease. Therefore another project concerns itself with the proliferation kinetics of erythropoiesis in various types of anemias calculating DNA synthesis and the ³H-TdR labeling index. Intramedullary cell-death or disappearance of precursor cells in certain stages of differentiation could be objectivated by this approach in pancytopenias. An extensive ineffective erythropoiesis restricted to the more mature cell type was found in thalassemia major.

Refined methods of hemato-morphology such as bone marrow biopsies were applied to a study on the long-term consequences of ³²P in polycythemia vera showing reduced hemopoiesis and marrow sinuses.

Ergebnisse des Projekts Nr. 4

Leiter des Projekts und wissenschaftliche Mitarbeiter:
S. Thierfelder, H. Rodt und M. Eulitz

Titel des Projekts:
Suppression of secondary disease with anti-T-cell
antisera.

Our approach to suppress the immunological complications of bone marrow transplantation (secondary disease) consists in the manipulation of the marrow of the donor before it is injected into the recipient. Heterologous antisera against the theta antigen on T-cells were raised in rabbits and thoroughly absorbed to avoid cytotoxicity against stem cells. We reported previously that complete suppression of acute secondary disease in (C57 Bl/6xCBA)_{F₁} recipients of C57 Bl/6 spleen marrow could be achieved. The present studies were concerned with the state of tolerance in these chimaeras. Using thymectomized recipients it was found that only recipients having their thymus were able to build up new T-cells. These cells were reactive against third party antigens but tolerant of the recipient's H₂ incompatible antigens. It was thus concluded that precursor thymocytes of the donor developed under the influence of the H₂ different semiallogeneic recipient's thymus. Using donor-recipient combinations differing in the theta allotype it could also be demonstrated that precursor thymocytes of the donor developed the donor type thetaalloantigen under the influence of the recipient's thymus carrying a different thetaalloantigen. Another study concerned the effect of anti-T-cell globulin on chronic secondary disease. Using the same approach anti-T-cell globulin induced chimaerism in recipients of marrow from allogeneic H₂ incompatible donors without secondary disease. The type of chimaeric tolerance is under investigation.

Ergebnisse des Projekts Nr. 5

Leiter des Projekts und wissenschaftliche Mitarbeiter:
S. Thierfelder , H. Rodt, E. Thiel und H. Huhn

Titel des Projekts:

T-B-cell concentration in normal athymic and
chimaeric rodents.

T- and B-antigens were measured by the following methods: cytotoxicity, light- and electronmicroscopical techniques using FITC or peroxidase labeled antibodies, complement fixation and immuneautoradiography using ^{125}I labeled antibodies. The last method was combined with photometric registration and permitted the measurement of the concentration of antigens on the single cell level. Thus the thetaantigen concentration on T-cells in the spleen was about 40% of that in the thymus. T-cells in the bone marrow carry an even lower antigen concentration.

The number of T-cells in athymic nude mice was of course reduced though less than expected. Still about 10% of the number of T-cells in thymus-bearing mice were found in the lymphnodes and spleens of nude mice. The graft-versus-host reactivity of these cells is under investigation.

Chimaeric mice did not differ significantly from untreated mice in their content of T-cells. Chimaeric mice thymectomized before bone marrow transplantation had a reduced number of T-cells. Immune-electronmicroscopy revealed no overlapping of T- and B-antigen carrying lymphocytes. The content of B-cells in nude mice appeared normal.

Ergebnisse des Projekts Nr. 6

Leiter des Projekts und wissenschaftliche Mitarbeiter:
P. Dörmer, W. Brinkmann und F. Hegemann

Titel des Projekts:

Erythropoiesis and kinetics of erythroblasts in
anemias and aplasias in man.

In the last year's report a method was presented which we developed for determining the DNA synthesis rate of individual cells. This method was applied to the determination of the kinetics of human hemopoietic cells in non-malignant states. Two distinct groups of diseases have been studied: a) various types of pancytopenia; b) thalassemia.

With few exceptions, intramedullary cell-death was observed in pancytopenias irrespective of the type of underlying bone marrow changes. It was found in erythropoietic as well as in granulopoietic cells. In pancytopenias accompanied by an ample bone marrow only the more mature stages of differentiation perish. This explains the compensatory hyperplasia of immature precursor cells, known under the term of "maturation arrest". On the other hand, there is evidence that in pancytopenias accompanied by an empty marrow already the earliest recognizable types of cells are submitted to intramedullary death. No compensatory hyperplasia can therefore take place. On the whole, cellular proliferation is reduced rather than enhanced, especially in cases with an empty marrow.

In thalassemia major there is also extensive ineffective erythropoieses which is restricted to the more mature cell types. Cellular proliferation is extremely reduced. Hyperplasia in this disease has to be considered therefore as a "pile-up" of cells before they die. This explains the strong tendency of the disease towards clinical decompensation. In thalassemia minor there is no ineffective erythropoieses in the proliferative compartment, but most likely in the maturation compartment. Cellular proliferation was only moderately reduced so that compensation of the anemia in this state is well explained.

Ergebnisse des Projekts Nr. 7

Leiter des Projekts und wissenschaftliche Mitarbeiter:
H. Grosse-Wilde, W. Mempel, B. Netzel, E.D. Albert und
S. Thierfelder

Titel des Projekts:

Tissue typing for bone marrow transplantation:
Definition of MLC-alleles in men and dogs.

Histocompatibility testing for allogeneic bone marrow grafts in man as well as in other mammals has become the prerequisite for the survival of the recipient. One important, genetically inherited system, the so called Major Histocompatibility Complex (MHC), has been found in different species like mouse, rat, swine, rhesus monkey, dog and man. Except for the rat the MHC of these animals consists of 3 linked but clearly separate loci or regions coding for determinants present on the cell surface. The products of two gene loci (HL-A Antigens) are detectable by serological tests, whereas one locus can only be detected until now by a short term culture method, the Mixed Lymphocyte Culture (MLC). This project has concentrated its investigations on the evaluation of the MLC-system in man and dog. We were able to develop a method to recognize the allelic expression of MLC specificities, using lymphocytes from individuals homozygous for one single MLC determinant in a one-way micro-MLC. The homozygosity was assessed by family studies. Five different MLC alleles are now identified by our group:

1	Type Pi	HL-A3-7/3-7
2	Pf	3-W5/3-W5
3	Sr	1-8/3-8
4	Ki	10-W18/10-W18
5	St	1-8/1-8

There is clear evidence that the polymorphism of this MLC system is as high as that of the HL-A system with at least 25 different alleles. According to the HL-A system the

MLC alleles show different gene-frequencies at the population level.

MLC specificities Pi and Pf are tested now against more than 300 unrelated healthy individuals in Western Germany. A significant linkage disequilibrium was found between HL-A 7 and Pi and for the whole haplotype HL-A 3-7-Pi. The same association was detected between HL-A 3 and Pf as well as for the HL-A haplotype 3-W5-Pf. This study was performed in cooperation with PD Dr. J. Bertrams/Gesamthochschule Essen (BRD). Specificity Sr is tested so far in a rather small group but it is evident that also this MLC allele is linked in favor to HL-A8. Recently LD determinant Ki and St could be identified by our group.

In cooperation with Prof. Dr. Rittner, Universität Bonn (BRD), a new polymorphic system could be localized on the same chromosome on the site of the MLC locus. This "Bf" locus codes for alleles of a protein called "C3-Proactivator", the starting enzyme for the alternate pathway of the complement.

Our group will participate as the German regional center for MLC typing in the next International Workshop of Histocompatibility Testing, Aarhus/Denmark, in July 1975.

The MLC system of the dog seems to be as polymorphic as the human MLC system. In cooperation with Dr. H. M. Vriesendorp, Radiobiological Institute TNO Rijswijk/Holland, the following 8 MLC determinants could be identified:

1	DL-50	DL-A2-4/2-4
2	51	2-5/2-5
3	52	9-6/9-6
4	53	3-b1/3-b1
5	54	10-5/7-12
6	55	3-b1/3-b1
7	56	1-13/1-13
8	57	3-b1/3-b1.

DL-50 to DL-56 were tested against a beagle population consisting of 64 independent haplotypes, a mongrel population with 65 independent haplotypes and a smaller group of labradors with 17 independent haplotypes. The genetic analyses

of the first two populations revealed that in the tested beagle population the linkage disequilibrium (expressed as delta-values) was higher and that more MLC specificities showed significant association to DL-A antigens than in the tested mongrel population. These data could be explained by selective breeding schemes ("founder" or "bottle neck" effect) in the beagle population affecting the polymorphism and the genetic association between MLC and DL-A determinants.

The delta-values in the mongrels, presumed to be not an inbred population, are higher than the ones found in human or primates. The most likely explanation is also a pronounced founder effect, however, some high delta-values could be still due to the influence of natural selection forces favouring special combinations of genes.

Our results were presented on the 2nd International Workshop on Canine Immunogenetics, Portland, Dec. 1974. At this meeting it was decided to have the next workshop 1977 in Munich organized by our group.

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Knochenmarksschäden durch akute und chronische Strahlenwirkungen.

"Strahlen, Blutgerinnung und Hämostase", "XVI. Hamburger Symposium über Blutgerinnung", F.K. Schattauer Verlag, Stuttgart, (1974), S. 73

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und E.D. Albert

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Transpl. Proc., (1974 in press)

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NEUTRONENDOSIMETRIE

NEUTRON DOSIMETRY

DOSIMETRIE DES NEUTRONS

Weitere Forschungsarbeiten zu diesem Thema werden auch in folgenden Jahresberichten beschrieben:

Further research work on these subjects will also be described in the following annual reports:

D'autres travaux sur ce thème de recherche sont également décrits dans les rapports annuels suivants:

094-BIAN ITAL, Wageningen (De Zeeuw)

101-BIOC Dosimetry Group

Biology Group Ispra

GESELLSCHAFT FÜR STRAHLEN- UND UMWELTFORSCHUNG MBH, MÜNCHEN
Institut für Strahlenschutz, Neuherberg

Vertrag: 113 BIOC

Leiter der Forschungsgruppe: Dr. Georg Burger

Allgemeines Thema des Vertrages: Calculation and Measurement
of Absorbed Dose and Neutron Spectra inside a Biological
Object with Reference to Fast Neutron Radiotherapy

The program is concerned with the determination of the biological effective neutron dose inside human phantoms. It includes neutron and gamma transport calculations and measurements with TE-proportional counters and various ionization chambers.

In addition to the scientific studies, the ENDIP intercomparison program, starting in 1975 was prepared. The main activities in this respect refer to the development and testing of the universal monitoring and data processing system MOSES (Monitoring System for the ENDIP-Service).

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2. Symp. Neutron Dosimetry Biol. Med., Neuherberg, 1974
- /3/ Burger, G. and E. Maier:
Mixed Field Dosimetry with the Twin Chambers Technique
GSF-Rept.-S 299, 1974

Ergebnisse des Projekts

Leiter des Projekts und wissenschaftliche Mitarbeiter:

F. Grünauer, G. Burger, E. Maier

Titel des Projekts: Calculation and Measurement of Absorbed Dose and Neutron Spectra inside a Biological Object with Reference to Fast Neutron Radiotherapy

Neutron spectra, and neutron and gamma-depth dose distribution have been calculated for the Neuherberg prototype collimator, and the irradiation facilities at the TNO-Rijswijk and the Hammersmith-Hospital. For the Neuherberg and Rijswijk arrangements the agreement between theoretical and experimental neutron, respectively total axial depth doses were excellent, for the Hammersmith-arrangement the results differ by 6,5 % in 10 cm depth. The source of discrepancy could not yet be solved.

In each case the gamma-components showed discrepancies. The influence of the environmental background of low energy neutron and gamma radiation offers a possible explanation. To assess this quantitatively, transport calculations have been performed for simplified spherical room geometries. The results indicate the important role of the background radiation component with respect to the gamma depth doses.

For the optimization of shielding arrangements as for example collimator shieldings, a computer program was developed, which determines by an iterative procedure the best arrangement of a given thickness or weight with given materials, in order to achieve the highest kerma-attenuation.

For the Heidelberg arrangement not only absorbed doses, but also RBE-doses on the basis of a reasonable RBE-function versus neutron energy have been calculated. Whenever the chosen RBE for *vicia faba* is not proven to hold also quantitatively for mammalian systems, the results indicate at least the possibility of severe changes in radiation quality, i.e. in the local RBE at the edges of the lateral dose profiles (see figure 1).

For the final computation of the INDI-results, correction factors for the kerma-ratios and W-values in the used TE- and C/CO₂-chambers inside the phantom have been calculated.

For the C/CO₂-chamber the relative deviation of the values in 20 cm depth to the free air case is 25% for the kerma ratios and 7% for the W-values, based on Dennis' assumptions.

An extend error analysis was performed for the use of the twin-chamber technique, which explains quantitatively the high uncertainties in the determination of small gamma doses in the presence of high neutron doses (see figure 2).

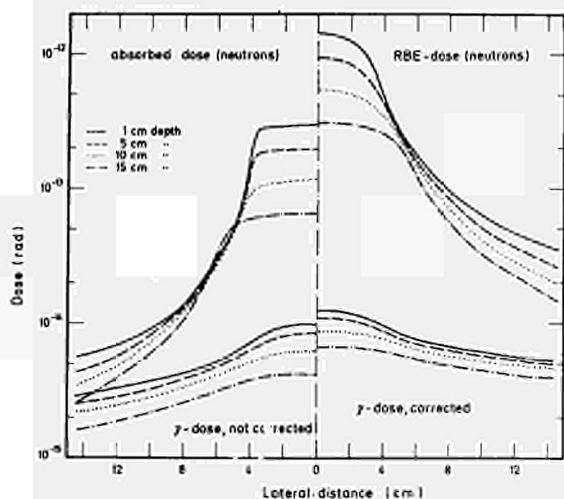


Figure 1:

Lateral profiles of absorbed dose and RBE-dose, calculated for the TNO-collimator

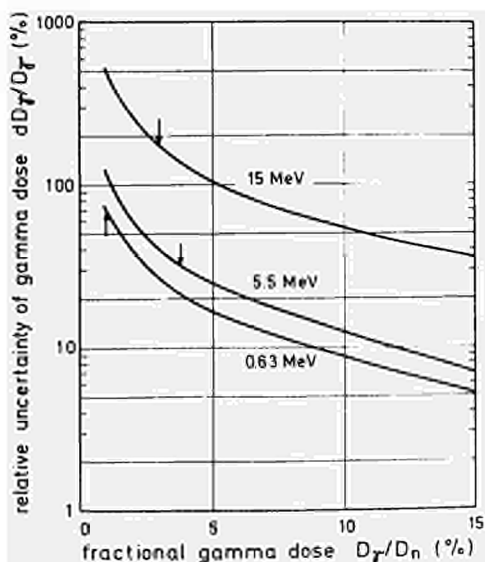


Figure 2:

Relative uncertainty in determination of the gamma dose component with TE/C-chamber pairs as a function of its fractional amount

Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung
München

Nr. des Vertrages: 113-72-1 BIOC

Leiter der Forschungsgruppe:

Prof. Dr. W. Pohlit

Allgemeines Thema des Vertrages:

Calculation and Measurement of Absorbed Dose
and Neutron Spectra inside a Biological Object
with Reference to Fast Neutron Radiotherapy

The average energy expenditure W to produce an ion pair in nitrogen was determined for protons in an energy range from 0.2 MeV to 0.56 MeV with an ionization chamber connected to a 600 kV BBC accelerator by a double pumping stage arrangement. The W -value is energy independent within the measured energy range. These experiments are concluded and will be extended to other gases such as tissue equivalent gas, carbondioxide, and methane. For the determination of dose back scattering factors in the vicinity of a polyethylene phantom different parallel plate extrapolation chambers have been developed and their characteristic features have been investigated. The dependence of the dose back scattering factor with phantom thickness can be described by a semiempirical equation using the relaxation length of the incident neutrons and an average scattering length.

Ergebnisse des Projektes

Nr. 113-72-1 BIOC

Leiter des Projektes und wissenschaftliche

Mitarbeiter: Dr.H.Kühn, Dipl.-Phys.T.Werba

Titel des Projektes:

Determination of the average energy expenditure to produce an ion pair in different gases for heavy ions

After some improvements regarding the double pumping stage arrangement connecting the ionization chamber with the BBC accelerator measurements for the determination of the average energy expenditure W to produce an ion pair in nitrogen irradiated with protons have been performed. The first arrangement had to be improved by inserting a Roots vacuum pump characterized by a high pumping speed to minimize scattering losses at the entrance diaphragm. Especially effort has been done for the determination of the saturation curves of the ionization current. During the measurements the proton current has been kept constant and the range was about 10^{-11} A. The saturation current has been determined by employing the theory of Jaffé, neglecting any volume recombination. The recombination loss at a polarizing voltage of 400 V has been about 1%. A dependence of the polarity of the chamber tension was within the experimental uncertainty and negligible. The ionization currents have been within the range 10^{-12} A to 10^{-7} A depending on the gas pressure within the chamber. This dependence has been linear with the pressure until the gas pressure has been reached at which the protons are stopped totally. At essentially higher pressures the ionization decreased according to the increasing scattering of the protons at the entrance diaphragm. Within the statistical uncertainty the value of W for protons in nitrogen was constant for energies ranging from 200 keV to 560 keV.

The uncertainties for the determination of the W value have been estimated and are summarized in the following list:

Energy of protons	ΔE_p	$\pm 0.5 \%$
Current of protons	Δi_p	$\pm 1.0 \%$
Ionization current	Δi_i	$\pm 1.0 \%$
Ionization current losses by recombination	Δi_r	$\pm 0.2 \%$
Further:		
Calibration of monitor	$\Delta i_p / i_{\text{mon}}$	$\pm 0.5 \%$
Current fluctuation	$\Delta i_p / \Delta t$	$\pm 0.5 \%$
Energy fluctuation	$\Delta E_p / \Delta t$	$\pm 0.5 \%$

Ergebnisse des Projektes

Nr. 113-72-1 BIOC

Leiter des Projektes und wissenschaftliche

Mitarbeiter: Dr.H.Kühn

Titel des Projektes:

Determination of back scattering factors
of neutrons

For the determination of dose back scattering factors in front of a polyethylene phantom with variable thickness cylindrical ionization chambers have been employed for the measurement at phantom-dosemeter distances equal or greater than 2.5 cm. The experimental results can be described by a semiempirical equation using the relaxation length λ of the incident neutrons and the average scattering length λ_s (e.g.: $\lambda = 13$ cm, $\lambda_s = 5$ cm for neutrons with an initial energy of 15 MeV). The average scattering length has been chosen in such a way that the experimental values are fitted best. For dose determinations at phantom-dosemeter distances less than 2.5 cm parallel plate extrapolation chambers with different wall materials have been developed. The distance between the high voltage electrode and the collecting electrode can be varied within a range from 0.02 - 1.00 cm by a thread ring. The uncertainty of the area of the collecting electrode is about ± 0.2 %, the accuracy of the electrode distance is about ± 0.001 cm. For the determination of the produced charge measurements with both polarities of the high tension have been performed. It followed from these measurements for the charge ratio $2(Q^+ - Q^-)/(Q^+ + Q^-) \leq 0.015$ for a tissue equivalent extrapolation chamber with electrode distances greater than 0.1 cm. For smaller electrode distances this ratio is increasing. The charges determined have been corrected to saturation values according to Jaffé. The increasing charge

ratio at very low electrode separations is not yet understood at the moment. At this stage dose back scattering factors have not been determined for phantom-dosemeter distances less than 2.5 cm.

References

Kühn, H.: Characteristics of different ionization chambers for fast neutron dosimetry. In: Second Symposium on Neutron Dosimetry in Biology and Medicine, Neuherberg, September 30 - October 4, 1974.

Contractor: Medical Research Council

Contract No. : 113-72-IBIOC.

Head of research team: Mr. D.D.Vonberg

General subject of Contract: Correlation of
physical and biologically effective
dose of fast neutrons.

Description of the work carried out.

Mammalian cells have been exposed to fast neutron beams generated by 16 and 42.5 MeV deuterons on beryllium, both in and out of a tissue-equivalent phantom. The cells were in suspension and were bubbled with air or nitrogen during irradiation.

The change in the neutron spectrum in the phantom compared with the spectrum with no phantom present has been measured with activation and fission detectors. In addition the results of these measurements have been compared with calculated spectra in the phantom.

Results of Project No. : 1.

Head of Project and D.K.Bewley
 Scientific staff: R.J.Berry
 B.Cullen

Title of Project: Measurement of biologically
 effective dose of fast neutrons.

Description of results

The results of the measurements made by R.J.Berry on lymphocyte leukaemia cells mentioned in last year's report are included in the table below. In addition, the work has been extended to neutrons generated at 42.5 MeV and to the response of mouse Erlich ascites cells studied by Miss B.Cullen.

These figures should be read in conjunction with the table included in the report for 1972. The OER for L.L. cells in the phantom fell steadily with increasing depth when neutrons of mean energy 7.5 MeV were used. At a mean neutron energy of 20 MeV a similar reduction in OER was seen in the phantom. With X-rays however the OER was the same in and out of the phantom. The EA cells gave different results; the OER with neutrons was slightly greater in the phantom than when no phantom was present, but the other way round with X-rays.

Cells	Radiation	Mean Energy MeV	In phantom				No phantom		
			Depth cm	Do		OER	Do		OER
				Aerobic	Anoxic		Aerobic	Anoxic	
L.L.	Neutrons	7.5	0	78	153	1.97 (1.58-2.48)			
E.A.	"	7.5	8.7	85	162	1.90 (1.74-2.06)	89	160	1.78 (1.61-1.96)
E.A.	"	7.5	8.7	55	110	1.98 (1.82-2.15)	64	115	1.81 (1.60-2.05)
L.L.	"	20	8.7	116	173	1.5	93	164	1.8
E.A.	"	20	8.7	99	193	1.95 (1.80-2.10)	114	197	1.73 (1.60-1.85)
L.L.	X rays	2.5	8.7	154	382	2.48 (2.02-3.05)	139	322	2.31 (1.99-2.67)
E.A.	"	0.1	8.7	127	340	2.68 (2.59-2.76)	131	354	2.79 (2.70-2.88)

Notes: L.L. = Lymphocytic leukaemia cells (R.J.Berry)
 E.A. = Erlich ascites cells (B.Cullen)
 The numbers in brackets are 95% confidence limits.

Results of Project No. 2.

Head of Project and	D.K.Bewley
Scientific staff:	C.J.Parnell
	D.E.Bonnett

Title of Project: Study of modification of
neutron spectrum by a phantom.

Description of results

A set of activation detectors and fission counters have been used to study the spectrum of neutrons from the M.R.C. Cyclotron at a depth in a phantom. An increase in low-energy neutrons (less than 1 MeV) has been found, particularly at larger depths in the phantom. This is in qualitative agreement with computer calculations. The magnitude of the fluence at low energies does not however look great enough to explain the biological results found by R.J.Berry. The measurements are continuing.

Radiobiological Institute TNO, Rijswijk (ZH), The Netherlands

Contract No. 113-72-1 BIOC

J.J. Broerse

Calculation and measurement of absorbed dose and neutron spectra inside a biological object with reference to fast neutron radiotherapy

Additional dosimetric data relevant to the experimental set-up employed for neutron irradiations of pulmonary metastases in patients have been obtained. The distribution of fast, thermal and intermediate neutron dose or kerma and gamma dose in phantoms have been measured, employing various experimental techniques. In order to determine the tumour dose and the exit dose for the irradiation of patients, depth-dose measurements have been performed for an inhomogeneous phantom containing lung-equivalent material. The measurements indicate a considerable difference in depth dose with respect to the dose distribution in a homogeneous phantom.

Results of project No. 1

J.J. Broerse and J.E. Broers-Challiss

Depth-dose measurements of d-T neutrons for radiotherapy applications

In order to study volume responses of pulmonary metastases, patients are being irradiated with collimated beams of 15 MeV neutrons produced by the d-T reaction (Van Peperzeel et al., 1974). For the assessment of the tumour dose, depth-dose curves and isodose patterns for different phantoms of the upper part of the human body have been determined. The relative contributions of fast, thermal, and intermediate neutrons and gamma rays to the total dose in the phantoms have been determined using tissue-equivalent ionization chambers, gold foils with and without cadmium cover, and a shielded Geiger-Müller counter, respectively (Mijnheer et al., 1975). The relative contribution of thermal and intermediate energy neutrons to the total dose amounts to 0.23% and 0.03% at maximum, respectively, for a 6 x 8 cm field for the clinical set-up at Rijswijk.

The beam profile studies indicate that the average whole-body dose outside the useful beam is significantly larger for 15 MeV neutrons than for megavoltage X-rays or ^{60}Co gamma rays. The increase in the dose distribution outside the beam with reference to free-in-air conditions was seen to be partly due to the interactions of the primary beam within the phantom; consequently, the problem of beam definition cannot be completely solved by the use of better collimators. From depth-dose studies at different source-to-skin distances (SSD) of 45 cm, 75 cm, and 100 cm, it is evident that depth-dose values could be improved by increasing the SSD; however, due to the limited fluence rates from d-T neutron sources, SSD values have to be restricted to 80 cm for therapy applications at present.

To assess the dose received by the tumours in the lungs, the dose distribution in an inhomogeneous phantom containing lung-equivalent material has been investigated with respect to the contribution from scattered radiation. The results are presented in fig. 1, in which the total absorbed dose rate and gamma-ray dose rate are plotted as a function of depth in the homogeneous and inhomogeneous phantoms for an SSD of 45 cm, and a field size of 6 x 8 cm. The measurements in the inhomogeneous phantom show complex depth-dose patterns and, at depths smaller than 6 cm, the total absorbed dose rate is lower than in the homogeneous phantom, indicating a reduced contribution from back-scattered neutrons. This dose reduction in the soft tissue in front of the lung will depend on the geometry

and composition of the lung tissue in the patient and will require careful consideration to arrive at an accurate tumour dose.

To provide further insight into the problem of monitoring the exit dose during patient irradiations by the use of threshold detectors, it was decided to compare the neutron dose in soft tissue determined from the TE ionization chamber and the GM-counter measurements at various depths in the water phantom with the neutron kerma derived from sulphur pellets and aluminium foils due to the $^{32}\text{S}(n,p)^{32}\text{P}$ and $^{27}\text{Al}(n,\gamma)^{24}\text{Na}$ reactions, respectively. The ratio of the neutron kerma to the neutron absorbed dose was plotted as a function of the depth in the phantom for an SSD of 45 cm and is presented in fig. 2. Curves a give the results assuming that all neutrons have an energy of 15.3 MeV and indicate that spectrum changes occur with depth along the central axis. Greene and Major (1972) observed a slight hardening of the neutron spectrum with depth. On the contrary, measurements of Hannan et al. (1973) indicate a decrease in the percentage of neutrons in the 15 MeV region for a collimated d-T neutron beam. Calculations of neutron spectra performed by Grünauer (1974) for the actual irradiation set-up at Rijswijk show a similar decrease in the contribution from 15 MeV neutrons with depth. The average kerma values calculated for the two spectra have also been compared with the neutron dose and are presented as curves b and c in fig. 2. It can be concluded that the variation in neutron kerma derived from the threshold detectors for neutron absorbed dose with depth is considerably reduced, when the calculated spectra of Grünauer are employed.

Greene, D. and Major, D., p. 463, In: Proc. 2nd Congress of Eur. Ass.

Radiology, Excerpta Medica, Amsterdam, 1972.

Grünauer, F., private communication (1974).

Hannan, W.J., Porter, D., Lawson, R.C. and Railton, R., Phys. Med. Biol. 18, 808 (1973).

Mijnheer, B.J., Broers-Challiss, J.E. and Broerse, J.J., In: Proc. 2nd Int. Symp. Neutron Dosimetry in Biology and Medicine, Munich, 1974, in press.

Peperzeel, H.A. van, Breur, K., Broerse, J.J. and Barendsen, G.W., Europ. J. Cancer 10, 349 (1974).

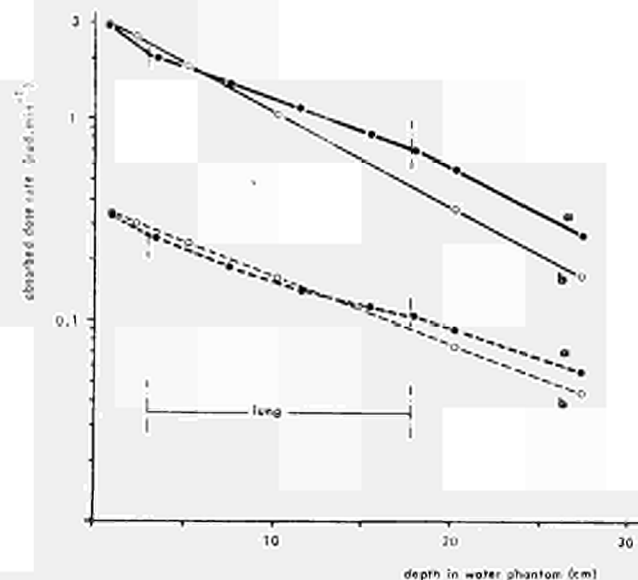


Figure 1. Depth-dose curves in an inhomogeneous phantom irradiated with 15 MeV neutrons. Measurements of total absorbed dose rate (solid lines) and gamma-ray absorbed dose rate (dotted lines) are given for a collimated 15 MeV neutron beam; SSD, 45 cm; field size, 6 x 8 cm, in a water phantom with (curves a) and without (curves b) lung-equivalent material.

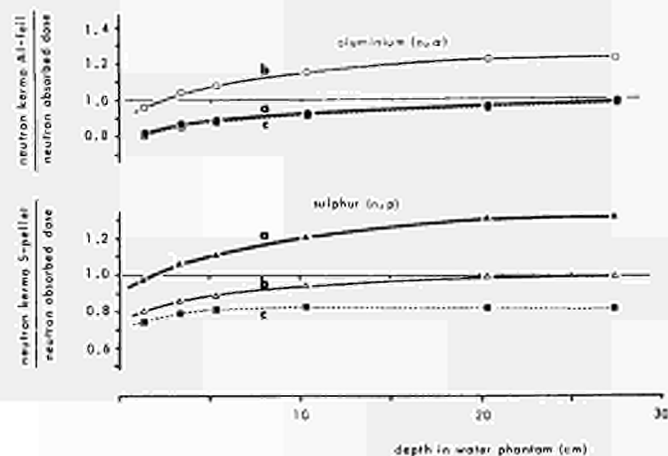


Figure 2. Ratio of neutron kerma, as determined by Al and S detectors, to neutron absorbed dose as a function of depth. The measurements were performed in a water phantom (SSD, 45 cm; field size, 6 x 8 cm). The kerma values have been calculated for monoenergetic 15.3 MeV neutrons (curves a) and for spectra derived by Hannan et al. (1973) (curves b) and by Grünauer (1974) (curves c).

Contractor:

International Commission on Radiation Units and Measurements (ICRU)

Contract No:

113-72-1 BIOC

Head of research teams:

Harold O. Wyckoff

General subject of Contract:

ICRU Neutron Dosimetry Intercomparison Project

During calendar year 1973, 14 groups from 6 countries measured selected radiations from the Radiological Research Accelerator Facility at Brookhaven National Laboratory. Monoenergetic neutrons of 15.5, 5.5, 2.1 and 0.67 MeV were made available as well as radiation from a ^{252}Cf fission source. Measurements were made of the neutron and gamma tissue kerma in free air for all sources and absorbed dose in tissue at two depths for the 15.5 and 5.5 MeV sources.

The analysis and report writing committee met in May 1974 for an initial consideration of the data, to develop an outline for the final report and to give initial consideration to the problems of analysis. Writing assignments were made for the report which is to be presented to the Commission for its consideration during its Fall, 1975 meeting.

A short meeting was held in October to consider the preliminary analysis of the intercomparison. Additional information is required from some of the participants and plans were made for further analyses.

Contractor: GSF - Projektgruppe Neuherberg
- Projektgruppe Frankfurt
MRC Cyclotron Unit
TNO Radiobiological Institute

Contract No.: 113-72-1 BIOC

General Subject: European Neutron Dosimetry
Intercomparison Project (ENDIP)

Heads of Project: D.K. BEWLEY, J.J. BROERSE, G. BURGER,
W. POHLIT.

The preparation of this project has been continued during 1974.

European Laboratories were invited to participate in the ENDIP; 20 laboratories met at Neuherberg in October 1974 in order to discuss the technical organisation of the intercomparison which will take place at Neuherberg during April - July 1975, and at Rijswijk during September - December 1975.

VI.

KOORDINIERUNGSTÄTIGKEIT

COORDINATION

ACTIVITES DE COORDINATION

VI. COORDINATION

Since the beginning of the programme "Biology - Health Protection" in 1960, coordination of research has always been accomplished by the Commission and its various contractual partners. A detailed scheme of the different levels of coordination, from the formulation of programme objectives to the implementation of joint projects, has already been published.*

One important tool is the "Study Groups"; those which held meetings in 1974 are listed in part A. of this chapter. Although they have different scientific objectives and different aims and working methods, one feature is common to them all, namely that they bring together scientists from a wide variety of national laboratories and the Commission in free and open discussions, exchanges of views and information, and that they create contacts between European laboratories and thereby foster meaningful and continuing cooperation.

Part B. lists the meetings which the Commission has organized itself or actively supported, within the limits of its financial resources. It is evident from the worldwide resonance that these meetings have encountered, that they play an encouraging and stimulating role for the European scientific community.

The Health Protection Directorate also organizes meetings of experts whose work has both the effect of coordinating and stimulating the research in the Commission's programme "Biology - Health Protection", especially with a view to practical measures for promoting radiation protection. Part C. lists these groups, particularly the Group of Experts on "Basic Safety Standards" mentioned in Article 31 of the Euratom Treaty.

* Annual Report 1972 "Biology - Health Protection", EUR 4864, chapter "Coordination", pages 747-751.

A.

Meetings of
Study Groups in 1974

Study Group "Personal Dosimetry"

Luxembourg, 19-20 February 1974

23 participants from 4 countries and the Commission.

Principal subject:

Discussion of the programme of research on personal dosimetry.

Study Group "European Neutron Dosimetry Intercomparison Project"

Neuherberg-München, 16 April 1974,

7 participants from 3 countries and the Commission.

Principal subject:

Preparation of the "European Neutron Dosimetry Intercomparison Project".

Study Group "Dosimetry"

Neuherberg-München, 18-19 April 1974,

31 participants from 5 countries and the Commission.

Principal subjects:

Transfer of radiation energy to matter.

Analysis and interpretation of dose-effect relationships.

External radiation dosimetry.

Internal radiation dosimetry.

Developments of methods and instruments.

Study Group "Marine contamination"

Rovinj (Yugoslavia), 9 - 10 May 1974,
35 participants from 7 countries and the Commission.

Principal subject:

- Physico-chemical and biological turnover of radionuclides and metals in marine environment.
- Effects of radionuclides and metals on marine organisms.
- Environmental parameters significant in the evaluation of the recipient capacity of the sea including the methodology for the evaluation of human exposure to radionuclides and heavy metals through the food of marine origin.
- Tritium and heavy alpha emitters in the marine environment.

Study Group "Primary Effects of Ionizing Radiation on Nucleic Acids"

Brussels, 11-12 June 1974.
19 participants from 6 countries and the Commission.

Principal subjects :

Studies of radical formation; properties and behaviour of radicals in nucleic acids in solid and liquid state and in complexes of nucleic acids and dyes or amino acids.

Study Group "Natural Radioactivity"

Luxembourg, 12 June 1974
28 participants from 9 countries and the Commission.

Principal subject:

Exposure of population to natural radiation.

Study Group "Thorotrast"

Heidelberg, 28 June 1974
16 participants.

Principal subject:

Discussion of toxicity of thorotrast.

Study Group "Personal Dosimetry"

Cadarache, 17-18 September 1974
3 participants from 2 countries.

Principal subject:
Coordination of research work on neutron dosimetry.

Study Group "European Neutron Dosimetry Intercomparison Project"

Neuherberg-München, 4 October 1974,
43 participants from 8 countries and the Commission.

Principal subject:
Preparation of the "European Neutron Dosimetry Intercomparison Project".

Study Group "Mutation Breeding"

Wageningen, 8-10 October 1974,
43 participants from 7 countries and the Commission.

Principal subjects :

- Incompatibility in higher plants.
- Protein improvement.
- Disease resistance induction.

The meeting was organized by the Association EURATOM-ITAL.

Study Group "Mutation Spectra and Modifications of Dose-Mutations Relations in Cell Cultures and Microorganisms"

Brussels, 5-6 November 1974,
20 participants from 6 countries and the Commission.

Principal subjects :

Discussion of individual results and programmes; elaboration of recommendations for future researches on dose-mutations relations in microorganisms and cultured cells and on the genetic control of repair processes.

Study Group "Radioentomology"

Wageningen, 6 - 7 November 1974,
21 participants from 7 countries and the Commission.

Principal subject:
Radioentomology in the frame on integrated control of noxious
insects: present state of knowledge, future research needs.

Study Group "Modifications of Dose-Mutation Relations in Higher Organisms
and Induced Aberrations of Chromosomes"

Brussels, 7-8 November 1974,
16 participants from 5 countries and the Commission.

Principal subjects :
Discussion of individual results and programmes; elaboration of recommen-
dations for future researches on the origin, frequencies, repair and
transmissibility of mutations and chromosomal aberrations in multioellu-
lar organisms.

Study Group "Personal Thermoluminescence Dosimetry"

Bologna, 11-15 November 1974
12 participants from 8 countries and the Commission.

Principal subject:
Comparison of principles of measurement.

Study Group "Marine contamination"

Brussels, 14 - 15 November 1974,
5 participants from 4 countries and the Commission.

Principal subject:
Radiological protection in the marine environment with respect
to nuclear energy: present state of knowledge, future research
needs.

Study Group "Biochemistry of repair"

Brussels, 19-20 November 1974,

20 participants from 8 countries and the Commission.

Principal subjects :

Discussion of individual results and programmes; elaboration of recommendations for future researches on DNA lesions and the repair of induced damages.

Study Group "Personal Dosimetry"

Luxembourg, 21 November 1974

12 participants from 3 countries and the Commission.

Principal subject:

Coordination of research work on neutron dosimetry (rem counter).

Study Group "Radioactive contamination of terrestrial and fresh water ecosystems"

Wageningen, 26 November 1974,

12 participants from 8 countries and the Commission.

Principal subject:

Quality of life, terrestrial and freshwater ecosystems, and nuclear energy: present state of knowledge, future research needs.

B.

Meetings organized or co-sponsored by
the Commission of the European Communities in 1974

First co-operative meeting on freezing thrombocytes and granulocytes

Reisensburg/Ulm, 8-9 February 1974.
10 participants from 4 countries.

European Late Effects Project Group (EULEP)

- Reisensburg/Ulm, 2-3 May, 1974
17 participants from 6 countries and the Commission.

Principal subjects :

Research proposals for the next five-years plans of Euratom in the frame of the Committees : Carcinogenesis, Dysplasia and Dystrophia - quantitative and qualitative cell changes, Point source effects, Dosimetry standardization, Animal standardization, Pathology standardization, Clinical pathology standardization.

- Brussels, 8-9 November 1974
20 participants from 6 countries and the Commission.

Workshops for the discussion of the different aspects of the research proposals : late effects in lungs and cancerogenesis (development of immune monitor subgroup, bladder, leukemia, liver).

5th International Congress of Radiation Research

Seattle, Washington, U.S.A., July 14-20, 1974.
About 150 contributions by 1944 scientists from 36 countries.

Second Symposium on Neutron Dosimetry in Biology and Medicine

Neuherberg/München, 30 September - 4 October 1974.
153 participants from 17 countries and the Commission.

This Symposium was held in view of the increasing application of neutrons for biological, medical and technical purposes, to evaluate the radiation risk and to assess the physical basis of radiological applications of neutrons.

Seminar on Chromosome Aberrations

Rome, 12-13 November 1974
40 participants from 7 countries and the Commission.

Principal subject:
Chromosome aberrations as indicators of irradiation levels.

Symposium on "Medical supervision of workers exposed to ionizing and non-ionizing radiation"

Brussels, 28-29 November 1974
100 participants from 9 countries and the Commission.

C.

Meetings of Experts

Chapter III of the Euratom Treaty in 1974

Group of Experts "Personal Dosimetry"

Luxembourg, 29 January 1974

22 participants from 9 countries and the Commission.

Principal subject:

Drafting "Basic Principles of Personal Dosimetry".

Group of Experts "Personal Dosimetry"

Luxembourg, 30-31 January 1974

13 participants from 9 countries and the Commission.

Principal subject:

Drafting technical recommendations for the use of thermoluminescence in personal dosimetry.

Group of Experts "Article 36" of the Euratom Treaty

Luxembourg, 4-5 April 1974

11 participants from 5 countries and the Commission.

Principal subject:

Radioactive contamination.

Group of Experts "Article 31" of the Euratom Treaty

Brussels, 18-19 April 1974

22 participants from 9 countries and the Commission.

Principal subject:

Review of basic safety standards.

Group of Experts "Thermoluminescence Dosemeters"

Arnhem (Netherlands), 25-26 April 1974
10 participants from 5 countries and the Commission.

Principal subject:
Preparation of technical recommendations.

Group of Experts "Article 37" of the Euratom Treaty

Luxembourg, 6-8 May 1974
27 participants from 9 countries and the Commission.

Principal subject:
Examination of estimates radioactive effluent discharge from
the nuclear power stations of Tihange and Phenix.

Group of Experts "Personal Dosimetry"

Luxembourg, 31 May 1974
22 participants from 9 countries and the Commission.

Principal subject:
Preparation of recommendations on the basic principles of
personal dosimetry.

Group of Experts "Article 37" of the Euratom Treaty

Luxembourg, 9-10 July 1974
13 participants from 9 countries and the Commission

Principal subject:
Comparison of iodine filters.

Group of Experts "Article 37" of the Euratom Treaty

Karlsruhe, 10-12 September 1974
40 participants from 9 countries and the Commission.

Principal subject:
Examination of estimates of radioactive effluent discharge from
the Biblis, Borssele and Caorso nuclear power stations.

Group of Experts "Personal Dosimetry"

Luxembourg, 8-9 October 1974

26 participants from 9 countries and the Commission

Principal subject:

Examination and approval of technical recommendations on personal dosimetry and thermoluminescence dosimetry.

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