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**ASSOCIATION**

**EUROPEAN ATOMIC ENERGY COMMUNITY - EURATOM**

**INSTITUUT VOOR TOEPASSING VAN ATOOMENERGIE IN DE LANDBOUW - ITAL**

# **APPLICATION OF ATOMIC ENERGY IN AGRICULTURE**

**(ANNUAL REPORT 1964)**

**1965**



**Work performed at the  
Instituut voor Toepassing van Atoomenergie in de Landbouw - ITAL  
Wageningen (Netherlands)**

**Association No. 003-61-5 BIAN**



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## INTRODUCTION

The facilities of the EURATOM-ITAL Association Institute at Wageningen were further extended in 1964.

By May 1964, construction of the following facilities had been completed : the radiobiological wing, comprising a 300 Ci Cs  $^{137}\gamma$ -source in a greenhouse with partial environmental control and a 5,000 Ci Cs  $^{137}\gamma$ -source shared by four plant cells with environmental control; a 2 MeV van de Graaff electron generator; a 250 KV X-ray machine; six plant growth chambers; four storage rooms for food products.

Construction of the extension to the radiochemical wing was practically completed, whereas the erection of a second greenhouse was started. This means that the original building plan had been fulfilled and, therefore, the official opening of the institute could take place at the end of 1964, namely on October 8.

Research projects under way progressed satisfactorily in the three main fields of interest of the Association, namely mutation breeding, food technology and the behaviour of specific nuclides in soils and plants.

Furthermore research in the field of dosimetry was started, the main forms of interest being energy deposition in plant cells.

In the framework of the Association's program for European cooperation two new subcontracts were considered, namely with the "Institut National des Recherches Agronomiques", in France and with the University of Louvain, in Belgium.

Besides participation in international working groups on mutation breeding and food preservation which were established in previous years, the Association staff has also participated in a Euratom working group on soil-plant-relations which started its activities in 1964.

The present annual report again integrates the scopes and results of the scientific research projects carried out directly by the Association staff and by that of the various Institutes linked to the Association by subcontracts. A new chapter on coordination of applied research has been included as well as a list of meetings, symposia etc. attended a list of governors and staff, a scheme of the research activities and a plan of laboratories and facilities. Details will be found in Appendixes I, II, III and IV.

Besides this main report, a short note on an improvement of the  $\beta$ -measuring technique is given in Appendix V as an example of the work of the instrument development group.

D. de Zeeuw,  
Director.

## RESEARCH ACTIVITIES

The EURATOM - ITAL Association research activities were, as in the previous years, directed to studies on the applications of atomic energy to agriculture. This meant that, on the one hand, practical problems in the fields of plant breeding, food preservation and contamination of the soil-plant-system were tackled in close cooperation with applied research institutes in the Community, and that on the other hand research was carried out to improve nuclear techniques and make them more suitable for application to agricultural research. In 1964, emphasis was again placed on studies concerned with problems of radiation induced changes in plants, food preservation by means of ionizing radiations, the behaviour of specific chemical elements in soils and plants and the improvement of nuclear techniques for agricultural research.

### RADIATION INDUCED CHANGES IN PLANTS

This program, which aims at making irradiation as effective as possible in order to produce the maximum of desired characteristics in plant material was carried out in three countries of the European Community, namely Italy, the Netherlands and the German Federal Republic. This coordinated program of research is closely connected through direct EURATOM contracts with projects in Belgium and France.

Since research should be oriented to practical applications, it is realised that in many cases a proper mutation research program can be based solely upon treatment of plant seeds. However, complementary mutagenic treatments are often required during other ontogenic stages. For instance, as is also stated by EHRENBERG and others, if the germline is represented by many initial cells in the embryo, the mutation will be obtained in a sectorial chimaera and may easily be lost in competition with non-mutated cells. Furthermore, irradiation during earlier stages of pollen or seed formation is necessary when the mutated plants must be homogeneously heterozygous for the mutation. In that case irradiation of whole plants in different stages of development is necessary. This treatment is also necessary when searching for stages where a minimum of diplontic selection occurs. Including both a practical mutation



breeding aspect and a fundamental radiobiological one, studies on radiation induced changes in plants concentrated on a number of test plants : beans (Phaseolus sp.), cereals (Triticum and Hordeum sp), peas (Pisum sp), potatoes (Solanum sp) and tomatoes (Lycopersicum sp) in the case of the mutation breeding program and included other species when necessary for the radiobiological studies. Comparative studies are in progress utilizing X- and gamma-radiations, neutrons as well as chemical mutagens such as ethyl-methane-sulfonate (EMS). Following completion, in 1964, of the facilities of the Association's Institute, possibilities are available for irradiation with X-rays under rather well controlled conditions, with neutrons from the "BARN" (Biological Agricultural Reactor Netherlands), and gamma's from 300 and 5000 C Cs 137 sources under carefully controlled conditions of illumination, temperature, humidity and growth medium for acute or chronic treatments. Details of these facilities are given in Appendix IV. The decision to make control of environment at conditions available at the Institute was prompted by the need to obtain a better assessment of radio-sensitivity of plants, seeing that it is no doubt influenced by their physiological conditions during and after treatments.

## MUTATION BREEDING

### ASEXUALLY PROPAGATED PLANTS

The induction of chimaeras is of obvious importance for the practical breeding of vegetatively propagated plants such as Chrysanthemum, roses and potatoes. Studies on these plants were carried out in order to obtain greater variation in flower colours. Although so far no new morphological aberrations were noted on potatoes, the fact that so many other traits were affected leaves hope for the induction of disease resistance or other beneficial physiological characters.

Finally, the prevention of chimaera formation in Saintpaulia gave some interesting results following treatment of leaf blades of stalks.

## STUDIES ON CHIMAERAS

C. BROERTJES (+)

Chrysanthemum :

The same total percent mutations were obtained in 1964 as in 1963 from the cultivar "Hortensien rose", namely 21 %. Irradiation was with 1.5 - 2.5 Krad X-ray. Recovery of the mutations was facilitated by improving rooting of the cuttings with hormones. A number of new experiments were started, using not only X-irradiation but also thermal neutrons from the "BARN" (Biological Agricultural Reactor Netherlands) of the Association. "Jacob Maris", var. plants were thus treated with 1500 rad X and with various thermal neutrons doses (ranging from 1.5 -  $7.5 \times 10^{14} N_{th}/cm^2$ ). Half of the plants were pruned back to study effects on the size of the mutated sector. Three other varieties, namely "Hortensien rose" "Breitner" and "Asta Lee" were irradiated with optimal X-ray doses, some of them being pruned back.

Roses :

Experiments reported in 1963 were continued and field grown plants showed some mutations. Rooted cuttings from the mutated branches have been planted. A new set of "Baccara" roses plants were irradiated with neutrons in the thermal column of the reactor at doses ranging from 1.5 -  $12 \times 10^{13} N_{th}/cm^2$ , before planting in experimental fields.

Saintpaulia :

Experiments were continued with this species. Irradiation with 2 - 4 KR X of the leaf petiole, leaf blade or the complete leaf showed that a higher mutation rate was scored when the complete leaf was irradiated than when only the petiole was treated. It was found that the mutations also occurred after irradiation of the leaf blade. Neither neutron nor ethyl-methane-sulfonate (EMS) treatments were successful at low doses. Further experiments are in progress consisting of acute and chronic treatments with neutrons and higher doses of EMS.

(+) Association EURATOM - ITAL - Wageningen, the Netherlands.



C. BROERTJES (+)

Application of extracts of mashed leaves irradiated with 3 - 10 KR X to untreated leaves and treatments with soft X-ray doses (50 KeV, 25 mA) of petioles were included as treatments to study further the above mentioned results.

#### THE INDUCTION OF POINT MUTATION IN POTATOES

F.P. FERWERDA (+)

The maintenance, multiplication and assessment of aberrant types obtained from X-ray treatments from 1961 to 1963 was carried out in 1964.

The clonal offspring of irradiation series representing respectively the  $M_4$ ,  $M_3$  and  $M_2$  were maintained and multiplied in the Experimental Farm of the Foundation for Plant Breeding, situated in the North East Polder of the Netherlands.

All individuals suspected of harbouring virus disease were eradicated. The fact that tuber lines continue to segregate into two or sometimes three distinct types was again noted. This segregation is not surprising if the mosaic structure of the initial individuals is considered. In advanced vegetative generations uniformity within clones increases. A logical consequence of this phenomenon is that to screen for disease resistance clonal material should be taken from tuber lines that have already been maintained for at least 2 years and are more or less stabilized. This policy was adopted when collecting seed tuber material for a screening test for disease resistance. So-called "light grown sprouts" of a large number of well stabilized tuber lines were raised and observed. It is generally recognized that the characteristics of these sprouts are very stable, used as a criterion for determination of clones. Among 43 tuber-lines derived from the "Bintje" variety, 6 showed a markedly aberrant light-sprout.

(+) Instituut voor Veredeling van Landbouwgewassen (IVL) - Department of Agricultural Plant Breeding, Agricultural University - Wageningen, the Netherlands.

In the variety "Burmania", 5 out of 77 tuber lines had a light-sprout considerably deviating from the normal type, mostly with regard to shape, hairiness and anthocyanin content. These facts clearly show that mutagenic treatments can bring about hereditary changes in various organs of a potato plant.

In 1964, emphasis was placed on irradiation of di-haploids since their diploid structure could facilitate the detection of mutants and chimaeras. Some aberrant types were observed and will be investigated. Since an effective mutagenic treatment practically always results in a chimaerical structure, the lateral buds that replace the inactivated main vegetative apex can be considered already too much differentiated. To avoid a chimaerical structure cells or tissues ontogenetically younger should be irradiated, probably very young lateral or adventitious buds just initiated.

Eye-excision experiments to uncover chimaerical structures have been continued in 1964. Two aberrant types with a variegated (yellow spashed red) tuber skin, one derived from the "Burmania" variety, the other risen spontaneously in the variety "Desiree", were submitted to a large scale eye excision test. Results indicate that all the adventitious buds regenerating from the inner part of the treated tuber developed into plants bearing red skinned tubers, while the untreated tuber halves developed into plants with variegated (yellow splashed red) tubers.

F.P. FERWERDA

It thus appears that the outer tissue layers differ genetically from the central part. The test cross data mentioned below support the assumption that the mutated part includes at least the sub-epidermal cell layer. Apart from the two aberrant types just mentioned a few more suspected of having a chimaerical structure are being investigated.

#### Genetical analysis of some aberrant types :

Some information about the genetical background of the "ivy-leaf" anomaly was obtained from a set of test crosses performed in 1963 and analyzed this year. The data obtained are summarized in table 1.



Table 1 : F<sub>1</sub> segregation ratios in test cross progenies of "ivy leaf" potato.

Type of pollination	<u>Number of plants</u>		Approximate ratio	
	Ivy	Normal	Ivy : Normal	
Ivy, selfed	236	118	2	1
Ivy x normal	140	160	1	1
Normal x ivy	139	151	1	1
Normal, selfed	-	493	0	1

The hereditary nature of the "ivy-leaf" type is thus obvious. The segregation ratios observed can be explained assuming the genetic constitution Iiii for ivy, IIii for normal leaf; a further postulate to be made is that iiii is non-viable. Additional evidence will have to be collected to support this hypothesis. The original assumption that "ivy-leaf" may result from uncovering a latent periclinal structure, has been disproved by a series of eye excision experiments which never gave back normal leaf type from eye-excised "ivy" tubers. The tuber skin anomaly indicated as "yellow splashed red" some years ago was obtained by irradiating "Burmania" variety. The experiments mentioned above prove that it is a periclinal chimaera differing from the original variety only by the genetical composition of its outer tissue layers. A series of test crosses, analyzed this year, provided additional evidence to support this conclusion. Both types had been crossed with "Ulster Knight" which has a colourless tuber skin. The F<sub>1</sub>-progenies segregated into yellow, pink and red as follows :

Test cross or selfing	<u>Number of plants with tuber skin colour</u>		Approximate ratio
	Yellow	Pink+red	
a) Yellow splashed red, selfed	108	289	1 : 3
b) Y.s.r.x "Ulster Knight"	301	216	3 : 2
c) Normal "Burmania" x "Ulster Knight"	239	226	1 : 1
d) Normal "Burmania", selfed	126	267	1 : 2

## .P. FERWERDA

The discrepancy between test crosses b) and c), too striking to be ascribed to mere chance, indicates that yellow splashed red and normal type "Burmania" differ genetically. However, the information available does not yet allow to define it by a genetic formula.

## M. MONTEZUMA DE CARVALHO (1,

Histological and cytological effects of mutagens on the apical shoot meristem of potato :

Histological investigations confirm the impression obtained from external inspection that the apical meristem is inactivated. From approximately 3 weeks after irradiation the typical arrangement of meristem cells gets more and more disturbed and the activity of lateral axillary born buds increases.

Dormant and non-dormant excised eyes of potato (var. "Bintje") were irradiated with a dose of 3500 R X. The cytological effects of irradiation were studied at anaphase by scoring the number of bridges and fragments. After irradiation the non-dormant eyes show a period of complete mitosis inhibition that lasts 24-48 hours. The maximum for damaged cells is reached on the 5th day. Fixations made on the 12th, 17th and 28th day show a 20-fold decrease in the mitotic activity. For dormant eyes the maximum for damaged cells is reached on the 7th day. In contrast to the non-dormant eyes only a two-fold decrease in mitotic activity was found. Results show that when dormant eyes are irradiated more cells are able to enter division for a longer period. This can be related to a lower sensitivity of the cells. It is in fact apparent when we compare over the period of 28 days the percent of cells (anaphase) showing abnormalities in the non-dormant and dormant eyes. For the non-dormant there are 81.1 % abnormal anaphases and for the dormant group 74.3 %. It is interesting to note, however, that the mean number of fragments per abnormal anaphase is about the same for both dormant and non-dormant eyes (i.e. 2.13 and 2.15 respectively). Thus there could be two populations of cells in dormant eyes : one with the same radio-sensitivity and the non-dormant ones and another more resistant.

(+) Instituut voor Veredeling van Landbouwgewassen (IVL) -  
Department of Agricultural Plant Breeding, Agricultural  
University - Wageningen, the Netherlands.



SEXUALLY PROPAGATED PLANTS

Despite unfavourable field conditions in 1964, most mutation breeding trials were carried to conclusion.

R.J. HERINGA (+)

## MUTATION RESEARCH ON BEANS

Results obtained following X-ray and EMS treatments of dry seeds of beans (Phaseolus vulgaris) var. "Beka", are not promising. No visible mutants were found in the  $M_2$  and  $M_3$  plants. A high amount of sterility was noted in recently treated seeds of the var. "Berna"; environment conditions were not particularly favourable in this last reported experiment and a lower than normal germination was noted in the controls.

## SIGNIFICANCE OF MICRO-MUTATIONS IN BARLEY AND WHEAT

H. GAUL, K. BENDER, C. U. HESEMANN (++)

The variability experiments with "Amsel" and "Haisa II" were completely transferred from the  $M_3$  to the  $M_4$  generation. During the growing season records were taken of the number of chlorophyll mutants segregating, heading date, the number of partially sterile spikes, and kernel yield per plot. Data collected are being analyzed. The analysis of the  $M_3$  generation was completed. One of the most significant findings on the induced variability of yield is a decrease of the mean value and at the same time an increase of the genotypic variance of the lines derived from radiation as compared to the corresponding controls.

Furthermore it was concluded that decrease of the mean, increase of the chlorophyll mutation frequency and increase of the genetic variance were correlated with each other.

(+) Stichting voor Plantenveredeling (SVP) - Foundation for Agricultural Plant Breeding, Wageningen, the Netherlands.

(++) Max-Planck-Institut für Züchtungsforschung - Köln - Vogelsang, W.-Germany.

The variability experiment with the winter wheat "Heine Stamm 2806/55-B20/60" considered the number of chlorophyll mutations segregating, the heading date, the number of culms and the yields per plot. Statistical analysis is in progress.

#### SIGNIFICANCE OF MACRO-MUTATIONS IN BARLEY

H. GAUL, K. BENDER, C. U. HESEMANN

The material consists of 1,010 families of the spring barley program and 196 families of the winter barley program. During the growing season a great number of records were taken with regard to the behaviour of the various characters of interest. The analysis of the  $F_2$  generation is in progress. A small, but highly interesting, part of the material is grown in the greenhouse in  $F_3$  generation at the present time and further crosses, particularly back crosses, are conducted along with selfings.

Some clear examples that the pleiotropic effect of macro-mutations can be modified are available from the data of the  $F_2$  generation.

A mutant which differs from its mother variety by two main characters, a shorter culm and a denser spike, was found. The cross of this mutant with a barley from Abyssinia, var. "Bulchi Gofa", resulted in some  $F_2$  plants with short culm but with a lax spike. Thus the two main characters of the mutant, short culm and dense spike, can be separated if new gene interactions are introduced by means of crossing.

#### DIPLOIDIZATION OF AUTOTETRAPLOID BARLEY

H. GAUL, K. BENDER, C. U. HESEMANN

The procedure of repeated mutagenic treatment and of multiple inter-crossing of  $F_1$  plants was continued on a large scale. A number of diploid lines were compared with tetraploids to study yield capacity of rough tetraploids. Results indicate that tetraploid barleys have only about 50 % of the yielding capacity of the corresponding diploids. Considering earliness and tillering, and using the pedigree method, lines could be selected which are already earlier than common tetraploid

barley and other tetraploid lines could be isolated which appear to tiller as much as common diploid barley. Colchicin treatment of a number of diploid varieties resulted in at least 6 new tetraploid barleys.

The three inversion lines found up to now in diploid barley, which are in the genetic background of non-adapted varieties, were transferred to three German varieties: "Union", "Volla" and "Wisa". The new inversion lines will be used for the diploidization program after doubling their chromosome number.

#### STUDIES ON THE EMS-EFFECTS IN BARLEY

H. GAUL, K. BENDER, C. U. HESEMAN

In addition to the above mentioned projects a study was carried out on the action of EMS to improve the control of its effects. The factors used in combination with EMS treatment include : washing of the treated seeds up to 36 hours later; drying back of the seeds; storage of the dried seeds in warm and cool temperature up to a period of 12 months. The criteria used for measuring the EMS-effects are : seedling height, survival rate, sterility and frequency of chlorophyll mutations in the  $M_2$  generation.

#### CYTOGENETICS OF DURUM WHEAT

G. T. SCARASCIA

A detailed idiogram of *Triticum durum*, var. "Cappelli" as comparison base for the caryotype of some mutants, has been made. The total length of single chromosomes and their arm ratios have been determined. The chromosomes, according to their arm ratios, are divided into three groups : median, submedian and subterminal. On one chromosome, not well identified, which could be derived from T. Aegylopoides, a very small satellite has been found.

Moreover cold treatment has put in evidence some heterochromatic zones in some chromosomes. The drawing of the idiogram of T. Aegylopoides (Genoma A) and of Ae. speltoides (Genoma B) is under way. As to cytogenetics of durum wheat mutants, cytological analyses of progenies of "Cappelli" and "Aziziah" have been made. The presence of an aneuploid with 30 chromosomes (Instead of  $2n = 28$  of "Cappelli") has been confirmed. An investigation is in progress to ascertain which chromosome or chromosomes are duplicated. Other mutants showing an irregular pairing of chromosomes in diakinesis are also under investigation to detect which type of translocation

was induced and which chromosomes are affected. The cytogenetical analyses of the several progenies put in evidence some other aberrations : desynaptic and diplochromosomes meiosis as well as translocations involving up to 8 chromosomes.

#### INVESTIGATIONS ON PLEIOTROPY AND EXPRESSIVITY OF MUTATED GENES

W. GOTTSCHALK, A. JAHN (+)

In 1964, the cytological evaluation of mutant genes of Pisum sativum influencing the course of meiosis in different respects was carried out. 14 different mutant types have been found so far showing a variable reduction of number of chiasmata during the first meiotic prophase. Considering different mutant types a direct relation between the degree of reduction of chiasmata and the number of univalents could be stated.

In some mutants of this group a relatively high fertility could be observed, because the number of univalents was small. In other case, nearly exclusively univalents were formed during the late stages of meiotic prophase and of metaphase I, causing full sterility of these genotypes.

One or two series of multiple alleles may be responsible for these meiotic disturbances.

A second group of mutant genes causes full breakdown of meiosis in different, but specific stages. These genes are primarily acting on tapetum tissue, causing exclusively male-sterility, while macrosporogenesis is completely normal, producing functionable egg cells. So far, 6 different mutant types of this group could be studied. One of them does not develop and archespore tissue within the stamens and no pollen mother cells are available; meiosis can therefore not take place.

In a third group of mutants, genes which cause more or less inspecific meiotic disturbances have changed, resulting in manifold irregularities which reduce the proportion of functionable germ cells.

(+) Institut für landwirtschaftliche Botanik der Universität Bonn- Bonn, W.-Germany.

A small group of mutants showed full sterility, but no irregularities in microsporogenesis could be noted .

During irradiation of seeds, a large number of chromosomal mutations were induced within the surviving initial cells of the growing points of the embryos. Development of these plants is more or less normal, but specific irregularities can be expected during meiosis. Exclusively reciprocal translocations could be found causing reduction of number of functionable germ cells. Usually, a ring of four chromosomes is present in the pollen mother cells during metaphase I. In one case, a ring of 6 chromosomes could be observed in every cell, formed by three different, non-homologous chromosomes.

Two genotypes showing a clear relation between the degree of expressivity of the mutant gene and the course of ontogenetic development have been studied. One has some sepals of the flower differentiated in the form of carpels; within the lower inflorescences of the plant the basal flowers often show completely normal structure and no action of the mutant gene can be observed. The morphology of the apical flowers however show clear irregularities, indicating a strong action of the gene. Considering the flower morphology with subsequent course of ontogenesis, a progressively increasing degree of expressivity of the mutant gene can be stated. Therefore the flower differentiation in the upper inflorescences of all plants of this genotype is extremely irregular in respect to the basal flower of the same inflorescences.

In a second mutant a relation between the expressivity of the recessive gene and the course of ontogenesis has been found in respect to leaf-morphology.

Some new groups of non-allelic polymeric genes of the genome of Pisum were found in former investigations. In 1964, five different mutant types were analyzed in respect to leaf morphology.

These mutants have extremely reduced but not agreeing stipules. After hybridization with one another, exclusively mutant plants arose showing reduction of stipules. It is therefore clear, that the differences are not caused by different polymeric genes. The mutants were simultaneously used to study whether these constantly appearing differences in leaf-shape are due to the action of multiple series or the non-identical genotypic composition.



A second group of nearly agreeing mutants of different origin is now under investigation and the first hybridizations have been made. Studies concerning the penetrance of a distinct mutant gene were continued. The action of the gene in question refers to a forking or a dichotomy of the stem. This mutant could be of great interest for practical breeding, because two pod-bearing stems are present in the upper part of the shoot, increasing the number of pods and the number of seeds per plant.

Unfortunately, only 40-60 % of all plants being homozygotic with respect to this gene are developing the forked stem, thereby reducing total yields. Four different mutants lines showed this dichotomy, but with a different proportion of stem-forked plants. Plants showing a relatively low situated point of dichotomy had a higher proportion of forked stems in their offsprings.

#### MUTATION RESEARCH ON PEAS

R. J. HERINGA

The work on micro-mutations was the most important part of this project in 1964. Progenies of the preselected  $M_3$  (variety "Pauli") were compared with each other and with the standard variety. Results available indicate that EMS had a strong negative influence. Some variations exist within the kinds of irradiations, a treatment with X-rays appearing favourable. A larger variability mostly negative, was found in lines originating from  $M_2$ -families in which visible mutants occurred than in those without macro-mutations. The effects of storage after irradiation were also examined. Seed lots with 7, 15 and 23 % moisture were irradiated with 12.000 rX and stored for periods of 0, 2, 4 and 8 days after treatment. Measurements on seedling height show a remarkable reduction of the groups with 15 and 23 % moisture. The group with 7 % moisture could not be measured due to lack of sufficient growth. The longest storage period showed a decrease of seedling height. Seeds of the variety "Pauli", treated with 10, 12, 14, 16, 18 and 20 Mr X showed when germinated a marked reduction in seedling height with increase in dose (26.5 - 7.0 mm; controls : 49.7 mm).

## MUTATION RESEARCH ON PEAS

G.J. SPECKMANN (+)

Seeds of the dun pea variety "Aureool" with 8, 14 and 20 % water content were treated with a 0.17 % EMS solution, in order to study the influence of the water-content of the seed on the effect of EMS treatment.

It appeared that the damage in the  $M_1$ , expressed by growth reduction and reduced fertility, increased with increasing water content of the treated seeds.

In the  $M_2$  generation the highest mutation frequency was found in the progenies of the material with the highest water content. No difference was found in the mutation spectrum. During the 1964 season a large number of flower buds of probable mutation heterozygotes, induced by EMS treatment, were fixed for cytological examination of the meiosis. No structural changes in the chromosome pattern were found so far.

## COMPARISON OF THE MUTAGENIC EFFECT OF X-RAYS, GAMMA-RAYS, NEUTRONS AND ETHYLENE-METHANE-SULFONATE ON PEAS

S.J. WELLENSIEK (++)

A large number of  $M_3$  lines were grown to reconsider the  $M_2$  results and to obtain data on the segregation ratios. A new experimental series has been initiated to include and compare EMS, gamma- and X-treated seeds as well as neutron treated ones. Data are being worked out. It has already been found that the winter-grown  $F_1$ 's suggest recessiveness of "fine" growth.

Miss B. WILDERVANCK (++)

The results of studies on sterility after mutagenic treatments can be summarized as follows :

- (+) Stichting voor Plantenveredeling (SVP) - Foundation for Agricultural Plant Breeding - Wageningen, the Netherlands.
- (++) Laboratorium voor Tuinbouwplantenteelt - Horticultural Department, Agricultural University - Wageningen, the Netherlands.



Treatment		Average number of seeds per pod	Seed setting % ovules giving full grown peas	% healthy pollen
Controls		4 - 4.6	66 - 72	100
EMS	0.1%	0	-	50
	0.2%	0	-	10
	0.3%	0	-	0
	0.4%	0	-	0
γ-rays	7Kr	3.2	51	80
	15Kr	3.2	43	50
X-rays	7Kr	3.2	56	50
Neutron	1hr	4.0	57	98
	48hrs	2.0	35	45

The cytological investigations of the pollen mother cells from the pea-plants treated with different mutagens, are finished. Of each treatment about 10 plants were investigated and about 3 flowers per plant. It appeared that on the average half of the plants were normal and of the other half only a part of the flowers had meiotic aberrations in their pollen mother cells. The most common aberration is the formation of micro-nuclei in the tetrad stage. Especially in the high EMS dosages many cells per flower can be disturbed and have nuclei up to 12 per cell. The micronuclei arise as a result of lagging in the first and/or second anaphase of chromosomes or chromosome fragments. A more severe form of this are dyads, sometimes triads, when dividing of chromosomes is incomplete in the second anaphase. The first metaphase is always looking quite normal, except for stickyness and dark staining of the chromosomes. Univalents and multivalents are observed only very occasionally. In both anaphases lagging has been observed and bridges also occur. Differences between treatments and doses could not be found. But all aberrations cannot explain the pollen sterility, because too many normal tetrads are found. Even the young pollen grains look well in general, but they are not able to complete their development. So the cause of the sterility must be very small, microscopically invisible deletions or a disturbance of the metabolism of the cells and their chromosomes leading to meiotic aberrations and pollen sterility.

## MUTATION RESEARCH ON TOMATOES

G.J. HILDERING (+)

Germination delay and mutation frequency studies considered 15 germination groups of  $M_1$ -plants treated in 1963 with EMS. During 1964 the  $M_2$  families of these  $M_1$  plants were screened for mutations, in the seedling stage. It was found that mutation frequency is a function of germination date, and the numerical shifts in chimaeric patterns can be ascribed to mutation frequency. Correlations of mutation frequency with  $M_1$  - fertility are being analyzed.

Chimaeric structure of the  $M_1$ -plants : in 1963  $M_1$ -plants germinated on the 6th day after 0.8 % EMS-treatment were grown and described, and their fruit and clusters harvested individually. During 1963-1964 topographical studies on the chimaeric constitutions of these  $M_1$ -plants were made using individual fruit progenies.

It appears that the sporocyte tissue of the treated tomato plant derives from 1 to 3 cells; X-rays give rise to less complex patterns than EMS-treatment, probably by killing more initials; the principal time of separation of components is between first and second cluster; finally harvested on these two clusters represent in general the whole array of mutations induced.

More detailed investigations to supplement these results, are in progress. Progenies of fully fertile 0.8 % EMS  $M_1$ -fruit, with unreduced germination, and containing no "visibles", nor showing reduced  $M_2$ -vitality or fertility, were selected for easy to score quantitative characters such as number of leaves under first cluster and time of flowering. A selection index was established on the basis of progeny heritabilities, obtained within and between experiments. Pretesting of selections in  $M_3$  indicated a significant response.

(+) Laboratorium voor Erfelijkheidslcer - Department of Genetics, Agricultural University - Wageningen, the Netherlands.

# COMPARISON ON MUTAGENIC EFFECT OF X-RAYS, GAMMA-RAYS, AND NEUTRONS ON TOMATOES

K. VERKERK (+)

Following treatment with ionizing radiation, the segregation in the  $M_2$  lines usually gives a percent of mutants close to the expected 25. EMS treatment however showed in general a pronounced deficiency of mutants in the  $M_2$  segregation ratio. It was therefore necessary to study the diplontic selection in tomato plants after treatment with chemicals and ionizing radiation.

Results indicate that after irradiation the germination of  $M_1$  plants decreased in speed. The total percent germination was almost equal after treatment with different doses.

Following treatment with EMS or X-rays plants flowered later and fertility decreased. These effects were less marked after EMS treatment than following X-ray treatment at 40 Kr dose. In the  $M_2$  the number of plants with mutations steadily increased from 9 to 26 for doses of 10 to 40 Kr and was 34 for EMS, when 50 plants were considered. The treatment with 0.8% EMS was more efficient therefore than that with 10, 20, 30 or 40 Kr X-rays.

The number of mutants found after treatment with X-rays was almost proportional to the dose used, most mutations being present in only one part of the plant, indicating the chimaera character of the  $M_1$  plant following mutagenic treatment of the seed. It must be concluded that from these results the proportion of normal tissue to mutated tissue is fixed below the first truss. From that point up the distribution of mutated and normal tissues will mostly, if not completely, be determined by chance and is therefore no longer a diplontic selection.

With X-rays, it was found that a 40 Kr-dose eliminates more cells of the sporocyte tissue in the seed than EMS does. The less severe X-ray treatments with 10 and 20 Kr should give relatively more chimaeric  $M_1$  plants than homogeneous ones in comparison to the 40 Kr treatment and this is just what was found.

(+) Laboratorium voor Tuinbouwplantenteelt - Horticultural Department, Agricultural University - Wageningen, the Netherlands.



The study of "non-germination" mutants after neutron irradiation of tomato seeds is progressing satisfactorily. In the  $M_3$  generation this selection appeared to be true in about half of the selected lines. There is a strong indication that partly or totally sterile mutants and "non-germination" mutants are distributed in the  $M_1$ -plants in the same way as "normal" mutants, so that they behave identically.

## R A D I O B I O L O G Y

The availability of the facilities at the Association's Institute, in addition to those available already at the sub-contract center of the "Comitato Nazionale per l'Energia Nucleare" in Casaccia, near Rome, Italy, will offer possibilities to fill a large gap in radiobiological experimentation through irradiation of metabolically active plants in different growth stages.

Much effort was devoted in 1964 to seeking plant species well adapted to the available facilities and to the research requirements. Tomatoes, even the variety "Tiny Tim", have not so far been most satisfactory; Peas, which have a high radiosensitivity, have a small number of seeds per pod making them less suitable for genetic studies. Arabidopsis thaliana is very radioresistant; Chenopodium rubrum is now under investigation.

## EFFECTS OF RADIATION ON DIFFERENT ONTOGENETIC STAGES IN HIGHER PLANTS

M. DEVREUX, G. T. SCARASCIA (+)

Studies conducted along this line have been concentrated on the action of gamma-rays from  $Co^{60}$  on the first stages of development : gametes, zygote and proembryo. The Nicotiana species was chosen as experimental material.

(+) Comitato Nazionale per l'Energia Nucleare, Centro di Studi Nucleari della Casaccia - Rome, Italy.

M. DEVREUX, G.T. SCARASCIA, B. DONINI

Cytogenetical and genetical investigations have been carried out on the  $R_1$  and following generations. Analyses on root-tip mitoses in seedlings after irradiation of gametes or zygote have allowed to determine the presence of bridges and fragments in anaphase, the index of chromosomal aberrations persisting over several cell-generations as well as the cause of abnormal chromosome and gene sets. Another indication of the induction and presistence of structural aberration was derived from the analysis of pollen fertility in  $R_1$  plants; many plants appeared entirely or partially pollen sterile particularly after gametes and zygote irradiation. The presence of persisting chromosomal aberrations was also shown through the analyses of the microsprogenesis in the  $F_1$  from sterile or semisterile  $R_1$  plants crossed with control<sup>1</sup> plants. 61  $F_1$  plants, deriving from 13 different crosses, have been analyzed and in 11 crosses one or more plants showed inversions or heterozygote translocations. Genetical analyses, carried out on 73  $R_2$  progenies totalling 3780 plants, showed that 1135 plants spread over 71 progenies were abnormal for different characters concerning plant habit and leaf and flower morphology. The mean number of "mutation" per  $R_2$  progeny was around 4. Several progenies from  $R_2$  mutated plants were analyzed in  $R_3$ ; only half of them bred true, while the rest segregated for the mutation together with new phenotypes. This finding reminds of similar cases of new mutations in  $R_3$  or  $R_4$  following seed irradiation and ascribed to small mutated sectors in the chimaeric  $R_1$  plant. Segregation was also observed in  $F_1$  from  $R_1$  plants crossed to the mother line, indicating the occurrence of  $R_1$  gametes with structural aberrations, big deletions or aneuploidy. Observations on the pollen grain development, from the tetrad stage to anthesis, has been started comparing binucleate and trinucleate pollen grain species. Microdensitometric measurements were pursued to ascertain when DNA syntheses occurs. Preliminary observations on the floral biology and histology of fertilization in Lycopersicum esculentum have been carried out : different genotypes carrying recessive marker genes of the cotyledons, leaves, inflorescence and fruit were sown in greenhouses.

Moreover,  $R_1$  barley and durum wheat plants deriving from 1500 r gamma-irradiation of male gametes in trinucleate stage, female gametes in gametophyte size and both gametes, have been grown. Analyses of seed germination, types and frequency of aberrant seedlings, as well as plant survival suggest that the male gamete stage is more radiosensitive. Due to unfavourable growth conditions it is difficult to ascertain through the analysis of chlorophyll mutations in  $R_2$ , the presence of the mutation in a chimaeric or heterozygote situation.

#### STUDIES ON RADIOSENSITIVITY, IN RESPECT OF SOMATIC CHARACTERS, FERTILITY AND $M_2$ -MUTATION FREQUENCY, OF DIFFERENT GROWTH STAGES OF TOMATO

R.G. CONTANT (+)

The induction of new genetic variability being the main practical aim of irradiating plant material, a study of the ontogeny of the plant at cell and tissue levels as well as the morphology and mutation frequencies has been initiated. Experiments carried out consider important stages in the plant life cycle; the comparison of different mutagenic agents, gamma- and/or X-rays, thermal neutrons, and chemical agents. One or more plant species are considered and environmental conditions are kept under control for light, nutrient medium, temperature and relative humidity. The initial experiments were carried out with tomatoes, var. "Money Maker" or "Tiny Tim", the last chosen because its size makes it a suitable material. Peas, var. "Aurora" and "Pauli" as well as Arabidopsis thaliana and Chenopodium rubrum are also used as test plants.

Preliminary results indicate that mutation frequency following neutron irradiation of germinating seeds is low compared to that obtained with irradiated dry seeds. In an acute neutron irradiation experiment of the "Money Maker" var. of tomato seeds, most aberrant  $F_1$  plants have been transplanted and are being grown to maturity. Seeds will be harvested for confirmation tests in  $F_2$ . Observations of a

(+) Association EURATOM- ITAL- Wageningen, the Netherlands.

general nature are made on the distinct mutant types. Preliminary results indicate that no noticeable reduction occurred in seed percentage showing the first germination stages up to  $38 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$ . Expansion of the cotyledons was hardly affected by  $4.5 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$  but an abrupt decline occurred immediately after that point reaching a 75 % reduction at approximately  $37 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$ . Leaf development was parallel but at a lower level reaching 75 % reduction at approximately  $27 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$ .

A linear response was noted in respect of plants reaching flowering, the same 75 % reduction being reached at  $35 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$ . Good linear responses were also found for time of ripening of the first fruit and percent of plants giving viable seeds. Mutants scored as percentage of surviving and fertile plants ranged linearly from 10 % at  $4.5 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$  to over 60 % at  $29 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$ . The average number of seeds per fruit could be related exponentially to dose with a 75 % reduction at approximately  $20 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$ . Very similar results were obtained in a repeat experiment. Here emergence however was not affected by a dose of  $103 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$ . The reduction in fertility although again exponential with dose reached 75 % at  $13 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$  against  $20 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$  in the previous test. The reason for this variability has yet to be ascertained. Chronic neutron irradiation of "Tiny Tim" var. tomato seedlings, has shown in preliminary results that fruit maturation was slow. The first cluster has not fully ripened 6 months after treatment on any plant. Chronic neutron irradiation of pea seedlings, var. "Aurora", in the "BARN" climate controlled room at doses of 0 -  $16 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$  rads, showed in preliminary results that 100 % mortality occurred above  $5.8 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$  treatment. An 80 reduction in pod number and an even higher reduction in number of seeds per plant was noted at  $4.5 \times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$  although this treatment was not lethal. An acute neutron irradiation test of dry pea seeds, var. "Aurora" carried out in the "BARN" thermal column giving doses of 0-66  $\times 10^{12} \text{ N}_{\text{th}}/\text{cm}^2$ , (measured with gold foils; at 5 % accuracy; Cd ratio approx. 170), until chemical composition of the seeds can be taken into account later sown in vermiculite within 10 minutes from termination of the treatment is in progress to determine cytohistological modifications in the shoot and root apices.

Preliminary results of an X-ray acute treatment of tomato seedlings indicate that plant survival was as follows :

treatment time (hr of day)	percent survival				
	100	75	50	25	0
01.30	1600 r	3200 r 4000 r	-- --	4800 r 5600 r	>5600 r
05.30	1600 r	4800 r		5600 r	>5600 r
	4000 r				
09.30	1600 r 4000 r		4800r		>4800 r
17.30	1600 r 4800 r			5600 r	>5600 r

It should be mentioned that all these plants when treated had their roots shielded with lead. A slight difference was noted when plants with unshielded roots were treated. In another X-ray treatment experiment, when roots and endosperm were shielded, root length was only slightly reduced at the highest dose of 2000 r whereas shielding of roots gave a 50 % root length reduction at the same dose. Unshielded roots showed a 50% reduction at doses smaller than 750 r and 74% reduction at 1000 r. Similar results were obtained for shoot development and fresh weight when shielding was carried out as mentioned before.

Experiments have been initiated to attempt to relate morphological effects of irradiation to cyto-histological changes in shoot and root apices of pea plants.

#### RADIOGENETICS OF GAMETOGENESIS AND GAMETOPHYTES IN HIGHER PLANTS

R.M. ECOCHARD

1. Use of climate rooms and liquid growth medium has afforded an accurate method for studying the stage sensitivity of Vicia faba, for irradiations "in-toto".



As the inflorescences develop regularly along the stem, an analysis of the location of various stages of flower bud development, in relation to a landmark easy to record externally, makes it possible to know the timing process of male-meiosis and pollen formation. Thus when observing a plant so many days after irradiation, one can relate any observed response to the stage irradiated.

For X-rays (unfiltered, dose rate of 50 r. per min.) the more sensitive phase is the young buds, before meiosis : some are affected by 25 r. so that they cannot survive at meiosis; their percentage increases with the dose, reaching 100 percent at 150 r. But up to 175 r. the apex is safe and able to produce viable buds later (but still with a number of aborted pollen grains increasing linearly with the dose).

Buds irradiated during meiotic prophase exhibit a number of chromosome disturbances, namely bridges, fragments and laggards in anaphase I and II, resulting in extra nuclei at tetrad stage, and in micro pollen grains later, their number increasing linearly with the dose.

The idea lying behind this investigation is that buds or pollen grains having just escaped lethal damage might have undergone a mutational process.

2. In an other series of experiments, attempts to incorporate  $^3\text{H}$ -thimidine in pollen-mother-cells to label their chromosomes have failed to give clear results, with the different techniques so far tried.

3. Flowering plants of Lyc. esculentum and of Lyc. peruvianum have been irradiated with X-rays with the aim of overcoming the genetic barriers to crossability.

For 100 r and 500 r no results have been obtained whatever the irradiated parent, and the development stage of buds during irradiation.

4. Preliminary irradiations have been carried out on Lilium, Tradescantia and Tomato resting pollen with X-rays (unfiltered, 1950 r/min.).

Neutrons (using the "rabbit tube" reaching down to the graphite elements of the reactor :  $8 \times 10^{10} \text{ n}_{\text{th}}/\text{cm}/\text{sec.}$ , contaminated with fast neutrons and gammas).

The technique used for measuring the percentage of inactivation is adequate (in-vitro tests of germination) and the curves are regularly sigmoidal, but the standard deviation of the LD<sub>50</sub> is rather large due to the lack of control of the environmental conditions, namely oxygen and water content of pollen.

That is why a special device has been designed and constructed at the Association and will be used in 1965.

So far, by plotting the data available for several replications, and assuming a dose-rate equivalent of 150 rad/min for the neutrons used, the RBE compared with X-rays is around 1,75.

And the pollen radiosensitivity is higher for Lilium than for the two other species : LD<sub>50</sub> = 600 Kr of X-rays.

#### TISSUE CULTURE AS A TOOL FOR RADIATION RESEARCH

G. SAUER (+)

Studies with tissue culture or cell suspensions in which all cells are in a certain phase have been continued. The single cell cultures according to STEWART's methodology have been used to solve practical problems, namely to investigate chemical changes occurring after irradiation of the living cells. The artificial synchronization of cell suspensions lasts long enough to enable irradiation of the separate mitotic stages. Following irradiation of Pathenocissus tissues, 3 unknown compounds were found in the hydrolyzed DNA. Five spots were found in the chromatograms after UV irradiation of the DNA bases in the solution. Different newly formed compounds have been chromatographically detected following irradiation of DNA bases in vitro as well as of tissue cultures. 5-hydroxy-cytosine and 5-hydroxymethyluracil have been identified; 3 other compounds were detected, one of them being rather unstable.

(+) Association EURATOM - ITAL - Wageningen, the Netherlands.

## FACTORS GOVERNING RADIOSENSITIVITY

S. AVANZI, G. MODUGNO

Studies on factors governing plant radiosensitivity have been continued in 1964, considering biochemical and cytological investigations.

In fact the roots of irradiated plants show a remarkable increase of fresh and dry weight and of total nitrogen content with respect to those of control plants. In all tests the nitrogen content per gr. remains constant. In general, it seems that the increase of weight of the roots compensates for the loss of weight of the aerial parts. Investigations on histological and biochemical modifications are in progress. Peroxidase activity in barley seedlings from seeds irradiated at 5 and 15 KR was determined to clarify some aspects of the possible correlation between peroxidase activity and growth.

Seedlings from seeds irradiated at lower doses were comparable in fresh weight and peroxidase activity to the control. After 15 KR treatment, seedlings showed at 50% decrease in weight and 40% increase in peroxidase activity. An extraction method and chromatographic separation on paper and thin-layer of reduced glutathione, oxidized glutathione, cysteine and cystine after the blocking of sulfurated compounds with N-ethylmaleimide have been developed to study the turnover of reduced glutathione and the effect of radiations and auxins on the metabolism of glutathione  $S^{35}$ . It was not possible to show the presence of indole-acetic acid in plants of pea, maize and bean, using the separation on silica gel column in the presence of IAA-  $Cl^{14}$ .

Results of the cytological investigations can be summarized as follows : The investigation on the radiosensitivity of two durum wheat varieties "Cappelli" and "Aziziah" has been extended : to the influence of the nucleocytoplasmatic volume ratio and the action of fast neutrons at chromosomal level, to determine which correlation exists between the cytological action and nuclear volume and between the cytological action and DNA content per nucleus. The study of the idiogram of the caryotype of the two varieties was also carried out. The evaluation of the nucleo-cytoplasmatic ratio indicated that in "Cappelli" it is 2.63 and in "Aziziah" 2.49; the cellular volume in "Cappelli" was 9.15 % higher than "Aziziah" and the nuclear one 6.34 higher. The idiogram of the caryotype of "Cappelli" and "Aziziah" has been completed.

The total length of single chromosomes of "Cappelli" and their arm ratios have been compared with the corresponding measurements of "Aziziah". From the collected data it appears that the idiogram of "Aziziah" differs from that of "Cappelli" only in the length of one satellite-chromosome ("Cappelli" has the longer one). The difference has been located in the short arm (satelliferous arm) and the statistical analyses showed a high significant difference. To correlate difference in radiosensitivity to nucleic acids content, chemical determination of DNA and RNA in "Aziziah" and "Cappelli" is now in progress from a quantitative and qualitative (with the technique of chromatographic separation) point of view.

The cytophotometric determination of DNA content of the two varieties "Grifoni" and "Russello" has been carried out and it has been ascertained that both varieties have a DNA content higher than that of "Cappelli", being respectively 1.6 and 5 %. The DNA determination of 23 different species of Nicotiana is in progress. Preliminary cytophotometric determinations showed that N. rustica var. "Brasilia" has a higher DNA content per cell than N. Tabacum.

#### EMBRYO-ENDOSPERM RELATIONS IN IRRADIATED SEEDS

P. MELETTI, C. FLORIS (+)

The screening of chlorophyll mutations in wheat plants coming from embryo transplantation of Triticum durum var. "Cappelli" has been carried out in 1963. The following transplants have been made : EM (i) EN (i), irradiated embryos grafted on to irradiated endosperms : EM (i), EN (i), irradiated embryos grafted on to unirradiated endosperms : EM (u)/EN (i), unirradiated embryos grafted on to irradiated endosperms : EM (u)/EN (u), unirradiated embryos grafted on to unirradiated endosperms, as transplant ("homotransplant") of control. The analysis of chlorophyll mutations has been carried out to assess the percent frequency of mutant spikes (mutations per spike progeny) and of mutant seedlings. Mutants have been classified according to GUSTAFSSON (1940). The following types have been observed : albina, virido-albina, chlorina, striata, tigrine. As to mutation frequency, no significant variation in the behaviour of the transplants EM (i) EN (u) and EM (u) EN (i) as compared with their controls (respectively EM (i) EN (i) and EM (u)/EN (u)) was noted.

(+) Istituto Botanico dell'Università - Cagliari (Sardegna), Italy.

It should be pointed out that results of X-ray treatments are preliminary and studies are still in progress. New  $R_2$  material grown in 1964, is being screened for chlorophyll mutation. Durum wheat plants from pre-soaked seeds X-rays treated with 2, 4, 6 12 and 24 KR and from dry seeds treated with 15, 20, 25 and 30 KR (for the graft EM (i) EN (u)) and 60 and 120 KR (for EM (u) EN (i)) are available. The analysis at genetical level, of wheat plants, now in  $R_1$ , coming from embryo transplantations in seeds X-irradiated, after pregermination, with 1500, 2000, 2500, 3000 and 12000 R (this dose only for the graft EM (u) EN (i)) will be carried out. In cytological studies, dry seeds irradiated with 500, 1000 and 2000 KR of gamma-rays from  $Co^{60}$ , were soaked in water and the following types of transplants made : EM (u)/EN (i) (unirradiated embryo on irradiated endosperm), 500, 1000 and 2000 KR; EM (u)/EN (u) (unirradiated embryo on unirradiated endosperm : "homo transplants", used as controls). The transplants were grown in petri dishes in sterilized sand at 20° C. Root tips were collected from the seedlings of the different transplants at 12-hour intervals from 72 to 120 hours after transplantation. It was found that the irradiated endosperm depressed significantly the mitotic index (percentage of cells in mitosis) in the root tips of the EM (u)/EN (i) transplants. Since the prophase index (prophase in percent of all dividing cells), was not changed, it may be concluded that the irradiated endosperm induced a general retardation in the mitotic cycle and did not interfere with any particular stage of mitosis. Research continued on the occurrence and biological activity of the germination and growth inhibitor discovered in the endosperm through the use of embryo transplantation. The extraction technique of the inhibitor has been developed and extraction is now carried out on a large sample of "Cappelli" durum wheat.



P R E S E R V A T I O N   O F   F O O D   B Y   M E A N S  
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The research carried out in this field aimed both at the practical possibilities offered by preservation methods involving irradiation of perishable food, and at basic physiological and biochemical studies of the after-effects of radiations on fruit and vegetables. The project studying the application of ionizing radiations for increasing keeping quality of horticultural produce has been extended to include spectrophotometric analysis of anthocyanin for colour changes and tenderometer measurements for texture. Optimal application doses for extension of shelf life are not necessarily the same as those offering the best preservation of taste and flavour. It is therefore obvious that maximum dose levels must in the future, to be worthwhile, consider as a sine qua non condition the maintenance of the organoleptic properties. Thus inhibiting the surface microflora is only part of the problem, which cannot be separated from the physiological studies on ripening process with and without irradiation, and biochemical changes occurring during this process. Finally, studies on the genetical hazards of irradiated food were continued; they included the effects of irradiated food on plant chromosomes, and genetical effects of irradiated full meal on mice by assessing the frequency of dominant lethal mutants, as well as a number of tests on Drosophila. The "Van de Graaff" electron generator of the Association has been installed and is now fully operational.

THE APPLICATION OF IONIZING RADIATION FOR INCREASING KEEPING  
 QUALITY OF HORTICULTURAL PRODUCE

O. L. STADEN (+)

In previous years the technical screening program considered a large number of horticultural products. From this catalogue of information may be concluded that radiation alone cannot

(+) Instituut voor Bewaring en Verwerking van Tuinbouwprodukten (IBVT) - Institute for Research on Storage and Processing of Horticultural Produce - Wageningen, the Netherlands.

always solve the problems encountered with the storage of horticultural produce. Much attention has therefore been paid to combined treatments. Furthermore research work on promising items was intensified in order to get a better evaluation of the practical merits of radiation processing. Soft fruit. The results obtained with strawberries are consistent. Several varieties have been tested and shelf life extension was found to vary with the variety. No differences could be observed between 1 and 3 MeV irradiated berries. Raspberries and Blackberries are very sensitive to irradiation. The dose, necessary for the retardation of mold, causes leaking of cell-sap out of the fruit, and discolouration; taste panels found loss of typical flavour. In previous years cherries offered little perspective for radiation preservation, but during the 1964 season much more encouraging results were obtained. Irradiated cherries were found to be firmer and insipid, the controls softer and more aromatic.

Shelf life and taste of irradiated peaches were investigated. No differences between treated and controls were observed after a storage period of 14 days. Peeling of irradiated peaches was difficult. Preference was given to peaches irradiated with 100 Krads.

#### Hard fruit :

With summer pears which deteriorate quite rapidly, no increase in shelf life or quality was found. The quality of the pear variety "Triumph de Vienne", which can be stored at low temperature for some months, was improved by a dosage of 60 Krads.

Mushrooms are very suitable for radiation preservation. This product has been studied thoroughly and the results obtained can be summarized as follows : The shortest time delay between picking and irradiation is preferable; storage time decreases at higher temperatures, up to 5 days at 20° C cause no damage; cold treatment before placing at higher temperatures is only slightly better for quality maintenance; packing requirements are not yet elucidated, perforated films may be necessary. Irradiation with 3 MeV electrons give better results than treatment with 1.5 MeV electrons. The taste of irradiated mushrooms is slightly better than that of non-irradiated ones.

Vegetables:

Combined treatments - dipping in chemical solutions and irradiation - were applied to the following cut-prepacked vegetables : carrots, red cabbage, onion, chicory, endive.

The best results, i.e. less discolouration, were obtained with dippings of PCE (pyrocarbonic acid diethylester), vitamin K, lecithine and ascorbic acid, followed by irradiation with doses of 50 to 100 Krad. The packaging material plays an important role. Some vegetables need perforated bags while other species give better results with unperforated plastic bags. The preliminary character of these studies do not allow to draw conclusions.

Following extended storage, irradiated tomatoes were found to taste better than non-irradiated fruit.

#### PHYSIOLOGICAL INFLUENCES OF IRRADIATION ON SOFT FRUIT AND VEGETABLES

J.G. VAN KOOY (+)

Preliminary studies have been carried out to ascertain changes in cell permeability following irradiation of fruit and vegetables. Bananas have been chosen as test material and discs of standard size treated with a mannitol solution of 0.1 - 0.5 M concentration giving a range of osmotic pressures adequate for the experiments envisaged. The method includes three steps : following immersion in water to attain full turgescence, the discs are placed in an osmotic solution in which the tissue loses water and takes up solute, it is finally transferred to water to regain turgescence and by re-weighing, changes in osmotic value can be ascertained.

To establish the physiological response of fruit to irradiation a number of respiration tests were carried out using banana discs. The pH of the medium was not found to change during the period in which oxygen uptake measurements were taken.

(+) Association EURATOM - ITAL - Wageningen, the Netherlands.

## BIOCHEMICAL STUDIES ON IRRADIATED FRUIT AND VEGETABLES

Miss H. L. TELJEMA (+)

The biochemical background of the fruit and vegetable softening during ripening is the main scope of investigation of this project. It aims at ascertaining whether the softening noted following irradiation is the same or a similar process as the one occurring naturally in the post ripening period. It is known that high dosage irradiation promotes softening of fruit, thus limiting the preservation potentialities. Strawberries and tomatoes have been chosen as test material. The irradiations carried out with the "Van Graaff" electron accelerator will include dosages up to and exceeding the threshold for texture changes. Preliminary tests on the effect of X-irradiation of an AIS (alcohol insoluble solids) preparation on pectins indicated that soluble pectin content rises at the expense of "residual pectin". This result has been reported in the literature for solutions; in the tests reported here, irradiation was carried out on dry pectin. Also preliminary tests on pectin solubilization have been carried out on tomatoes, var. "Money Maker" irradiated with 800 Krad (MeV electrons) at two ripeness stages, namely mature green and red ripe, and stored for a long period in a deep freeze. The solubilization of pectin was significantly promoted when AIS (alcohol insoluble solids) preparations were used.

## GENETICAL HAZARDS OF IRRADIATED FOOD

J. MOUTSCHEN (++)

Powdered irradiated strawberries and potatoes irradiated with beta-rays at respectively 0.5 and 5.0 Mrads at 3 MeV and 10 Krads at 1 MeV, as well as wheat and soja flour treated with gamma-rays from Co<sup>60</sup> at 200 Krads were used.

(+) Association EURATOM - ITAL - Wageningen, the Netherlands.

(++) Université de Liège - Centre Interfacultaire des Sciences Nucléaires - Laboratoire de Génétique - Liège, Belgium.

Barley seeds were germinated in solutions of these irradiated powder, and roots studied during the first three mitoses following germination. No chromosome aberration was noted. It should be pointed out that practical conditions were considered is so far that treated potatoes were first boiled and peeled, and wheat and soja seeds were used dry or germinating. Due to the fact that, under certain experimental conditions, local centromere lesions may occur in plant chromosomes, the primary non disjunction of X-chromosomes of Drosophila test was used on irradiated potato. No significant difference could be noted between flies fed with irradiated or non-irradiated potatoes. Flies with vestigial wings were found to be phenocopies and not mutants. No sex-linked lethal recessive mutants were found. The Drosophila test was also carried out on gamma-irradiated wheat and soja flcuer which may contain peroxides induced by the treatment and are perhaps mutagenic in action. Results obtained were similar to those obtained for potatoes even using the SWAMINATHAN test, differences not being significant. The MULLER-5 test gave similar results to the ones mentioned above. Long range tests were carried out over 3 and 6 months periods to assess the possibilities of cumulative effects over many generations of flies fed only irradiated food. No increase in morphological aberrations has been observed from one generation to the next. When these existed, in general, they were not mutations. It appears that an increase in lethal recessive mutants exists after 3 and 6 generations. Tests for the induction of lethal dominants in mice fed gamma-irradiated food gave no conclusive results, although some positive reactions were noted in long term experiments on animals of both sexes.

#### THE BEHAVIOUR OF SPECIFIC NUCLIDES IN SOILS AND CROPS

The introduction of radio-activity into the biosphere slightly increased in 1964. Furthermore fast development of experimental and industrial power plants has created more real problems owing to possible disasters and radio-active waste products.

However, so far situations have not occurred where the contamination of food has passed the safety level. Yet, it is of paramount importance to ascertain qualitatively and quantitatively residues of contaminants.

The prevention of accumulation of these contaminants in soils is an important consideration. Thus, the accumulation and distribution in the soil profiles and the actual uptake and translocation from leaves of intact plants were given particular attention in 1964. Studies carried out at the Association's Institute made use of the carefully controlled environmental conditions available for growing plants in an effort to allow a safer extrapolation if circumstances required. This aspect of preventive research is linked with studies on the elimination of the contaminants from important foods intended for human consumption or feeding stuffs for animals.

#### STUDIES ON FOLIAR PENETRATION

E.C. LEVI (+)

The penetration of nuclear fall-out or airborne waste products through the cuticular layers present on the outer surfaces of plants appears to be the first part of the process of foliar uptake. The subsequent distribution and accumulation in the various plant parts and the various stages of growth form complementary studies essential to the possible evaluation of the problems raised by atmospheric contamination of plants.

A project concerning caesium penetration and retention by bean leaves and its subsequent distribution in the plant initiated in 1963 was continued under controlled conditions. At constant light intensity as well as at day and night temperatures, yet at a relative humidity twice higher in one case than in the other, the amounts of  $\text{Cs}^{134}$  washed off application spots varied slightly, with a time-lag of approximately one hour. The amounts held in the treated leaf were similar. Preliminary results indicate that no  $\text{Cs}^{134}$  is held on the outer wax layers of primary leaves of beans. During the 1 - 12 hours following treatment, most of the caesium retained is in the application region.

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Three years' results indicate that at 40% relative humidity, the fraction washed off the leaf surface by water after periods of up to 96 hours decreased steeply at first, remaining relatively constant thereafter. Of the amount retained by the plant, a very large and relatively constant percentage was found in the treated leaf even after a five weeks period during which the plant had reached maturity. This constant accumulation indicates a probable cuticular fixation of  $\text{Cs}^{134}$ , which has to be confirmed. The other leaves or the fruit of the bean plant accumulated a very minor fraction while the stem and roots as a whole had an intermediate activity.

Autoradiograms of freeze-dried plants indicated that following application of elements such as  $\text{P}^{32}$  or  $\text{Cs}^{134}$  to only one primary leaf of a bean plant, transport was initially to only one fraction of the root system. An accumulation was noted in that region for relatively long periods. This constant leafroot distribution was further investigated by feeding the root system or part of it for short periods of time and determining the distribution in the leaves. Results show that for  $\text{P}^{32}$ , absorption from only one group of adventitious roots was followed by an accumulation in either one primary leaf, one half of each primary leaves or in most of one primary leaf, the complement area being present in the opposite leaf. In general the total amounted to one whole leaf, and the further distribution was in thirds of half leaves considered across the main vein. Following cutting of the treated root fraction, and a subsequent growth period of 7 days, the  $\text{P}^{32}$  was found throughout the plant, yet the initial accumulation areas were clearly visible. Absorption in the same conditions of  $\text{Ca}^{45}$  showed that the initial accumulation areas were the veins, presumably the xylem system, starting from the largest. After complete labelling of the vein system, the accumulation in the same fashion as described for  $\text{P}^{32}$  was noted. However when the treated root was cut and the plant allowed to grow, the Ca was found in all the aerial parts, but not in the rest of the root system as was the case for  $\text{P}^{32}$ .

## MOVEMENT OF STRONTIUM IN SOILS

M. J. FRISSEL and P. POELSTRA (+)

Fall-out or waste products enter the plants via the root system following uptake from the soil. This indirect contamination is influenced by soil properties. In the case of  $\text{Sr}^{90}$  the soil Ca content is the main factor and the relationship between it and the  $\text{Sr}^{90}$  accumulated in plants is of importance to predict contamination levels in given situations in the field. The possibility of decreasing uptake by plants from the Sr pool in the soil by proper cultivation practices can also be envisaged owing to the chemical similarity between the two elements. Obviously the exchangeable Ca, i.e. that fraction available to plants is the one of paramount importance.

The relationship between the soil exchangeable Ca and the  $\text{Sr}^{90}$  contamination level of plants under normal agricultural field conditions has been investigated in 4 localities of the Netherlands. Experimental sites were chosen to cover over a wide range of soil-Ca content. Results available from samplings taken regularly from 1958 to date indicate a good agreement between soil Ca content and  $\text{Sr}^{90}$  in plants. The  $\text{Sr}^{90}/\text{Ca}$  ratio in grass was found to follow the equation

$$y = 0.66 Q \cdot X^{-1.21} + 1.24 v$$

for the mean contamination level in which

$$y = \text{pC } \text{Sr}^{90} / \text{g Ca};$$

$$Q = \text{mC } \text{Sr}^{90} \text{ per km}^2;$$

$X$  = exchangeable Ca content in the soil ( $\text{kg}/\text{km}^2$ ), and

$v$  = amount of  $\text{Sr}^{90}$  ( $\text{mC}/\text{Km}^2$ ) deposited in the preceeding month.

Results further indicate that vertical transport of  $\text{Sr}^{90}$  in Ca rich soils was more rapid than in Ca deficient ones. An extension of this project to total 7 experimental sites has been initiated, and sampling of the 0 - 5 and 5 - 10 cm layers continued.

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The investigation of Sr distribution in soil profiles has also been studied in the laboratory. Columns of Dowex 50W - X8 resin of 0.2 - 0.4 mm particle size range, mechanically mixed with sand of same particle size have been set up. These columns are 38 cm long topped by a 2 cm layer consisting of sand and Ca resin, the last being labelled with  $\text{Sr}^{90}$ ,  $\text{Sr}^{85}$  or  $\text{Ca}^{45}$ , and are air free to prevent bubble formation. Percolating liquid, normally  $\text{CaCl}_2$ , is carefully dripped on the top of the columns to allow very low flow rates which can be maintained for several months on the same level. A quantitative survey of the distribution pattern in the column is then carried out for each cm layer. This experimental set up which is now operating satisfactorily, has also available a column scanner developed for direct measurements of the distribution pattern at the Association's Institute. Slight modifications of the setup described will allow to include studies on : the influence of flow rate on the distribution pattern of radio-active Sr in columns consisting of resin and sand mixtures; the influence of salt concentration; the influence of the cation exchange capacity and the influence of particle size on distribution pattern, as well as the difference in behaviour between radio-active Sr and radio-active Ca under the same conditions.

Results obtained are fitted to the equations established by GLUCKAUF, THORNTHWAITE et al and SAID, to give parameter values applicable in general cases. Simultaneously a completely theoretical approach mentioned in the previous annual report was continued.

#### ROOT UPTAKE STUDIES

##### RINGOET (+)

Studies on the uptake by plants of calcium and strontium supplied to the root system of different species have been continued. The influence of various physiological statuses in relation to well defined environmental conditions, in particular the effect of soil moisture stress, were investigated. Contrary to some published results, data available

(+) Association EURATOM - ITAL - Wageningen, the Netherlands.

show that moisture uptake is not the determining factor for the uptake of calcium and strontium by oat plants. A possible uptake and translocation scheme of calcium in plants has been worked out. It implies that absorbed calcium ions are not immediately fixed upon absorption but can move in the water stream and be temporarily held by metabolically produced exchange sites in the various plant parts. It is assumed that more sites are available in young tissues and their number increases from the leaves to the leaf bases or stems, and to the roots. Thus an important fraction of the absorbed calcium ions move from one exchange site to another, upwards and downwards.

A number of experiments were also carried out to check the high mobility reported above. They consisted in supplying  $\text{Ca}^{45}$  to one half of a split root system of oat plants. Results available show conclusively that, although some capillary movement of Ca occurred on the root surface, Ca ions in the roots and probably in part of the aerial tissues, i.e. leaf bases, are easily exchangeable for Ca ions in the culture solution. Ca absorbed during the first growth period is redistributed over the different plant parts during the subsequent growth. A fraction of this initially absorbed Ca moves out of these plant parts to younger ones, which thus receive Ca from the mineral solution and from other plant parts, in particular the leaf bases of older leaves, the stems, and the roots. To account for the total exchange between roots and solution and for the partial Ca feeding of the young tissues from the roots and other plant parts a basipetal movement of Ca ions from the earlier developed aerial plant parts is accepted. Results also indicate that only a very minor fraction of the Ca ions initially taken up by one part of the root system move to the other roots.

#### REMOVAL OF $\text{Sr}^{90}$ FROM MILK

J.F. STOUTJESDIJK (+)

$\text{Sr}^{90}$  is under certain circumstances, among the radio-active isotopes produced in the nuclear fission of heavy atoms, the most dangerous source of contamination of human food. It is very similar to the metabolically important Ca-ion

(+) Association EURATOM-ITAL - Wageningen, the Netherlands.

and this similarity, while it offers a possibility of deposition of Sr in the human skeleton, also allows a possible removal or decontamination method for every system where Sr is exchangeable with Ca.

Milk is the most important source of both elements for humans and the hazards involved from  $\text{Sr}^{90}$  ingestion, particularly by children, are obvious. Much research has already been carried out in various countries, and a number of decontamination methods proposed. They are the ion exchange method using cation exchange resins, developed in 1954 by LIBBY and co-workers, or Ca phosphate and phosphate containing minerals suggested by ARMSTRONG and SINGER or SILVERMAN and co-workers; and the electrodialysis method of GREGOR.

A new project started in 1964 aims at further improvement of the known methods. Both the direct stirring of milk with calcium phosphate and the use of calcium exchange columns have been considered. Studies in progress consider a number of parameters, in particular temperature, contact time, amount of calcium phosphate contained in mineral, chemical composition of the calcium phosphate containing material and the effect of added inactive strontium.

Preliminary results confirm that in vitro tests, involving the stirring of calcium phosphate in milk reduced the strontium content of milk by 70% within 2 minutes; second treatments brought this figure to more than 98%. Difficulties were met in the column experiments due to particle size of available calcium phosphate in the Netherlands.

Plastic balls coated with calcium phosphate powder were found to reduce the  $\text{Sr}^{85}$  content of milk substantially.

1 - 2 mm pellets of calcium phosphate reduced the strontium content of milking passing through a 26 cm column by approximately 70 %. Longer columns of 0.6 - 1.0 mm pellets removed up to 90 % of the bound Sr. Approximately half of the strontium remaining in the column was fixed in the top 10 cm of the 45 cm column, and most of it could be eluted with a 20 %  $\text{CaCl}_2$  solution.

Experiments are in progress to ascertain whether such regenerated columns could be efficiently used again and again.

# I M P R O V E M E N T   O F   N U C L E A R   T E C H N I Q U E S

## RADIO-ISOTOPE METHODS

Direct determinations of pressed dry grass, powdered or not, for a number of fall-out nuclides were carried out in a gamma-scintillation counter with a large well-type (NaI (Tl))-crystal fitted to a 200 channel analyzer. Samples of grass or vegetables pressed at 800 - 1000 atm were used and indicated the presence of  $\text{Cs}^{137}$ ,  $\text{K}^{40}$ ,  $\text{Mn}^{54}$ ,  $\text{Ru}^{106}$ ,  $\text{Ce}^{144}$  and perhaps  $\text{Zn}^{65}$ .

Some inaccuracies still exist due to the rather high content of  $\text{Mn}^{54}$ . Gamma-determinations of liquid samples in a well-type scintillation counter with automatic sample changer were found to vary due to differences in tube size when the solution to be counted was measured by volume. This could be avoided by keeping the height of the aliquot constant and, for accurate determinations, by correcting for the weight differences.

### A. RINGOET, A.J. GIELINK (+)

The micro-autoradiographic technique for determining radioactive tracers within plant tissues has been applied in a number of experiments during the past year in an effort to improve the preparation of the sample, as well as the emulsion pouring technique. Comparison of freeze dried preparations using an apparatus specially designed and constructed at the Association's Institute with those obtained from standard freeze substitution method have been made.

### R. V. RECHENMANN (†)

Systematic studies have been carried out comparing emulsions for the pouring technique. The drying process has been improved by using a dessicating chamber specially designed and constructed at the Association's Institute. The very attractive method of the electron-microscopical observation of autoradiograms is developed by a careful choice of the numerous parameters involved. For this, a systematical and, from a fundamental point of view, critical approach had to be undertaken.

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## R. V. RECHENMANN

The biological application of semi-conductor detectors to the quantitative evaluation of radio-isotopes in vivo, in plants, has been continued. Units using Au-Si surface barriers have been used to detect beta emitting isotopes, in particular  $\text{Ca}^{45}$ . A study of calcium movement in oat-leaves is in progress.

Detection of  $\text{C}^{14}$  labelled sucrose has also been possible following foliar application of  $10 \mu\text{C}$  of sodium acetate. Improvements have been brought to the detection assembly with respect to noise and stability.

At  $15^\circ$  detector temperature the maximum noise level of the assembly corresponds to an energy of  $18 \pm 0.5 \text{ KeV}$  with detectors from the CRN in Strasbourg.

Improvements are expected with a new assembly using home-made charge-pre-amplifiers, which are in development.

## J. G. DE SWART

The instrument development department worked on the following items :

Because the temperature drift in spectrometer-systems, together with the thermal noise in very sensitive detectors (e.g. semi-conductor detectors) is important in radiation measurements., an investigation into temperature control has been started with the following results :

Temperature measuring and recording instrument

An instrument was developed to measure temperatures of  $-200^\circ\text{C}$  -  $+150^\circ\text{C}$  with an accuracy of  $0.1^\circ\text{C}$  and a reproducibility of better than  $0.01^\circ\text{C}$  using subminiature detectors (it is applied with good results for the dosimetry of an electron beam). For temperature calibration a system with a reproducibility better than  $0.01^\circ\text{C}$  has been developed.

A thermoregulator unit to control temperatures, having the same specifications as the temperature measuring and recording instrument, has been developed.

A rectifier system for cooling of semi-conductor detectors, amplifiers, freeze-microtome, etc. has been constructed to form, together with PELTIER elements and built in regulators of the thermoregulator type, a complete temperature controlled system.

Soil-column-scanner

For the measurement of the amount and position of gamma-activity in soil - columns, a step scanner has been developed with the following specifications:

- a. Collimator with a lead shield and an adjustable slit;
- b. Step-size adjustable
- c. Number of steps/minute adjustable
- d. Soil column turns on turntable in front of the detector head, to give an average measurement from the cross-section;
- e. Scanning accuracy  $0.2\% + 1 \text{ step}$ .

J.G. DE SWART

A special high voltage supply for operation of scintillation detectors has been developed, because the existing commercial apparatus did not give sufficient guarantees for the safety of the (expensive) detectors.

D O S I M E T R Y

K.H. CHADWICK, W.F. OOSTERHEERT

In order to compare accurately experiments involving ionizing radiations a new project on dosimetry was set up in 1964. It aims at calibrating the dosimeters in accepted units (the Rad measured in soft tissue) and the development and calibration of the ConRad dosimeter for use with neutron irradiation.

For x and  $\gamma$  radiation, the dosimeter will be calibrated against a secondary standard, a Fricke ferrous sulphate dosimeter which will be previously calibrated calorimetrically using electron irradiation. Standard ionization chambers will be purchased and compared with the ferrous sulphate dosimeter. Although it can be used directly for neutron measurements, a new dosimeter using lithium fluoride crystals which will have a tissue equivalent response will be developed to allow more accurate determinations of neutron dose. It will be calibrated against tissue equivalent ionization chambers.

Finally the possibilities offered by semi-conductor detectors for neutron spectrometry will be investigated.

The following results have been already obtained :

X and gamma-radiation

The ConRad LiF phosphor has been checked against the Fricke ferrous sulphate dosimeter in the large Cs<sup>137</sup> facility. The results were

Fricke	830 rads/hr	at 1 meter
ConRad	850 rads/hr	at 1 meter $\pm 25 \%$

Further more detailed measurements will be made following the absolute calibration of the Fricke ferrous sulphate dosimeter against a calorimeter.

2. Electrons

It has been realised that it is not possible to calibrate the LiF absolutely, using electrons due to the mechanism by which the LiF is used to measure dose. However it is possible to obtain a series of calibration curves which depend upon the energy of the electrons and only one dosimeter capsule will be used.

The Fricke dosimeter will be calibrated absolutely against the calorimeter for electrons of 1.2 MeV. The calorimeter has been prepared during the last months and the electrical power measurement is accurate to 0,3 %. The accuracy of the temperature measurement is 0,3 % also.

Electron and  $\gamma$  ray dosimetry

Two miniature thermistors were prepared by the instrumentation group, tested for the same response to temperature change and mounted in the base of each calorimeter disc.

Electrical connections were made directly to one of the graphite discs and electrical calibration of the calorimeter system was made by passing an electric current through the graphite at a known voltage.

Using a stabilized low voltage supply which gave up to 4.5 amps it was possible to calibrate the calorimeter over the required range.

Voltage and current were measured using a digital voltmeter and a standard resistance of the same order as the graphite disc. A straight line relationship between the thermistor reading and the electrical power in joules was obtained with a correlation coefficient of 0.9985. The radiation calibration will take place early in the new year.

### 3. Neutrons

Various liquids having the same elemental constitution as muscle, S. Paulia tissue, and one with an intermediate constitution have been made up which are compatible with the LiF; they will be used to look into the response of these tissues to neutrons.

Neutron irradiations will be carried out in Quartz glass containers which have a low cross-section for neutrons.

#### Neutron spectrometry

A preliminary investigation into the fast neutron spectrum in the irradiation room of the reactor was made using a  $\text{Li}^6$  filled semiconductor sandwich counter.

The results obtained did not agree fully with measurements made by a group from R.C.N. using the Hankins Bonner Ball system (1).

The resolution of the neutron spectrum below 1 MeV is subject to considerable doubt and an attempt to gain insight into this part of the spectrum using an  $\text{He}^3$  filled semiconductor counter failed because further modification of the electronics proved necessary.

Further measurements will be made during 1965.

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# COORDINATION OF APPLIED RESEARCH

## MUTATION BREEDING

### C. BROERTJES

In order to promote the mutation breeding research with a practical aim also in 1964 much attention has been paid to :

1. Organization of work-contacts via the Mutation Breeding Contact Group Wageningen and its subgroups; these contacts are mainly between scientists of the Association, including the subcontract partners;
2. The setting up of cooperative programs with research-institutes, experimental stations and private breeders in the field of mutation breeding.

A survey of these programs is given in the following list :

<u>Self-pollinating plants</u>	<u>Aim</u>	<u>Organization</u>
Bean	Improvement of productivity	Various
Pea	Higher yield	Private firm + Institutes
Tomato	Induction of desired characteristics (quality, early ripening lower light requirements)	Various
Canary seed	Induction of desired characteristics	Department of Plant Breeding
Colza	Induction of desired characteristics (late-opening pod)	Private firm
Salvia	Induction of desired characteristics	Private firm
<u>Cross-pollinating plants</u>		
Brassica rapa	Improvement of productivity	Foundation for Agricultural Plant Breeding
Onion	Induction of desired characteristics (disease resistance)	Experimental Station
Sugar beet	Induction of desired characteristics (monogerm seed)	Foundation for Agricultural Plant Breeding

Vegetatively propagated  
plants

A. Ornamental plants

Bulbs

.....

Tulip	Other flower colours	Private firm
Hyacinth	Other flower colours	Private firm
Iris	Other flower colours	Bulb Research Department

C. BROERTJES

Ornamental shrubs  
.....

Azalea	Other colours (flower or needles) + habitus	Boskoop Expe- rimental Stat.
Buddleia	Other colours (flower or needles) + habitus	Boskoop Expe- rimental Stat.
Cytisus	Other colours (flower or needles) + habitus	Boskoop Expe- rimental Stat.
Hydrangea	Other colours (flower or needles) + habitus	Boskoop Expe- rimental Stat.
Laburnum	Other colours (flower or needles) + habitus	Boskoop Expe- rimental Stat.
Potentilla	Other colours (flower or needles) + habitus	Boskoop Expe- rimental Stat.
Rhododendron	Other colours (flower or needles) + habitus	Boskoop Expe- rimental Stat.
Syringa	Other colours (flower or needles) + habitus	Boskoop Expe- rimental Stat.
Conifers :		
Chamaecyparis	Other colours (flower or needles) + habitus	Boskoop Expe- rimental Stat.
Juniperus	Other colours (flower or needles) + habitus	Boskoop Expe- rimental Stat.
Picea	Other colours (flower or needles) + habitus	Boskoop Expe- rimental Stat.
Azalea altacla- rensis	Other flower colours	Private firm.
Roses	Other flower colours	Various Private firms.

Florist crops  
.....

Alstroemeria	Induction of desired characteristics	Private firm
Chrysanthemum	Other flower colour(s)	Private firms
Dahlia	Other flower colour(s)	Private firms
Saintpaulia	Induction of desired characteristics (flower colour, morphological variations)	ITAL

B. Fruit

Apple	Red fruit, larger fruit	Experimental Stations and private firms
Pear	Increase of fertility	Experimental Station

C. Forest crops

Ulmus	Improvement of growth rate	Experimental Station
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## C. BROERTJES

D. Apomicts

Poa pratensis	Induction of desired characteristics	Private firm
Rough-stalked meadow grass	Larger seeds and breaking apomixus	Various private firms

E. Agricultural crops

Potato	Improvement of productivity	Department of Plant Breeding and Foundation for Agricultural Plan Breeding
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## F O O D P R E S E R V A T I O N

## J.G. VAN KOOY

Applied research was promoted in 1964 by the organization of seven meetings of the Study Group on Food Irradiation and by cooperative programs with the following institutes :

Institute for Research on Storage and Processing of Horticultural Produce at Wageningen (subcontract):

Continuation of fruit and vegetable irradiation program (see page 26);

Institute of Dairy Research, Ede:

Surface-irradiation of prepacked cheese;

Institute for Research on Storage and Processing of Agricultural Produce, Wageningen:

Studies on the shelf life extension of prefried French fries, and on the inhibition of sprouting in potatoes;

The problem of decay of onions by Botrytus was discussed with the Institute of Phytopathological Research, and a large experiment on this subject was carried out by this Institute. The results obtained were negative;

Institute of Marine Products :

A small program was started on irradiation of shrimps in order to try to extend their shelf life.

Much attention was paid to the development of a pilot plant for food irradiation. A report "The preservation of food by means of ionizing rays; pilot plant studies in the Netherlands", was prepared and its main consequence was the introduction of the pilot plant concept to the Dutch Government via the Agricultural Corporations.

## F A L L O U T

A number of joint research projects are under way with the Institute for Biological and Chemical Research on Field Crops and Herbage (IBS) to study the possibilities of decontaminating feedstuffs during treatment or processing. Another project with the Institute for Rural Home Economics Research considers decontamination methods of edible plant products. The laboratory of Animal Physiology of the Agricultural University of Wageningen is pursuing a project dealing with the influence of Ca-addition to pasture grass in relation to Sr-contamination of milk.

A joint research project with the Netherlands Institute of Nutrition and the Institute of Agricultural Research of Biochemical Products in Wageningen has been initiated to

study the influence of Calcium and/or stable Strontium on the elimination of  $\text{Sr}^{90}$  from/pigs, whose physiology is very similar to that of men. These different research projects consider practically the major aspects of applied research that can be carried out at present within the framework of the Association.

Finally, all scientists of the Association working in this field participate in a Euratom Contact Group on soil-plant relationships.

## O T H E R   A C T I V I T I E S

### H. HEKMAN \*

The activities of the health physics group during 1964 covered mainly the implementation of regulations of the radiation protection service in the reactor and other irradiation facilities as they became operational. The supervision of the installations themselves as well as of the working areas and ventilation system of the radiochemical laboratories was carried out, together with the film badge service and the disposal of solid waste.

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De toepassing van straling voor voedselconservering.  
(Atoomenergie en haar toepassingen).

ZEEUW, D. de:

Les radiations ionisantes au service de l'agriculture.  
(Encyclopédie Le Progrès Scientifique).

ZEEUW, D. de:

European radiation preservation of fruits and vegetables.  
(Proceedings of the International Conference on Radiation Preservation of Foods - Boston).



# A P P E N D I X    I

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## M E E T I N G S,   S Y M P O S I A,   S T U D Y   V I S I T S, E T C.

Scientists of the Association have attended the following symposia and conferences :

FAO-IAEA Technical Meeting on Use of Induced Mutations in Plant Breeding, Rome, Italy: S. AVANZI, O. BANGA, A. BOZZINI, C. BROERTJES, T. CERVIGNI, R.B. CONTANT, M. DEVREUX, B. DONINI, R.M. ECOCHARD, F.P. FERWERDA, H. GAUL, W. GOTTSCHALK, G. GUERRIERI, C.U. HESEMAN, G.J. HILDERING, H. LAMBERTS, M. MARIANI, R. PRAKKEN, G. SAUER, G.T. SCARASCIA MUGNOZZA, J. SYBENGA, K. VERKERK, S.J. WELLENSIEK, D. de ZEEUW.

The Xth International Botanical Congress, Edinburgh, Scotland: E.C. LEVI and D. de ZEEUW.

The International Conference on Radiation Preservation of Foods, Boston, USA: D. de ZEEUW.

The Dutch food industries meeting, The Hague, the Netherlands: J.G. van KOOY and D. de ZEEUW.

Meeting of the Food Technology Students of the Agricultural University, Wageningen, the Netherlands: J.G. van KOOY.

FAO/IAEA Panel on the Use of Isotopes and Radiation Techniques in Soil Moisture and Irrigation Studies on Irrigated Land, Vienna, Austria: M.J. FRISSEL.

VII Session of the OECD Study Group on Food Irradiation, Paris, France: J.G. van KOOY and D. de ZEEUW.

A meeting was organized with the Dutch Physical Society at the Association's Institute.

The meeting of the Mutation Breeding Contact Group was organized at the Association's Institute.

A congress was organized with the "Nederlands Atoomforum" on food preservation by irradiation.

A 4 week Training Course on the Applications of Atomic Energy in Agriculture was held at the Association's Institute.

A P P E N D I X I (Cont.)  
 =====

R.G. CONTANT has attended the "Advanced Interregional Training Course on Cellular and Molecular Aspects in Radiology", Rehovoth, Israël.

Miss H.L. TEIJEMA and G. MODUGNO have attended the EURATOM-Course on Molecular Biology and Radiobiology, Brussels, Belgium.

H. GAUL has visited several institutes in Japan during 1964.

## A P P E N D I X    II

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### ASSOCIATION    EURATOM - ITAL

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 J.G. DE SWART  
 H.L. TEIJEMA (Miss)

#### ADMINISTRATIVE STAFF

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 P.H. van NIERCP (Finance and Personnel)  
 H.J. SONIES (Public relations and  
                   documentation)

STAFF OF OTHER SCIENTIFIC INSTITUTES ASSOCIATED WITH  
EURATOM - ITAL BY SUBCONTRACT:

- BELGIUM : Université de Liège - Centre Interfacultaire des Sciences Nucléaires - Laboratoire de Génétique - Liège: J. MOUTSCHEN, M. MOUTSCHEN-DAHMEN.
- ITALY : Comitato Nazionale per l'Energia Nucleare - Centro di Studi Nucleari della Casaccia - Laboratorio per le Applicazioni in Agricoltura - Rome: S. AVANZI, A. BOZZINI, T. CERVIGNI, M. DEVREUX, B. DONINI, M. GIACOMELLI, B. GIORGI, G. GUERRIERI, G. MODUGNO, L.M. MONTI, G.T. SCARASCIA MUGNOZZA.  
Istituto Botanico dell' Università - Cagliari (Sardegna): C. FLORIS, P. MELETTI.
- NETHERLANDS : Instituut voor Bewaring en Verwerking van Tuinbouwproducten (IBVT) - Institute for Research on Storage and Processing of Horticultural Produce - Wageningen: O.L. STADEN.  
Instituut voor Veredeling van Landbouwgewassen (IVL.) - Department of Agricultural Plant Breeding, Agricultural University - Wageningen: F.P. FERWERDA, J.M. MONTEZUMA DE CARVALHO.  
Instituut voor de Veredeling van Tuinbouwgewassen (IVT) - Institute of Horticultural Plant Breeding - Wageningen: A.E. ZEILINGA, L. ZELLES.  
Stichting voor Plantenveredeling (SVP) Foundation for Agricultural Plant Breeding - Wageningen : R.J. HERINGA, G.J. SPECKMANN.  
Laboratorium voor Erfelijkheidssleer - Department of Genetics, Agricultural University - Wageningen: G.J. HILDERING, K.R. NARAYANAN.  
Laboratorium voor Tuinbouwplantenteelt - Horticultural Department, Agricultural University - Wageningen: K. VERKERK, S.J. WELLENSIEK, B. WILDERVANCK (Miss).

GERMANY

: Institut für landwirtschaftliche Botanik  
der Universität Bonn - Bonn: W. GOTTSCHALK,  
A. JAHN, F. MÜLLER.

Max-Planck-Institut für Züchtungsforschung -  
Köln : K. BENDER, H. GAUL, C.U. HESELMANN.

## GUEST SCIENTISTS AT ASSOCIATION EURATOM-ITAL:

A. van Es- Instituut voor Bewaring en Ver-  
werking van Landbouwproducten -  
Institute for Research on Storage  
and Processing of Agricultural  
(I.B.V.L.) - Wageningen, the  
Netherlands.

J.P.W. NOORDINK - Instituut voor Planten-  
ziektenkundig Onderzoek -  
Institute of Phytopatholo-  
gical Research (IPO) -  
Wageningen, the Netherlands.

H. VEEN - Centrum voor Plantenfysiologisch  
Onderzoek - Plant Physiological  
Research Centre (CPO) - Wageningen,  
the Netherlands.

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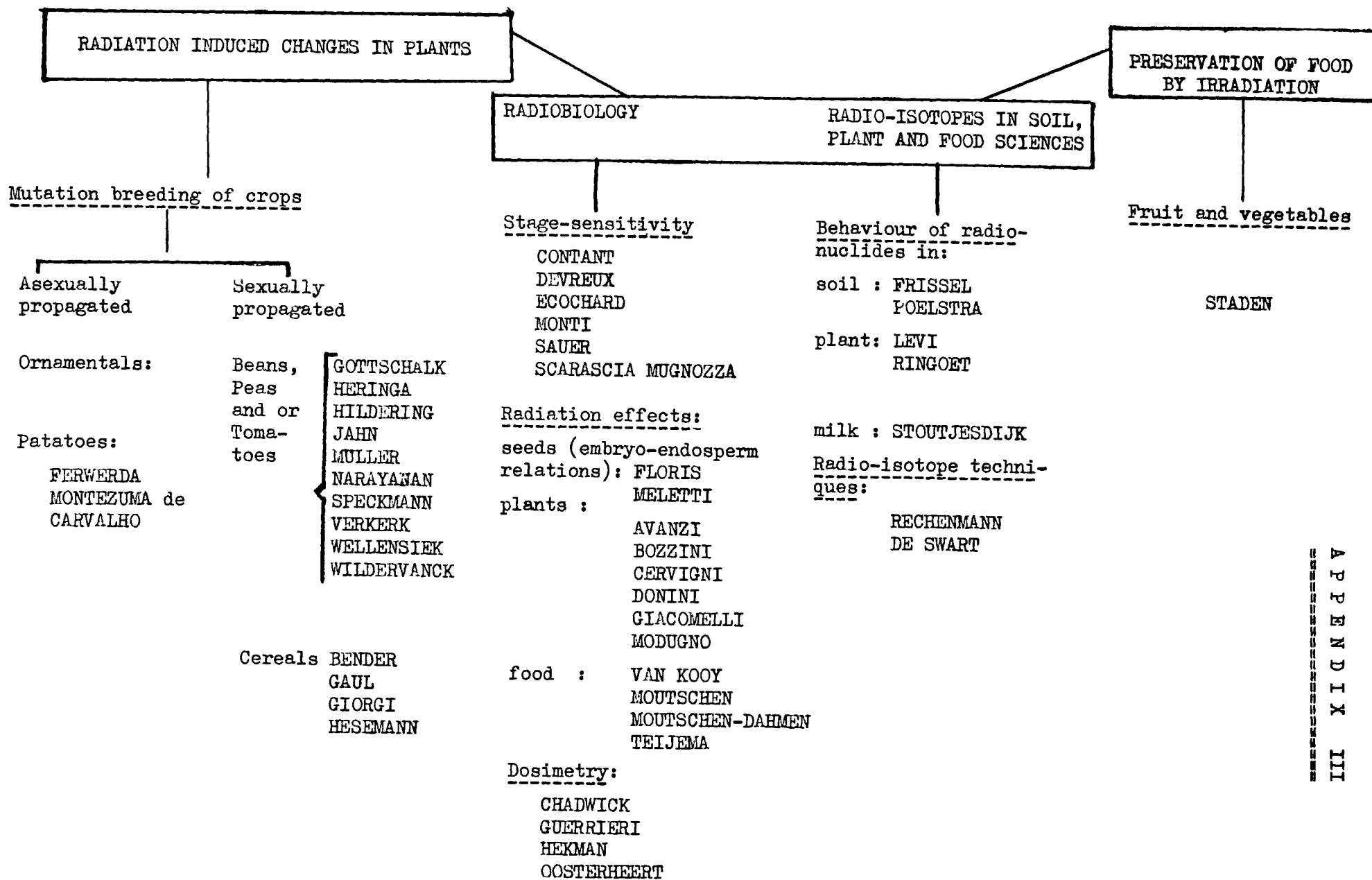
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# S C H E M E O F S C I E N T I F I C A C T I V I T I E S (1964)

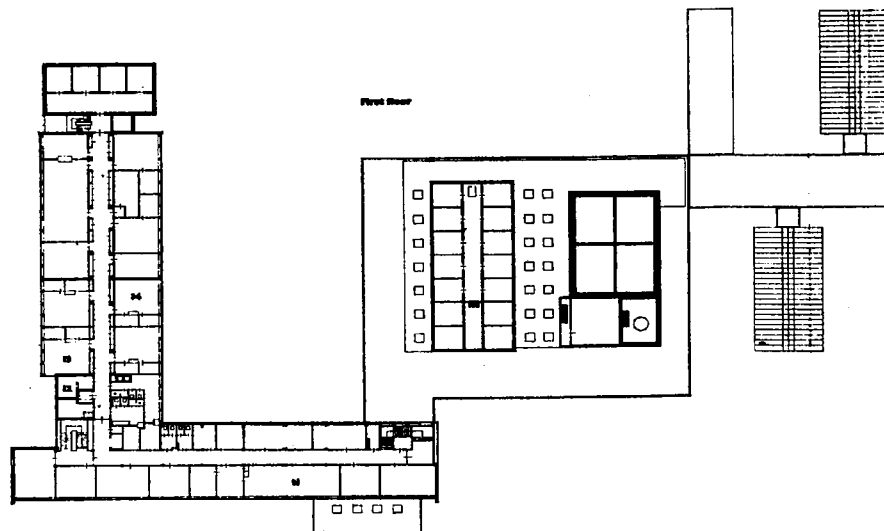
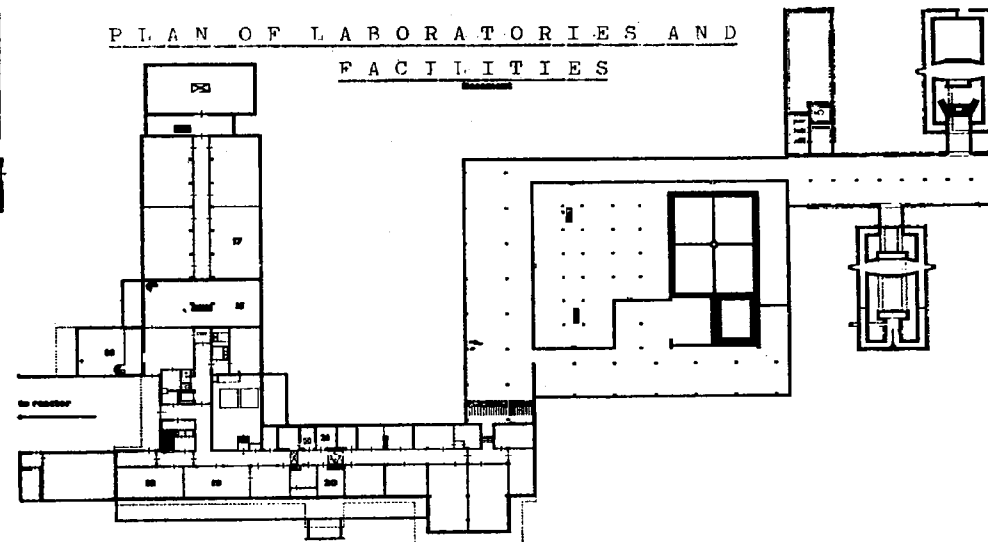
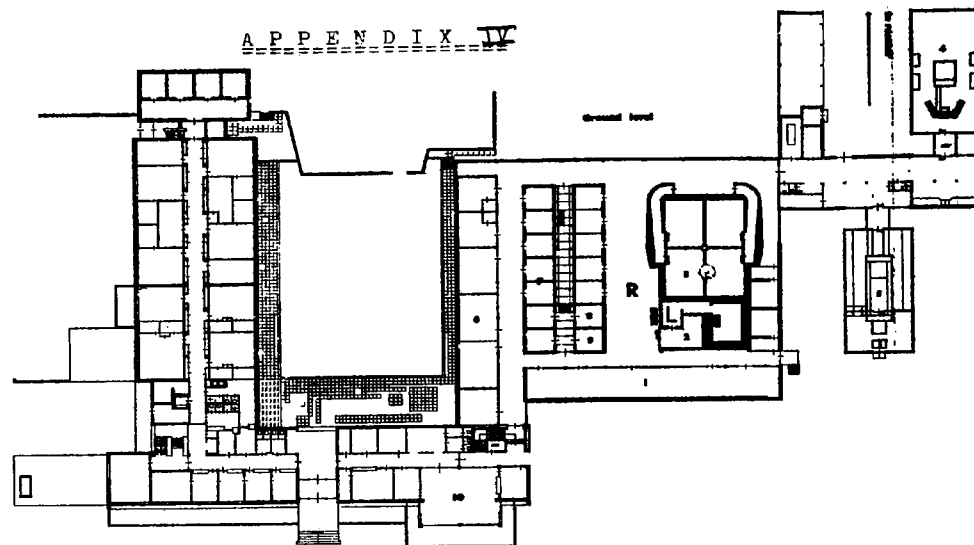
CARRIED OUT AT THE INSTITUTE OF THE EURATOM - ITAL ASSOCIATION (WAGENINGEN) AND AT THE SUBCONTRACTING INSTITUTES





# APPENDIX IV

## Plan of facilities



Some aspects of the research carried out at the Association EURATOM-ITAL Institute and some of the facilities can be seen as follows:

- R. Reception hall
1. Exhibition
2. Electron generator for food irradiation
3. Indoor gamma field; acute irradiation of plants with  $\gamma$ -rays
4. Gamma greenhouse with partial controlled climatic conditions: chronic irradiation of plants with  $\gamma$ -rays.
5. Greenhouse: X and neutron irradiated ornamentals.
6. Respiration measurements of fruit-tissues in Warburg-apparatus.
7.  $\gamma$  and neutron irradiated tomato-plants grown in controlled environmental conditions.
8. Respiration measurement of irradiated fruit in low temperature controlled room.
9. Containers for storage of irradiated food products in cold storage room.
10. Canteen.
11. Library.
12. Measurement of fallout Caesium in hay samples by counting in  $\gamma$ -spectrometer.
- Counting of  $\beta$ -activity in liquid scintillation spectrometer.
13. Radiochemical laboratory.
14. Experiment to study transport of inorganic chemical salts in soils.
15. Radioactive waste disposal.
16. Investigation of size, shape and density of particles on segregation of dry blended granular material.
17. Installations for microautoradiography of plant material.
18. Instrument development.
19. General workshop.
20. Mass-spectrometer.
21. Penetration and transport of radiolotopes in plants in environmentally controlled rooms.





A P P E N D I X V

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A.C. unit for  $\beta$  measurements

by

J.G. de SWART

The design was based on the following requirements: -

- a. the unit should be suitable for measuring radioactive strontium in fall-out;
- b. it should be as reliable as possible;
- c. it should have a built-in time measurement from a few minutes to at least 24 hours with an accuracy of at least 0.5% for all settings;
- d. the measurement results should not be affected by supply breakdowns with warning;
- e. in emergencies it should be capable of being fed by simple petrol-driven units;
- f. compact and space-saving design;
- g. simplicity of operation.

### General description.

This unit is intended for the measurement of emitters in small amounts, for which purpose the zero effect is reduced to an extremely low value (about 0.9 account/min.)

This low zero effect is due to: -

- a. the use of two GM tubes in anti-coincidence connection, the counting tube being housed in the guard tube (Philips system).
- b. a lead shielding, at least 160 years old and a minimum wall thickness of 10 cm surrounding the entire detecting system.

The counting tube usually has a window thickness of about  $10 \text{ mg/cm}^2$  and an effective surface area of  $0.08 \text{ cm}^2$ , but if necessary can be replaced by other tubes with a thinner window.

The sample to be measured is introduced by hand via sample slides. The pulses are counted on an apparatus specially designed for low-activity measurement and almost entirely transistorised electronic tubes with heated cathode or filament, are not applied, to increase the reliability. In view of the low counting rate, the counting register is designed with three electro-mechanical decades preceded by an electronic decade. This electronic decade is so fast that the dead-time of the entire installation is determined by the dead-time of the recorder, which is negligible in low-activity measurements. The instrument is designed for preset time measurements. For this purpose a clock unit for long measuring times is built in (adjustable from 0 -  $10^6$  mins in steps of 1 minute).

The accuracy of the time setting depends on the stability of the mains frequency, which according to information given by the Electricity Company varies from 0.1% under normal conditions to 0.3% under heavy conditions.

The built-in high-tension unit is adjustable from 0 - 1500 Volt in calibrated 85 V steps. The calibration error is about 10 Volt and the unit is stabilised against mains-variations of about 10% with a stabilisation factor of 60. Since this type of measuring instruments often operate at night and week-ends, in order to economise on the counting tubes the high tension can be switched off automatically at the end of a measuring time.

Since an unnoticed breakdown in the mains may give a wrong measurement, a warning system is installed which indicates whether there was a breakdown in the mains during the measurement.

When there are asymmetric variations in the load on star-connected mains circuit, noise pulses occur in most counting instruments. To prevent these a special filter has been built in.

As the time measurement is not sufficiently accurate in a mains circuit of instable frequency (e.g. a gasoline power unit) it is possible to measure in manual operation with a stop-watch.

A testing facility on the frequency of the mains circuit is built in.

For the GM- and AC unit a Telefunken AC measuring head is used, which is commercial as a transistorised unit.

The measuring head is demounted into two parts and adapted for use in our unit.

The actual counting is done by means of a system made of separate units manufactured by Metronic AG. The counter unit has its own power supply and for the AC unit, a separate 12V power supply is built in.

The timer is based on a pre-adjustable register operated by pulses obtained from a micro-switch is coupled to a synchronous motor via a cam disc. After reaching the presetting on the register a relay is switched off, so that the count channel is blocked.

In order to be sure that the cam disc is always in the same position when a measurement is started, the motor can only stop in the starting position of the cam disc.

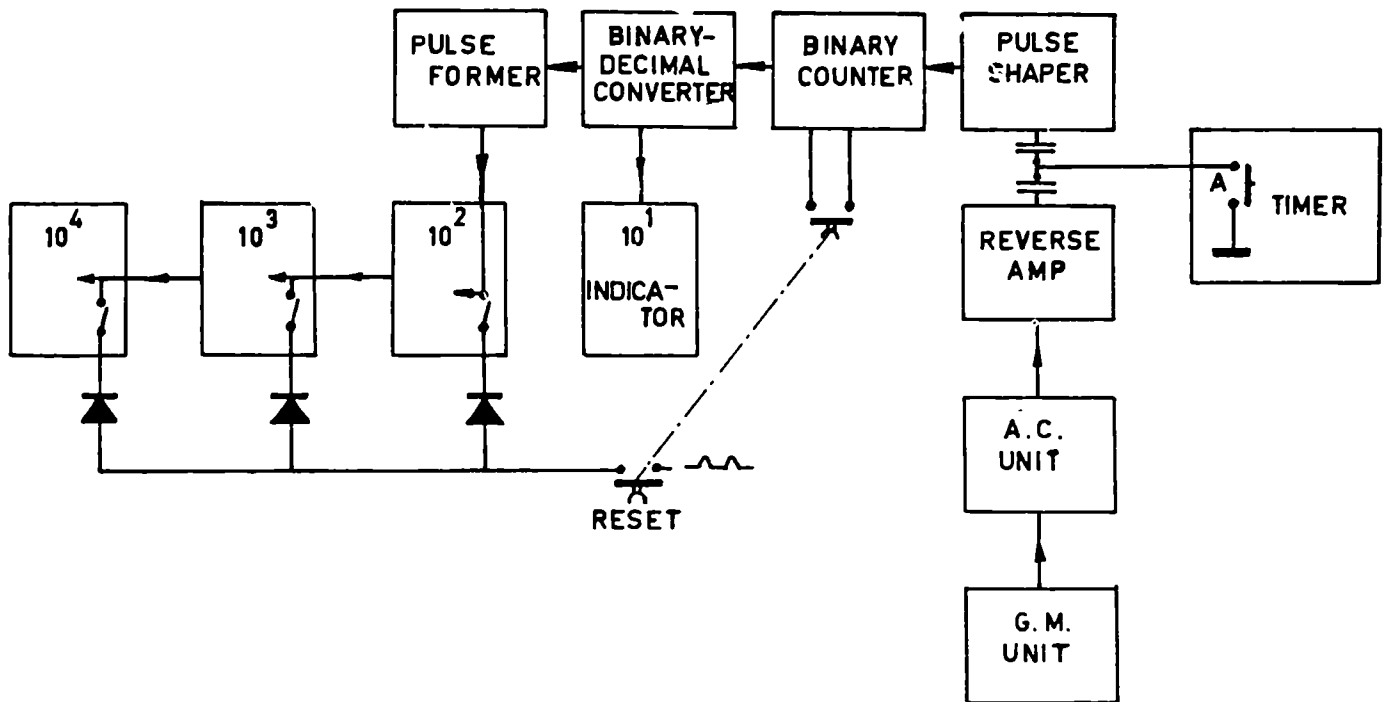
### Conclusion.

The price of this system is such that four of these instruments can be purchased instead of one unit with automatic sample changing. In view of the long measuring times more samples can be measured and the trouble-sensitivity of the measuring department is considerably reduced.

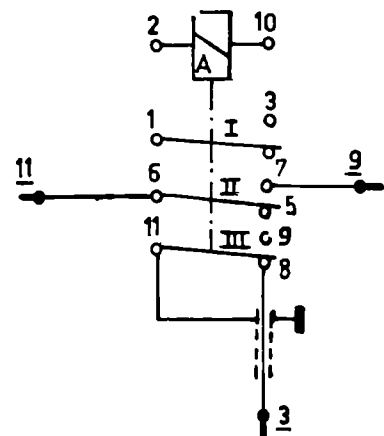
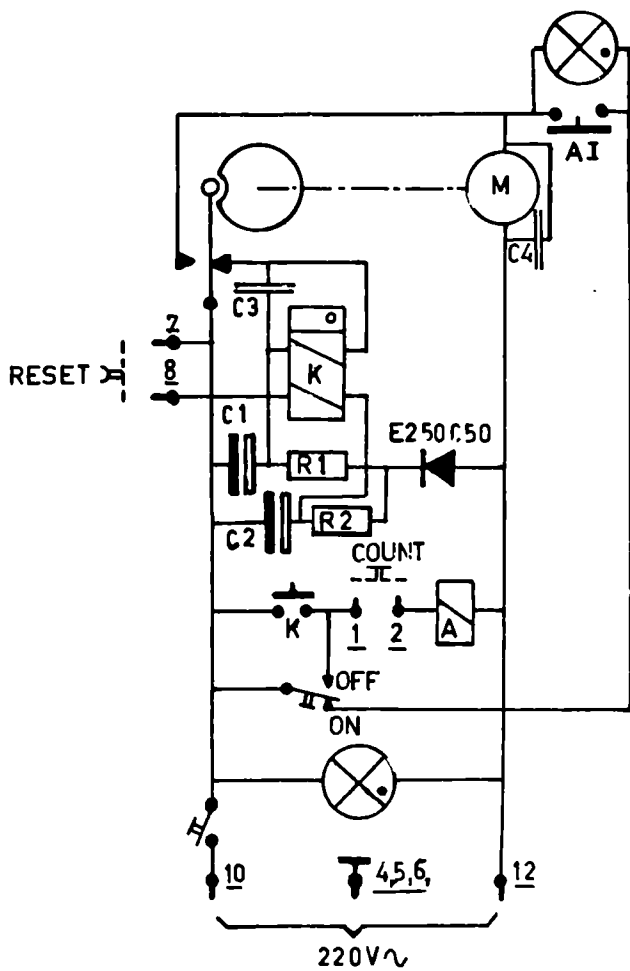
The first test measurements made by ir. Poelstra on the prototype showed that

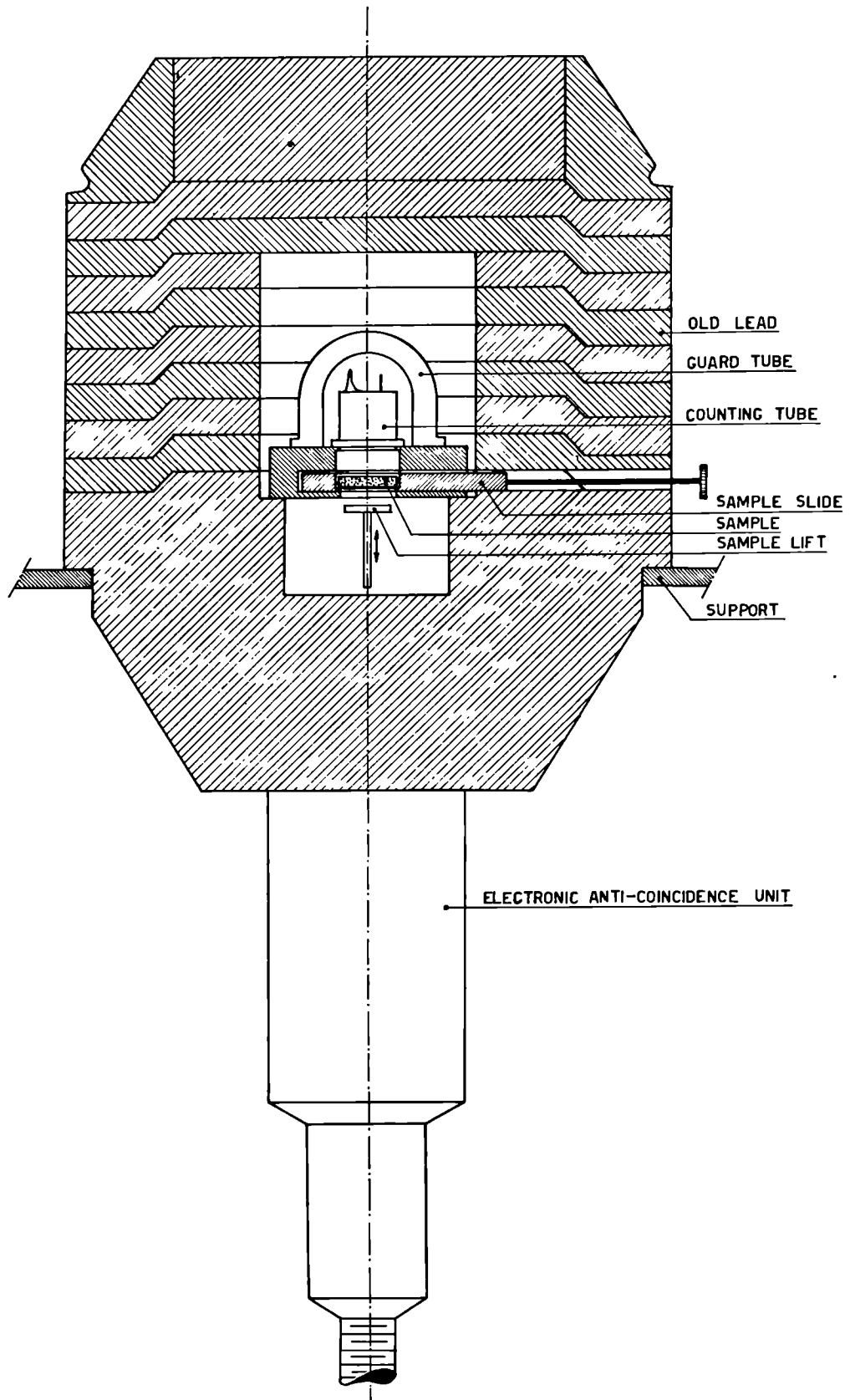
- a. the background is 0.8 ct/min this means 10 to 20% lower than commercial instruments of comparable design;
- b. the stability may be considered very good;
- c. the apparatus is very easy to handle.

# COUNT CHANNEL



# TIMER



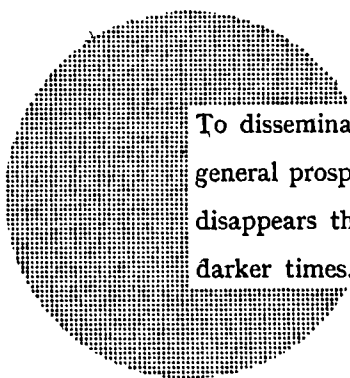


A-C LEAD SHIELD.

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To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel



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51 - 53, rue Belliard  
Bruxelles (Belgique)