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

APPLICATION OF ATOMIC ENERGY
IN AGRICULTURE
(ANNUAL REPORT 1963)

1964



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

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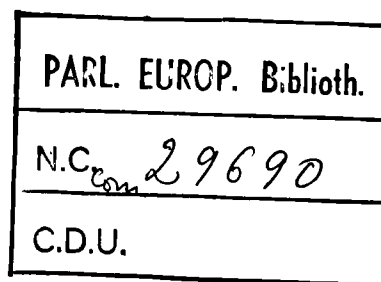
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EURATOM - ITAL

Wageningen, July 1964

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I N T R O D U C T I O N

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The research facilities of the EURATOM - ITAL Association Institute have been further extended in 1963 within the original building program.

Construction of the 'Biological Agricultural Reactor Netherlands' (BARN) which is the neutron radiation source of the Institute was completed. The reactor reached criticality for the first time on April 9th and was handed over to the Institute on November 13th.

Erection of the first greenhouse with partially controlled environmental conditions was also completed.

Construction of the radiobiological wing which includes two γ -sources, one in a greenhouse and the other in plant environmental controlled rooms, as well as a number of such plant growth rooms and the Institute Van de Graaff electron generator for food preservation studies, has been pursued satisfactorily. Excavations for the extension of the radiochemical wing have also been initiated.

Notwithstanding the fact that, in particular in the radiobiological research field, some facilities have not yet been completed research projects under way have progressed satisfactorily in the three main fields of interest of the Association.

In the framework of the European cooperation program of the Association an important subcontract was allocated to the Comitato Nazionale per l'Energia Nucleare, Centro di Studi Nucleari della Casaccia, Laboratorio per le Applicazioni in Agricoltura in Italy. This subcontract is of importance to the research program of the Association concerning mutation breeding of crops and in particular radiobiological investigations. The total number of subcontracts allocated so far for joint research programs to other Institutes was thus brought to eleven. International working groups in mutation breeding and food preservation have also been established in 1963 and fruitful cooperation initiated. A similar working group to consider problems involved in the assessment of the fate of radio-nuclides in the biosphere has been planned by the Biology section of EURATOM.

The present annual report 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 list of staff and an organogram of the research activities will be found in Appendices III and IV.

Besides the main report of scientific research an attempt has been made to summarise the prospects offered by food irradiation, one of the main fields of interest of the Association.

D. de ZEEUW
Director.

P R O S P E C T S I N F O O D I R R A D I A T I O N

by

J.G. van KOOY and D. de ZEEUW

When considering the prospects in food irradiation one has to realise that the development of industrialization in modern society led to such groupings of populations that the place where foodstuffs are produced or manufactured are now more or less separated from consumer areas. Agricultural and horticultural products have to be transported over long distances, this inevitably requires their preservation in some way or other. A large number of preserving methods are already available and research into the improvement of existing ones and the development of new ones is very active.

For instance, the necessity of supplying armies with food of good quality and storage stability have led in recent years to intensive research work being carried out in the field of food preservation. For example, during the last world war great progress was made in food dehydration, with the consequent result of a considerable improvement in the quality of dehydrated products. The Quartermaster Food and Container Institute of the US Army has played an important part in the latest developments in food technology. It was also at this institute that the first investigations on the possibilities of ionizing radiation for food preservation were carried out. The work begun on a small scale in the USA has now, some 20 years later, spread to other countries in the world.

Two different trends may be distinguished in radiation research: sterilization which aims to produce a stable product, and pasteurization, the purpose of which is to improve the storage stability for a certain time. The sterilization of foodstuffs by irradiation is of comparatively little importance; the high radiation dose required, i.e. more than 1 Megarad, often causes various undesirable changes in flavour, aroma and consistency of the product. On the other hand, radiation pasteurization affords excellent prospects. The main feature of the process is that the preservative effect is obtained without any change of temperature in the product. This property is extremely important since the ultimate aim of food technology is a preservation method which keeps intact as far as possible the original character of the foodstuff. This is especially important for products consumed fresh. It is obvious that for a fresh product, a longer storage stability is of great importance for its efficient distribution and utilization. To the agricultural producer it is important that a given product retains certain qualities for a longer period to prevent price reductions. This would also increase sales possibilities since the products can be transported over greater distances.

On the other hand the consumer is interested in a preservation method which reduces spoilage during commercialization and may thus provide a brake to rising prices of the sold products. The modern consumer requirements call for high standards of quality, keeping properties and packing. This implies a distribution organization offering a greater number of articles attractively packed to allow easy display, and which do not always have to be made up from stocks located elsewhere. These factors assume increasing importance as small specialized foodshops are replaced by large self-service stores and supermarkets based on the sale of prepacked products. Sales of fresh and/or live products are extremely difficult in this kind of commercial business. Irradiation is a possible appropriate method to give these products a longer storage life, since it usually enables the fresh character of the product to be retained and improves the stability of prepared products. We have so far commented only on the economic aspects of the possible use of radiation pasteurization in food technology. This is due to the fact that fundamental research in general and radiation biology research in particular requires the expenditure of large sums of money which can ultimately be justified by the economic advantages resulting to man from radiation research.

Food preservation research has already achieved some important practical results in the sphere of food preservation. This is due to the fact that macroscopic effects induced by ionizing radiation are by their very nature more readily ascertained than biochemical and physiological ones occurring in cells. Thus the effects of radiation on rapidly perishable products have been effectively studied by means of accurate visual observations: it has been found that fungus growth on soft fruit is increasingly inhibited with increasing doses of radiation; discolourations have been observed in such pigment-containing products as strawberry, salmon and tomato, increasing as the pigment in question is more sensitive to radiation, etc. An impression of the effect of radiation on consistency of products was also readily obtained. Moreover, taste panels allow to obtain information on flavour and aroma of irradiated products.

It is however much more difficult to gain an insight into the mechanism of these processes. Research carried out at the Association EURATOM - ITAL Institute to study the retarded ripening of live vegetable products following irradiation indicate that respiration of fruit is accelerated provided the irradiation is carried out during the development stage of the fruit which is characterized by an active metabolism. The carbon-dioxide evolution increases with radiation dose and respiration is maintained at this higher level for a certain time. If the radiation dose exceeds a value at which the fruit shows visible injury, a rapid decrease in carbon-dioxide production occurs after treatment. It is not yet possible to offer an explanation for this phenomenon. This shall only be possible when the physiological processes of ripening and senescence are known.

Products with a high protein and fat content, i.e. usually products of animal origin, are more sensitive to ionizing radiation than vegetable ones. Radiation sensitive components such as protein and fats are split off by interaction with ionizing radiation and the resultant chemical substances give the irradiated product characteristic anomalies of flavour and aroma. Although these phenomena occur only with high sterilization doses required to kill sporeforming bacteria, methods are being sought to reduce or eliminate these undesirable effects.

Some success has already been achieved using techniques such as: the irradiation of products in a frozen state or in an inert atmosphere; very high dose-rates; combining radiation with other methods of preservation (heat treatment, antibiotics); the addition of stimulants for the germination of spores or materials which make radiation-resistant micro-organisms more sensitive. The success of a more general application of radiation sterilization is in fact closely linked to the question as to whether these problems can be solved. In this connection it should be born in mind that for every method of preservation the ultimate determining fact is a compromise between quality, storage stability and cost.

The use of low radiation doses, pasteurization doses, causes no change in flavour and aroma of irradiated products, but requires the solution of some problems, among which:

The development of radiation sources suitable for carrying out experiments on a semi-industrial scale and in which care should be taken to insure as uniform a distribution of dose as possible in the product irradiated.

A dosimetry system should also be developed to record accurately the doses given to the products treated.

The specifications which public health authorities will prescribe with regards to the commercialization of irradiated food are dependent on one hand on accurate determination of radiation doses used and on the other on the wholesomeness of the treated products.

Four questions come to mind when the problem of food irradiation is considered. They could be stated as follows:

1. Does irradiation lead to the formation of toxic and/or carcinogenic substances?
2. Is the food still bacteriologically reliable after irradiation?
3. Is radio-activity induced in the food by irradiation?
4. Is the nutritive value altered?

Following considerable research undertaken to answer these questions, conclusions reached were stated at two FAO-WHO-IAEA meetings, held in Brussels in 1961 and in Rome in 1964*.

* 'Expert Committee on the Technical Basis for Legislation on the Wholesomeness and Microbiological Safety of Irradiated Food' - Rome, April 21st - 28th, 1964.

It appears that so far no formation of toxic or carcinogenic substances has been noticed following radiation treatments currently used.

Microbiologists experience the greatest difficulties in setting standards to determine bacteriological reliability of irradiated food and feeding stuffs. However there are no indications that this reliability is impaired by irradiation more than by other methods of food preservation.

Whenever radiation energies of less than 10 MeV have been used, induction of radio-activity was negligible compared to the maximum permissible concentration in drinking water. Moreover, radio-active materials that could be induced in food products have an extremely short half-life.

The nutritive value is hardly if at all affected by irradiation although, at any rate for the present, it should be remembered that some destruction of vitamins, e.g. vit K and vit E, occurs also with other methods of preservation.

The Rome conference recommended that governments concerned be advised to lay down regulations relating to the production, import, export and distribution of irradiated foodstuffs, supervision being based on individual permission on the principle of the positive list.

Finally it should be mentioned that the following irradiated products have been certified fit for consumption:

USA	: bacon, cereals and cereal products
Canada	: potatoes
USSR	: potatoes

The following are being investigated:

USA	: potatoes, oranges, strawberries
UK	: cereals and cereal products, eggs (Salmonella)

Applications to be submitted in 1965^{*}:

USA	: cherries, apricots, peaches, onions, carrots, fish
-----	--

The present possibilities offered by radiation pasteurization and the situation of food irradiation research in various countries, could be summarized as follows:

Potato - sprout inhibition: Radiation process is not exclusive; sprouting can be inhibited by chemicals as isopropylchlorophenyl-carbamate (ClIPC) and nonylalcohol. The health hazards of chemicals may reduce their value. On the other hand radiation treatments are economically attractive since low dosages are required. Commercial application is in an advanced stage in Canada, France and the USSR. Studies are also in progress to attempt extension of storage life of prefried potato chips.

^{*} so far as known.

Onions - sprout inhibition: sprouting during long distance transport, especially in early spring, is a big problem when temperatures cannot be controlled. Commercial application is only attractive for exports; neck rot control is doubtful.

Soft fruit - storage life increase as a fresh product is economically attractive owing to the high value of the product. The radiation process is exclusive. A pilot plant is under construction in the USA; widespread commercial interest in France, Sweden and Italy.

For hard fruit results are somewhat variable as regards increasing the storage life of the fresh product. Much research is still required, also to control a number of storage diseases by means of radiation.

Vegetables - increasing the storage life of the fresh prepacked product with radiation was found to have remarkable effect on mushroom flavour. The radiation process is exclusive. Commercial application is attractive in view of all year round production, with the possibility of increasing exports. The process is economically attractive owing to the high value of the products.

Insect control in cereal and flour products - Radiation is not exclusive as insects can also be controlled with insecticides and fumigants. A drawback of the latter method is the problem of residues. A pilot plant for irradiation treatment is in advanced stage of construction in India.

Milk products - at present some prospects are offered by the control of mould formation on prepacked cheese.

Salmonella control in eggs, egg products and fishmeal - the irradiation method is exclusive except for liquid egg products. Commercial applications are in an advanced stage in the UK.

Fish and fish products - combined treatments of radiation and cold storage offers very good prospects to increase the storage life of the fresh product. A pilot plant is under construction in the USA and considerable interest in many countries. Good prospects also exist for fried and smoked fish.

Good prospects appear to increase the storage life of only a small number of meat and meat products. Commercial applications exist in the USA for bacon. Improvement of the quality of canned salted ham by means of radiation is in an advanced stage of research in Denmark.

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R E S E A R C H A C T I V I T I E S

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The EURATOM - ITAL Association research program in 1963 dealt as in the previous year with problems of radiation induced changes in plants, food preservation by means of ionizing radiations, the behaviour of specific elements in soil, plant and animal, and the development of radio-isotope methods for agricultural research.

R A D I A T I O N I N D U C E D C H A N G E S I N P L A N T S

The principal aim of this program is to carry out research in order to make irradiation as effective as possible for inducing desired mutations in plant material.

The majority of studies in radiobiology have been made utilizing X- and γ -rays which have a low linear energy transfer (LET)-value. To understand more fully the biological effects of radiations one should however, in comparative studies include also higher LET-radiations, such as protons, α -particles and neutrons. Because of their limited penetration protons and α -particles can only be used for small volume material.

The important consideration in an analysis of relative biological effectiveness (RBE) is the fundamental physical concept of LET, although RBE will also vary with material, physiological conditions and parameters used. It is necessary therefore to use for the experimental purposes of this investigation radiations of different LET-values. The relative usefulness of slow and fast neutrons is still a matter of conjecture. It is clear that because of the various kinds and energies of radiations depending on the elemental distribution in biological material resulting from slow neutron irradiation, accurate dose measurements are difficult if not impossible. In plant tissue of average mineral content, approximately 600 times as much energy goes into the 2.2 MeV capture γ -ray of hydrogen as into the protons from nitrogen. Furthermore if 1 ppm boron is present in the tissue it would contribute already more energy of higher LET than the n,p reaction in nitrogen. This means that slow neutrons irradiation creates many uncontrolled elements. The use of fast neutrons allows on the other hand good dose measurements of high LET-irradiation. Although - from the points of view of total energy release and dosimetry - fast neutrons seem to be favoured it should be kept in mind that the physiological and genetic effects in plants are probably not primarily dependant on total energy release but on specific and local high LET-events in the biological material. It has been suggested that boron and nitrogen are concentrated to some degree in the chromosomes. This could therefore explain the fact that slow neutrons show more selective damage to the nucleus and chromosomes, resulting in more genetic than physiological damage and more mutations per neutron than fast neutrons.

The number and perhaps the nature of mutations is affected by the radio-sensitivity of the plants and the environmental, therefore physiological conditions of the plant during and after the irradiation. For these reasons much attention has been paid to these factors.

In the past year, the neutron irradiation facility of the Association's Institute, the BARN (Biological Agricultural Reactor Netherlands), has been completed and made ready for use in 1964. Both slow and fast neutrons will be available.

M U T A T I O N B R E E D I N G

ASEXUALLY PROPAGATED PLANTS

STUDIES ON CHIMAERAS

Begonia:

Broertjes^H

The following preliminary experiment was carried out: Of the 24 irradiated leaves, giving 77 young plants of the red-flowering variety 'Marina', 8 plantlets turned out to be mutants (spickled leaves, smaller flower, irregular growth, dwarf type plants, etc.). The white flowering variety 'Weisser Schnee' gave 1 mutant with smaller greenish-white flowers. Most plants were uniform for the mutated character which could mean that like Saintpaulia the aerial parts of every young plant comes from one cell. Since propagation via leaf cuttings appears to be difficult and very slow, experiments with this test plant will be discontinued.

Chrysanthemum:

The 4 varieties X-rayed showed a fairly high number of mutations. In the 64 pink-flowering 'Asta Lee' (1000 and 1250 r) 6 mutations were found: 2 (very) pale pink, 2 yellow-bronze and 2 (dark) yellow. A creamy budspot of the pink-flowering 'Breitner' was irradiated with 1500, 2000 and 2500 r (124 plants in total) resulting in many pink or partly pink flowers caused by uncovering. In addition 10 mutations were scored: 7 (dark) yellow, 1 bronze and 2 white. The pink-flowering 'Hortensien rose' gave the most striking results: 164 irradiated plants (1500, 2000 and 2500 r X-rays) showed 34 mutations: 11 (very) pale pink, 3 dark pink, 5 bronze, 6 yellow (bronze), 4 bronze-red, 1 brown red, 2 semi double, 1 single flowering and 1 dwarfish growing plant.

The pink-flowering 'Jacob Maris' showed no mutations at all in the 88 plants irradiated with 1250 and 1750 r X-rays. All shoots with mutations were rooted following treatment with 2% indolylbutyric acid. Before and during rooting the plants were kept under long-day light conditions to prevent flower bud initiation.

^H Association EURATOM - ITAL, Wageningen, the Netherlands.

Pelargonium:

Broertjes

Two mutations were found in the Pelargonium varieties irradiated: one in 'Rubin' (flower colour lighter red) and one in 'Byhouwer's Glorie' (the same lighter red flower colour). No indications were found that the latter variety was a periclinal chimaera of 'Rubin' (no uncovering found). The program covering these varieties has therefore been discontinued. Instead, other Pelargonium varieties and a number of induced mutations (from a cooperative investigation by S. Shapiro and C. Broertjes) will be propagated and used for this part of the program.

Roses:

Out of 277 irradiated plants (various acute X-ray dosages) 73 mutations were found, most of these being flower colour mutations, viz. 1 white, 33 pink, 29 lighter red and 10 darker red.

There was no clear-cut relation between number of mutations and dose in the roses irradiated in 1963:

Dose (r)	Number of mutations per 100 irradiated plants: (55 plants per treatment)
2000	22
2500	15
3000	18
3500	18

However, the plants of the same variety irradiated in November 1962 and cut back in the spring of 1963 gave the following results:

Dose (r)	Number of mutations per 100 irradiated plants: (10 - 20 plants per treatment)
1000	60
2000	45
3000	60
4000	100

These plants were much less radio-sensitive because they can stand a higher acute dose. They gave a considerably higher mutation rate.

Saintpaulia:

Final results obtained in 1962 indicated that: The production of young plants depends very much on the time of rooting: earlier in the autumn gives better results. In the first experiment X-raying of leaf petioles with 3000 r gave the highest number of mutants. In the second experiment 4000 r to petioles gave 40%, and to complete leaves 83% mutants in the young plants. Among 200 mutants, only 2 chimaeras were found, viz. most young plants seem to be genetically uniform. The mutation spectrum was very wide. The greatest number of mutations affected leaf and growth habit (leaf size, petiole size, leaf and petiole colour, leaf shape, dwarf and semi-dwarf plants, etc.). A smaller number of flower mutations was seen: colour, size and shape of the flowers. Ex-

Broertjes

cept for one case, flower mutation was always accompanied by a change in leaf or growth (more than one gene changed or pleiotropy).

THE INDUCTION OF POINT MUTATION IN POTATOES

Ferwerda^{*}

During 1963 the maintenance, multiplication and assessment of aberrant types obtained from irradiation treatments in 1961 and 1962, the genetical analyses of aberrant types and examinations of the vegetative apex for histological and cytological changes following irradiation were carried out.

Maintenance and multiplication of aberrant types:

The vegetative progenies of tubers treated with mutagens in 1961 and 1962 were multiplied in a nursery in the western part of the province North-Holland to prevent virus infection. Fortunately only 5% of the clones showed positive symptoms of virus infection.

This year the aberrant types were represented by large numbers of individuals and this made the contrasts clearly stand out, particularly in the oldest clonal offspring which, in 1963, represented the 3rd propagation cycle. The anomalies with respect to leaf shape and tuber colour mentioned in the previous year maintained themselves in the subsequent 2nd and 3rd propagation cycles.

Irradiation experiments in 1963 gave some additional information as to optimal radiation dose and dose rate. The optimal dose fluctuated within rather narrow limits around 3.5 Kr. Some striking examples of chimaerical structures occurring after irradiation and especially with regard to tuber skin colour were observed.

Whether radiation is to be considered as the causal agent of this chimaera or simply as a help to uncover latent chimaerical structures cannot yet be ascertained.

Genetic analysis of aberrant types:

One of the leaf anomalies ('ivy-leaf') was found to be clearly transmitted on generative propagation. A large proportion (60%) of a seedling progeny of this mutant consisted of 'ivy-leaf'-types whereas among the seedlings of check clones derived from non-irradiated bud-patches of the same original tubers not a single 'ivy-leaf'-type could be detected. A series of test crosses to determine the genetic structure of this 'ivy-leaf' mutant were performed.

The yellow splashed red tuber type obtained after irradiation of a red skinned clone was also included in the test crossing program.

Eye-excision experiment to prove or disprove the postulated periclinal structure of some commercial varieties failed to give decisive evidence and will have to be repeated in 1964.

^{*} Instituut voor Veredeling van Landbouwgewassen (IVL) - Department of Agricultural Plant Breeding - Wageningen, the Netherlands.

Histological and cytological changes in the vegetative apex after mutagenic treatments:

Montezuma de
Carvalho^x

Evidence gathered up to now indicates that radiation doses equal to or higher than 3 Kr completely inhibit activities of the terminal vegetative apex and that lateral buds take over their function. It is still uncertain whether these lateral buds have already been initiated before irradiation or have regenerated afterwards. From which elementary cell layer they originate, is also open to question; this and the overall reaction pattern after treatment with ethyl-methane-sulfonate (EMS) will constitute the main subjects of further anatomical investigations.

Studies on the changes occurring within the cells of the vegetative apex after irradiation have been started. The preliminary impression is that radiation doses as used in the trials cause a considerable proportion of chromosome breaks, chromatin bridges etc. indicating that the impact has been rather drastic.

It is still unknown whether these evidently damaged cells survive and are capable of further divisions.

SEXUALLY PROPAGATED PLANTS

MUTATION RESEARCH ON BEANS

Heringa^{xx}

A study of the practical applications of mutations following treatment with chemical mutagen and irradiation was continued.

An attempt to infect with Phaseolus virus 2 the M₂ generation of seeds of the variety 'Beka' treated with EMS has failed, visible mutations occurring very sporadically.

Seeds of the variety 'Berna' irradiated with X-rays at 12, 14, 16, 18, 20, 22, 24 and 26 Kr X have shown a decrease in germination of 1 - 7% from the lowest to the highest treatment; the number of plants coming to maturity and fertile decreased to 0 for 20 Kr treatments.

Hildering^{xxx}

The study of the resistance to yellow bean mosaic in the 'Widusa' variety of dwarf French bean obtained by EMS treatment has been further investigated. During the summer of 1963 the remaining M₂ material was sown and tested, as well as a considerable amount of M₃ material from M₂ plants unaffected in 1962. Infection of all plants of the M₂ and M₃ material was obtained. The difficulties in infecting and evaluating the resistance were found to be considerable.

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

^{xx} Stichting voor Plantenveredeling (SVP) - Foundation for Agricultural Plant Breeding - Wageningen, the Netherlands.

^{xxx} Laboratorium voor Erfelijkheidsleer - Department of Genetics, Agricultural University - Wageningen, the Netherlands.

THE USE OF ARTIFICIALLY INDUCED CHROMOSOME TRANSLOCATIONS IN THE BREEDING OF PHASEOLUS VULGARIS L.

Zeilinga*

The aim of this project is to collect some reciprocal chromosome translocations in bean which could be of value for breeding purposes. Such translocations are recognizable in different ways. Firstly by semi-sterility of the fruit and pollen, caused by a decreased viability of the gametes produced; this semi-sterility is only recognizable if the translocations are present in a heterozygous condition. Secondly, because the reciprocal translocation in the heterozygous condition leads to a special type of pairing of the chromosome in the meiosis (reduction division during pollen formation).

The mutagenic agent used for the induction of the translocation leads, as a result of physiological disturbances to a reduced fertility in most properly treated plants. Any selection of semi-sterile plants in the M_1 generation, i.e. the generation in which nearly all mutations exist in a heterozygous condition, is therefore unfruitful, except by examination of the meiosis of each treated plant. However, in the trials reported here, treated plants never produced more than an average of 15 seeds per plant. For a good analysis of the meiosis of a plant thus treated more than twice the number of young flowers are needed and consequently selection is difficult.

It seems feasible to score for semi-sterility, or better, for reduced fertility by evaluating the pollen quality and the number of seeds in the pods in the second generation - the M_2 . Such a procedure, which requires much specialized labour, can only be fruitful if the mutation agent is sufficiently effective.

The first aim of the project was to seek the most effective agents and the best ways to use them. This had to be done empirically by different treatments of seeds, growing plants and seeds from them and looking for variations in the second generation (M_2). In the following generations (M_3 and M_4) these variants must be examined for their mutational origin. In this work advantage is taken of the fact that mutations are sometimes coupled with reciprocal translocations; it is intended therefore to examine every mutation collected for such translocations.

In 1963 an M_2 generation, neutron irradiated in 1962 and including 130 progenies, has been examined for mutants. So far 7 fertile and 16 sterile mutations have been isolated. The mutants and their 'sister' plants have been harvested separately.

Much attention and time have been given to three M_3 generations comprising 10 progenies of neutron treated material, 23 progenies of X-rayed material and 188 progenies of material treated with EMS. These progenies are composed of the offsprings of all the sister plants of those with deviating habits in the M_2 generations grown in 1962. These progenies have been tested for segregation of these deviating characters. These trials are especially important for the sterile variants because they are the only means to preserve such types of mutations. The following results were obtained:

* Instituut voor de Veredeling van Tuinbouwgewassen (IVT) - Institute of Horticultural Plant Breeding - Wageningen, the Netherlands.

Zeilinga Neutrons : In 10 progenies, 6 segregating mutations were observed 5 of which were fertile.

 X-rays : In 23 progenies, 14 segregating mutations were observed 7 of which were fertile.

 Ethyl-methane-sulfonate : In 188 progenies, 102 segregating mutations were found 55 of which were fertile.

62 new variants have also been observed, 55 of these were fertile.

An attempt has also been made to multiply the fertile mutations, and, at the same time try to describe their characters and classify them. Fifty-six fertile mutations have been described, belonging to 9 groups. The majority viz. 28 have been grouped as viridis, 6 were bullata and 6 were nana types; 4 eliator types have been observed. Plants with mottled leaves have been grouped according to the precise characters and classified as costata (5), maculata (5) and necrosis (1).

In 1962 reciprocal crosses with fertile mutations and normal 'Widusa' variety have been made, which gave rise to 125 fertile plants and the same number of progenies for the next year. These plants will be used to detect the reciprocal translocations, after a survey of the meiosis in the young anthers, the pollen fertility and the seed set in the pods.

SIGNIFICANCE OF MICRO-MUTATIONS IN BARLEY AND WHEAT

Gaul*

This project has been started with the barley variety 'Amsel' which has been treated by X-rays and with the barley variety 'Haisa II' which has been treated by Co⁶⁰ γ -rays during pre- and postmeiotic stages. The material of both varieties was grown in 1963 (M₃ generation) in a special block design with 2 replications and the variability of some quantitative characters has been studied. The characters under investigation were (1) heading date, (2) culm length, (3) spike length, (4) kernel yield, (5) kernel size, expressed as 1.000-kernel weight. In these studies emphasis is put on yield characteristics. All the material could be harvested normally and records of the heading date and the kernel yield are given in tables I and II.

Another experiment was started with a 'pure line' of winter wheat namely 'Heine Stamm 2806/55 - B20/60' which has a high performance. The material was X-irradiated for 2 years and the M₂ generation was grown in 1962/63. The experiment itself was started this fall by sowing the material in a special block design in two replications. Similar records, as in the barley experiments, will be taken in 1964.

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Table I - Average kernel yield and heading date of barley, variety 'Amsel'.
M₃ derived from X-irradiated seeds.

Treat- ment	No. of M ₃ lines	Mean value	Mean square lines	error	Genotypic variance	t-test for mean square lines	F-test for mean square lines
<u>k e r n e l - y i e l d</u>							
(per row in grs)							
CI	300	57.1	111.7 ⁺	87.8	11.9	CI	CI
M	300	48.5	221.8 ⁺⁺⁺	76.5	72.6	M ⁺⁺⁺	M ⁺⁺⁺
<u>h e a d i n g - d a t e</u>							
(No. of days)							
CI	300	15.2	1.19 ⁺⁺	0.85	0.17	CI	CI
M	300	15.4	2.35 ⁺⁺⁺	0.71	0.82	M ⁺	M ⁺⁺⁺

CI = control individual.

M = derived from mutagenic treatment of seeds (X-irradiation).

Table II - Average kernel yield and heading date of barley, variety 'Haisa II'.
 M_3 derived from γ -irradiated (Co^{60}) spikes during pre- and post-meiotic stages.

Treat- ment	No. of M_3 lines	Mean value	Mean square lines	error	Genotypic variance	t-test for means of lines				F-test for mean squares of lines			
<u>k e r n e l - y i e l d</u>													
(per row in grs)													
CB	150	56.8	107.4 ⁻	99.9	-	CI	ML	MP	MZ	CI	ML	MP	MZ
CI	150	56.5	141.9 ⁺⁺⁺	87.1	27.4	CB	-	-	++	CB	+	+++	++
ML	150	57.2	154.4 ⁺⁺⁺	75.6	39.4	CI	-	-	++	CI	-	++	-
MP	150	55.0	219.0 ⁺⁺⁺	80.3	69.4	ML	-	-	+++	ML	-	+	-
MZ	150	52.2	167.8 ⁺⁺⁺	72.6	47.6	MP	-	-	-	MP	-	-	-
<u>h e a d i n g - d a t e</u>													
(No. of days)													
CB	150	16.4	0.83 ⁻	0.94	-	CI	ML	MP	MZ	CI	ML	MP	MZ
CI	150	16.5	1.06 ⁺	0.74	0.16	CB	-	-	-	CB	-	+++	+++
ML	150	16.3	1.37 ⁺⁺	0.89	0.24	CI	-	-	-	CI	-	+++	+++
MP	150	16.3	2.06 ⁺⁺⁺	0.79	0.64	ML	-	-	+	ML	-	++	++
MZ	150	16.6	2.11 ⁺⁺⁺	0.72	0.69	MP	-	-	-	MP	-	-	-

CI = individual control.

CB = bulk control.

ML = derived from irradiation of leptotene-zygotene stage.

MP = derived from irradiation of three nuclei-stage of pollen.

MZ = derived from irradiation of zygotes.

Gaul

SIGNIFICANCE OF MACRO-MUTATIONS IN BARLEY

In this project crosses of macro-mutations from the winter barley variety 'Atlas' and crosses of macro-mutations from the spring barley variety 'Haisa II' with a great number of different barley varieties were made. There are about 1,200 cross combinations and about 1,500 spikes were emasculated and pollinated. In two sets most of the F_1 material was and is grown in the greenhouse at the present time. Back crosses are made in part of the cross combinations. Growing the F_1 generation in the greenhouse will permit to sow the F_2 generation in spring 1964 in the field. The behaviour of the mutations in the different genetic backgrounds will be studied in the F_2 generation. A detailed morphological investigation of the mutants and of the varieties used in this program has been made to find out appropriate characters for the study of the variability of the pleiotropic effect.

DIPLOIDIZATION OF AUTOTETRAPLOID BARLEY

In this project additional tetraploid material has been collected from other countries, particularly USA, Canada, Sweden and Japan. During the summer a large number of crosses have been done. Most of these crosses follow the multiple crossing program among F_1 -tetraploid plants the parents of which have been irradiated year after year at least five times. Selections were made for (1) fertility, (2) tillering and (3) earliness. The F_1 material is growing in the greenhouse and intercrops of F_1 plants are done at present to speed up this program.

EXPERIMENTAL MUTAGENESIS IN TRITICUM DURUM

Scarascia^{*}

A comparative study of different mutagenic agents for the induction of mutations in durum wheat is in progress. The advantages offered by this test plants are: its relatively large spike progeny which offers in controls and treated plants a number of individuals sufficient for statistical evaluation of mutation frequencies and spectra; its tetraploid character which makes it more resistant to chromosomal damage; its good frequency of chlorophyll and morphological mutations. In addition, economical considerations are involved. Durum wheat is grown particularly in Italy and the Mediterranean area. New lines with reduced size, increased spike and spikelet fertility and higher lodging resistance would be an immediate contribution to cereal production in that part of the world.

From an analysis of the mutations induced in the chlorophyll apparatus (more than 1 million M_2 seedlings from approximately 6,000 M_1 plants analysed) it has been possible to obtain data concerning: dose-effect relationships; size of the mutated sector in the M_1 spikes; relations between mutation frequencies and sterilizing effects of treatments; and mutation spectra.

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Scarascia

The relative frequencies of the various types of chlorophyll mutations induced by chemical mutagens (ethyl-methane-sulfonate, diethylsulfate) appeared to be significantly different from those induced by radiations; within radiations (X-rays, thermal neutrons, fast neutrons) no significant difference in mutation spectra was found. A characteristic common to the three types of radiations used is that high frequencies of mutations are induced by doses which generally produce a 50 - 80% reduction in the average number of seedlings per M_1 spike. On the contrary, with chemical mutagens, the highest frequencies are observed with treatments producing a reduction from 30 to 40% in the average number of seedlings per M_1 spike.

Another considerable difference in action between the physical and chemical agents used, lies in the size of the mutated sectors in the M_1 spikes, as revealed by M_2 segregation ratios. On the whole, the segregation ratios found for the chemical mutagens are considerably lower (average 11.8%) than those found with radiations (average 15.9%).

Concerning mutations for prominent phenotypic characteristics, more than 300 independent mutants have been isolated. These mutations involve both single and several characters (complex phenotypes), i.e. simultaneous changes in several morphological and/or physiological characteristics. The genetic study of some 15 mutants has shown that such mutations have a monogenic recessive type of inheritance.

Avanzi,
Giorgi*

The work carried out under the subcontract refers only to cytological observations conducted in the various mutant lines. Up to now, the analysis at meiosis of mutant lines has proved the presence of reciprocal chromosomal translocations in 25 out of the 50 progenies analysed.

MUTATIONS RESEARCH ON PEAS

Heringa

'Pauli' variety irradiated with X-, γ -rays and neutrons and treated with EMS have been studied throughout the year. The number of progenies of M_1 plants which had mutated varied very much although each treatment was made with the same number of seeds. Exact comparisons are therefore difficult but results indicate that high doses of X-rays and strong concentrations of EMS gave a decline in fertility, in particular for the EMS. The percent mutants noted was higher at lower doses; this may be due to the fact that at higher doses a smaller number of plants survived and that mutations which could have occurred did not appear. No differences were found in the mutation spectrum following X-ray, neutrons and EMS treatments. The mutation frequency after EMS treatment was higher than after irradiation. An effort to discover micro-mutations for increase in yield was not successful. M_2 plants found abnormal were resown and these abnormalities appeared to be no mutants, other were homozygous or segregated once more. Further to the tests of 1962 seeds were irradiated with 11.000 r X to ascertain the percentage of cross pollination. No indication could be found that cross pollination with long straw and yellow seeds existed.

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Speckmann*

Studies on the mutation of peas in 1963 concerned also the sensitivity of different varieties to irradiation and EMS treatments. Two wrinkled-seeded and two smooth-seeded varieties were considered and their responses to X-irradiation and EMS treatment studied. Although final conclusions cannot yet be drawn, results available indicate that the wrinkled varieties are more sensitive to the EMS treatment than the smooth-seeded ones. This is apparent from comparisons in plant survival, growth and sterility which was found higher with the same EMS concentration treatments. Differences in sensitivity between the two pea types are not as clear in the case of X-ray treatment.

The influence of temperature and of water content of the seed during treatment with EMS was studied and results indicate that treatments at higher temperature cause more sterility in the M_1 generation and a higher mutation frequency but a smaller mutation spectrum in the M_2 , as compared to treatments at lower temperature. First results of experiments with pea seeds treated at different water content level suggest a greater sensitivity to EMS treatment for those with higher water content. The M_2 material will be studied in 1964.

Cytological studies of structural changes in chromosome pattern after EMS and X-ray treatments have been initiated.

COMPARISON OF THE MUTAGENIC EFFECT OF X-RAYS, γ -RAYS, NEUTRONS AND ETHYLENE-METHANE-SULFONATE

Wellensiek**

Studies on the comparative mutagenic effect of X-rays, γ -rays, neutrons and EMS on peas indicate that fertility decreased in the M_2 with increasing dose treatments of EMS; this decrease was very slight following γ - and X-irradiation and absent following neutron irradiation. The percent mutants increased with increasing concentrations of EMS if expressed as total number of M_2 lines. No significant difference could be found between M_2 lines from M_1 plants with few or many seeds. The average number of mutations per mutating M_1 line varied from 1.6 to 2.5 for EMS; they were exceptionally higher than 1.0 for the radiations. For EMS, γ , thermal neutrons and X respectively the average percent mutants were 126.3; 17.8; 27.8 and 15.9. The mutants were approximately 7 times more numerous in the 'chlorophyll' group than in the 'other' mutants for the EMS treatments than for the irradiations which equalled one another. EMS gave approximately five times as many 'other' mutants as the radiations from which neutrons gave a relatively higher percent. From the plant breeding point of view, the 'other' fertile mutants showed after EMS, γ , neutron and X treatments 33.3; 8.1; 13.9 and 4.7% mutants for the total material considered. The general conclusion drawn from the experiments carried out is that EMS has given the most promising results with however a high degree of sterility while neutrons were next best with the advantage of a much lower sterility.

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Wildervanck^{*}

Material collected in 1962 showed very few aberrations in the meiosis of EMS treated plants. Tetrads with as many as 9 micro nuclei have been noted.

A study to compare the sterility in M_1 plants after treatment of the dry seeds with X-rays and EMS has been carried out. Crosses were made in the greenhouse between a genetically and cytologically wellknown line 'Weitor' and 'Dominant' and 'Elfjespeul' lines to compare their karyograms in the meiosis of the M_1 flowers with regards to translocations between them. Crosses were also made between the 'Dominant' and some mutants originated from it for the same purpose. No flower with completely fertile pollen was found at concentrations higher than 0.08% EMS although good germination followed that treatment even at high concentrations; seedlings however developed poorly in the last mentioned phase. On the other hand X-ray treated seeds germinated poorly following high dosage treatments; seedlings developed normally and the percent fertile pollen was approximately the same in one plant; all treatments had plants with completely fertile pollen. Seed setting of the treated plants was poor and lower for EMS treatments than for X-ray treated seeds. Average pollen fertility was lower for EMS. Cytological investigations showed no differences between EMS and X-ray treatments. Both cases had micro-nuclei in the tetrads of the meiosis of poorly fertile plants. Micro-nuclei arise from lagging in both first and second anaphase.

INVESTIGATIONS ABOUT PLEIOTROPY AND EXPRESSIVITY OF MUTATED GENES

Gottschalk^{**}

Quantitative pigment analyses on more than 50 different mutants of Pisum were carried out. A mutant with only 12% of the normal chlorophyll content was found fertile and produced a small quantity of seeds. Some mutants did not contain any chlorophyll b but showed a high physiological efficiency. The investigations on the cytological causes of sterility or of reduced fertility were initiated. Two different genes causing the full breakdown of meiosis leading to a complete male sterility were found. In four other mutants the number of chiasmata during the pachytene was strongly reduced. Consequently many univalents occur causing a high reduction of the portion of fertile germ cells. These abnormalities are controlled by recessive genes. In a large group of other mutants reduction of fertility is due to the presence of rings of four or six chromosomes, respectively.

Two new groups of polymeric genes in Pisum could be found following the study of several groups of mutants showing nearly the same abnormalities. These pleiotropic genes show about the same broad field of activities.

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Gottschalk

A very small chromosome region could be found showing a strikingly high susceptibility towards X-rays. It contains one gene causing female-sterility besides a series of three multiple alleles influencing the size of all organs of the organism. During one trial with X-rays four mutation processes occurred within this region. By crossing these mutants with one another it could be shown that a definite effect, which morphologically seems to be the result of the pleiotropic influence of a single gene, has in fact been caused by the simultaneous mutation of two neighbouring genes. 'Pleiotropy' in this case therefore is absolute linkage. The qualification of a stem-forked mutant for plant breeding was tested. The value of this mutant is highly influenced by the penetrance conditions of the mutant gene. The term 'penetrance' illustrates the proportion of individuals within the offspring of a homozygous mutant, which show the mutant character phenotypically. In the case of the stem-forked mutants studied here a proportion of only 40-60% of all daughter-plants were forked, and these individuals showed a better yield, compared with the standard-form. As a result a general decrease in yield occurred. Consequently, the total yield of these useful mutants is dependent on the proportion of unforked plants, i.e. on the degree of penetrance of the mutant gene. In several mutants the dependance of the expressivity of the mutated genes on internal and external conditions was studied. A correlation analysis is being made using 10 different mutants. Some mutants were crossed with corresponding genotypes of other workers to clear up the genetic relations between these mutants.

MUTATION RESEARCH ON TOMATOES

Hildering

The research on the tomato variety 'Moneymaker' was continued in 1963.

Experiments reported previously on the action of Ethylene Imine (EI) have been terminated and results published (see p. 39). Attention in 1963 was focused on the effects of EMS. The M_3 families of an initial series of EMS treatments (0.7, 0.8, 0.9 and 1%) were studied and compared with the M_2 results. Good agreement was found, the 0.8% concentration being most efficient. Work in progress concerns more detailed investigation of the first EMS experiment now completed. Particular attention is given to several points: only the most efficient concentration (0.8%) is used; a large number of plants per treatment is used; the fertility of M_1 and M_2 plants is better ascertained; important positive correlation found between retarded germination and mutation frequency is further examined; chimaeric structure of the M_1 plants is being analysed; and the M_2 and M_3 investigations are being partly directed to the study of micro-mutations.

In the study of the correlation between retarded germination and mutation frequency the development of M_1 plants has been followed and the aberrant specimens, often chimaera-like, have been described. When possible, fruit from the lowest 3 clusters were harvested, each plant and cluster considered separately. Seeds of each cluster were collected and counted and since the number of fruit harvested from each cluster is known it is possible to calculate for each plant the average number of seeds per fruit per cluster and per fruit per plant as an estimate of fertility.

Hildering

The seed obtained from nearly 3000 clusters will be used for obtaining nearly 3000 M_2 families to be used for making more accurate calculation of the previously found correlation between day of germination and mutation frequency. For the study of chimaeric structure of M_1 plants, 121 plants, germinated on the 6th day after the 0.8%¹ EMS treatment, were taken and cultivated and described separately. Only two of these plants failed to produce seeds. Results indicate that the number of seed bearing fruit per cluster in all the material varied from 1 to 18 and the number of seeds per fruit from 1 to 224. During winter and spring 1963-1964 about 2800 F_2 -families derived from individual fruit were studied.

Narayanan^{*}

The morphological investigation of the normal tomato chromosomes was carried out using the variety 'Moneymaker'. A method for the examination of the somatic metaphase chromosomes of the root apex has been developed. The identification of the 12 pairs from the criteria of total length; centromeric position, length of the chromatic and achromatic parts has advanced but is not yet complete. An achromatic part has been observed in most of the long arms but not in the short ones. In this respect there is a difference from the pachytene picture in which the terminal part of both arms is distinctly achromatic (except the short arm of chromosome 2). This difference in the presence of achromatic region in the short arms between pachytene and somatic metaphase chromosomes, along with the fact that the numbering in pachytene is mainly with the help of the difference in length due to achromatic regions, makes it difficult to homologize the pachytene and metaphase chromosomes. It is proposed to make use of the trisomics to overcome this difficulty. A preliminary examination of the cytological effects of 0.4% EMS, alone and in combination with copper and zinc ions, at three different pH levels of 5, 7 and 9 was carried out. 200 to 300 metaphase cells of the root tips of each treatment were analysed. The results show that, contrary to certain published evidence, EMS alone, without copper or zinc, causes a considerable number of breaks. The reported interaction of copper and zinc ions with EMS in the production of breaks is confirmed.

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

Verkerk^{**}

A genetical segregation study of non-germinating mutants was carried out in the M_3 generation. 3 'potato-leaf' mutants were found and crossed reciprocally and with the 'potato-leaf' type, from an old collection of the variety 'Yellow Globe'. One of the mutants was sterile. All 3 mutants segregated 1 : 3. All M_1 and M_2 plants of two types of the crosses had 'potato-leaf' showing that the same gene was involved.

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Verkerk

3 'green stem' mutants were found and it appeared that two of them were caused by the same gene, different from the third and from the 'green stem' types of the old collection of 'Yellow Globe' variety.

3 fruit colour mutants under investigation show that a yellow mutant has the same recessive gene as the variety 'Yellow Globe'. A 'dirty yellow' mutant had reddish strips inside the fruit and is probably caused by the 'Apricot' gene in recessive form. An 'orange-red' mutant, also single recessive, not reported in the literature has been found. The fruit colour is orange-red on the outside and looks more or less as a double recessive for white and orange, on the inside.

Harvest of the M_1 generation of the EMS or X-ray treated dry seeds has been completed.

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

It is doubtful at present, whether a more or less directed mutagenic effect will be realised by means of the above-mentioned investigations.

In order to direct the mutagenic effect it is necessary that fundamental research concerning the interaction between radiations and biological material receive more attention. Much of this fundamental work requires considerable scientific specialization. Therefore in general a concentration on a limited number of subjects is necessary. The different phases which exist between the administration of radiation and the end result, the mutant, are difficult to study because of the very great complexity of the processes which via the primary lesions finally result in a mutation. The study of stage sensitivity appears to be a more effective approach.

To study the radiobiological and genetic aspects of irradiation and chemical mutagen treatment on different ontogenetical stages, and to overcome the limitations - in the expression of the mutation - caused by the myxochimaerical state of organisms developing from treated pluricellular organs, the phenomena induced in particular stages of the haplophyte, of the embryogenesis, of the pollen and of the growing plant are studied.

Available information on the effects of the irradiation of different ontogenetical stages is scarce in plant radiobiological literature, it is therefore of special interest to investigate:

- a. the effect of mutagenic treatments of gametes, zygotes and pro-embryos on the development of the embryo and endosperm;
- b. the radio-sensitivity of the various stages;
- c. the influence of intrinsic factors (for example: genotype, level of ploidy, biochemical substances) as well as extrinsic ones (environmental and physical: type and energy of the radiation, dose-rate and total dose, etc.).

It may be assumed that a better knowledge of the mutagenic treatment of the above-mentioned ontogenetic stages may make the use of these treatment methods possible for genetical purposes. This would be the case if the negative consequences of the chimaerical state and of the diplontic selection, which are unavoidable in common practice of seed treatment, could be reduced or

eliminated. In this way, an increase in mutation frequency and or changes in mutation spectra could be obtained, with also obvious advantages in mutation breeding programs.

EFFECTS OF RADIATIONS AND CHEMICALS ON DIFFERENT STAGES OF THE ONTOGENETIC CYCLE IN HIGHER PLANTS

Devreux,
Scarascia^M

Studies conducted along this line have been concentrated on the action of γ -rays from Co^{60} in the development of the embryo of Nicotiana rustica and Nicotiana tabacum (tetraploid species) and Nicotiana sylvestris (diploid species). The Nicotiana species have been chosen because:

- a. it is possible to irradiate, under identical conditions, a considerable number of ovules per flower and several flowers per plant;
- b. the embryogenesis is wellknown;
- c. the ease of histological observations permits also to determine the study of embryo development after the treatment itself.

Investigations have been carried out on the effects of γ -irradiation of different intensities on gametes, on three stages of the resting phase of the zygote (1) and on proembryos, observing the different radio-sensitivity of such stages in relation to dose-rate and total dose, as well as the anomalies of embryo development, the effects on seed germinability and morphological characteristics of the seedling in M_1 .

The morphological and cytogenetic characteristics as well as the fertility of the M_1 plants are being studied at present. Moreover, a first attempt to assess the potential genetic effects of the zygote irradiation has been carried out with a line of Pisum sativum, bearing various markers for characters detectable in the seedling stage, in the mature plant and in the seed.

Donini^M

With the same purposes in mind the male and female gametophytes of barley have been irradiated, and in M_1 the following effects have been studied: germination, survival at the seedling and mature plant stage and fertility of the ear. It has been found that the depressing effect of irradiation is greater when the male gamete is irradiated.

Finally, preliminary attempts on in vitro culture of ovules have had positive results. This technique should permit the treatment of ovules also under special experimental conditions as well as their exposure to neutron fluxes.

Contant,
Ecochard^{MH}

Similar studies with different ionizing radiations on the different stages of development of tomato have been started in 1963.

It has been decided to study the radio-sensitivity during gametogenesis on the following plants and for the following reasons:

Tradescantia paludosa, swedish clones having determinate genes of the S series, to be cross-fertile;

Vicia faba minor (pure strain) blooming within one month and having a chromosome recognizable at meiosis;

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^{MH} Association EURATOM - ITAL, Wageningen, the Netherlands.

Contant,
Ecohard

Lycopersicum esculentum, more light-requiring, but having both self-fertility and vegetative propagation, and an interesting floral biology, for instance unilateral incompatibility in crosses with other species;

Lilium pumilum, also autogamous and clone-propagated, very early and seed-fertile. Later, probably L. longiflorum, which is easier to grow in a greenhouse. The flower buds of lilies are very suitable for cytologic and radiologic research.

Meanwhile, the best microtechniques for meiotic observation of these different plants have been investigated; with the broad-bean, for instance, very fine preparations can be obtained after fixation in Carnoy, then in alcohol 70°, maceration, staining in Feulgen and squashing in acetic fast-green.

Plants of Vicia species at given stages of microsporogenesis were X-rayed at different doses. First results indicate a very great sensitivity of the buds, with two peaks. A number of characteristic behaviours are recorded in the generative cells, some developmental abnormalities varying with the dose. This experiment will be continued for several months, to investigate the influence of a number of parameters and elucidate with the best accuracy the relationship irradiated stage with response.

Sauer*

In this connection work with tissue culture or cell suspension in which all cells are in a certain phase is important. The number of plant varieties of which tissue culture and cell suspension can be made and the possibility of regenerating normal plants from them has been increased considerably during the last few years. This provides another opportunity to irradiate cells which are in a specific stage, increasing the chances to obtain genetically homogeneous mutants.

The species now in culture are Daucus carota, Parthenocissus tricuspidata, P. quinquefolia, Helianthus tuberosis and Chrysanthemum indicum. Experiments are carried out with these tissues to study the histogenetic effect of a number of chemicals, a necessary step to be able to grow mature plants from tissues. A good control of the induction of roots and in particular of buds is then possible. Some difficulties have been met keeping mono-cell-cultures free of micro-organism infection.

RADIO-RESISTANCE OF PLANT SPECIES

Avanzi,
Cervigni,**
Scarascia

Various parameters have been chosen, as indications of radiation effects, namely: morphological, cytological (chromosomal aberrations at meiosis, pollen fertility), physiological (fresh and dry weight, duration of the vegetative cycle, time of flowering) and biochemical (peroxidase, catalase, polyphenoloxidase activity, glutathione and ascorbic acid content).

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Avanzi,
Cervigni,
Scarascia

Other data collected include fruit set, number of seeds per fruit and seed germinability. Taking the genus Nicotiana, as an example of the several plants investigated, the effects of chronic γ -irradiation have been examined on 23 species (2n, 4n and aneuploids). On the basis of the parameters used, the species investigated could be divided into two groups, one comprising the more radio-sensitive species and the other the less radio-sensitive ones. It appears that the less radio-sensitive species have under natural conditions higher peroxidase activity values. This is in agreement with the suggestion that peroxidase - at least in the genus Nicotiana - may have a protective role against peroxides eventually produced by radiation. Results from an experiment on Vicia sativa, irradiated for the whole of the vegetative cycle at different dose-rates (from 6 to 42 r/h), indicate that the pattern of peroxidase activity is typically physiological; irradiation causes an anticipation - of a different degree according to the dose-rate - of the peak of maximum activity of the enzyme, corresponding, in the control material, to the period of full flowering. This seems to indicate that the plants react to the energy absorbed by regulating the level of peroxidase activity in such a way as to achieve rapidly the mature stage levels. Likewise, of interest are also the patterns of ascorbic acid content (decrease) and of dehydro-ascorbic acid content (increase); the abnormal increase of the oxidized form (dehydro-ascorbic acid) constitutes one aspect of a phenomenon generally observed in the irradiation of plants: the decrease in growth rate.

Remarkable growth reactions (increase of the average number of culms per plant and increase of the dry weight of the mature plants) have been noticed in various cultivated varieties of hard and bread wheat subjected to chronic irradiation. Interesting information on the effects of different intensities of chronic γ -irradiation has emerged from an experiment, which is under way in the γ -field since 1960 on different species of Conifers. If different dose-rates are studied, radiation effects such as damage to the apical meristems, inhibition of the apical dominance, new growth after semi-chronic irradiation and necrosis of tissues, occur after accumulation of quite distinct total doses.

Finally, in the framework of the action of radiations on biologically important chemical substances, studies on the radio-sensitivity of beta-indolacetic acid (IAA) can also be included. It has been found that IAA is a very radiation sensitive molecule (at a concentration of 5×10^{-6} M, in vitro, 30% of its biological activity is destroyed by 100 r only) and that radiation effect on IAA varies with concentration of the solution. These observations seem to support the view that auxin level may be an important factor in radiation sensitivity.

EMBRYO-ENDOSPERM RELATIONS IN IRRADIATED SEEDS

Melleti,
Floris*

The development of this project considered a series of treatments with radiations on the Triticum durum variety 'Cappelli'.

The following embryo transplantations (12.000 seeds) were made:

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Meletti,
Floris

X-rays : doses: 2, 4, 6, 12 and 24 Kr on presoaked seeds; unirradiated embryos on irradiated endosperms - EM(u)/EN(i) - and, vice-versa, irradiated embryos on unirradiated endosperms - EM(i)/EN(u);
Fast neutrons : doses: 300, 600, 900, 1200, 2400 and 3600 rads unirradiated embryos on irradiated endosperms and, vice-versa, irradiated embryos on unirradiated endosperms.

Pot sown transplants were transferred in the field to observe the development of the plants as well as their survival, earing, flowering and ripening. This will allow an analysis of some possible physiological effects induced by radiation on the embryo transplantation.

Single plants were harvested and the morphology and fertility of spikes analysed. The material was then stored under laboratory conditions for its utilization for the genetical study: screening of chlorophyll mutation in R_2 to see whether, in the graft EM(u)/EN(i), irradiated endosperms induce mutations on unirradiated embryos and, whether unirradiated endosperms are able to modify the frequency and spectrum of mutation in the graft EM(i)/EN(u).

The series of embryo transplantations on seeds treated with X-rays (2, 4, 6, 12 and 24 Kr) has been repeated to have new material available for further observations, at physiological and genetical level, during 1964.

A new series of embryo transplantations on dry ripe seeds irradiated with increasing radiation (X-rays) doses up to the lethal ones has been cultured: the doses were 15, 20, 25 and 30 Kr for the graft EM(i)/EN(u), 60 and 120 Kr for EM(u)/EN(i).

An investigation begun in December 1961 on the growth of durum wheat seedlings obtained from embryo transplantation EM(u)/EN(i) (X-rays: 4, 6 and 8 Kr) at different stages of ripening and after-ripening of seeds has been completed. During dormancy no inhibitor is present in the 'Cappelli' endosperm; when 'Cappelli' seeds have attained complete after-ripeness an inhibitory condition occurs in the endosperm. Since this inhibitor is destroyed - or neutralized - by radiation, EM(u)/EN(i) seedlings grow better than EM(u)/EN(u) ones. This point may be important for in general it seems probable that the increased growth of seedlings observed in several occasions after seed irradiation may result from the destruction of an inhibitor present in the seed.

As to the cytological work, an analysis was performed on the mitotic index in the radicle of seedlings obtained from unirradiated embryos transplanted on irradiated endosperms. For this work the technique described by MELETTI and D'AMATO in 1961 was used: namely, irradiation of dry seeds with 500 and 1000 Kr of γ -rays and transplantation of the unirradiated embryo on the irradiated endosperm (EM(u)/EN(i)). These transplants were analysed in comparison with the homotransplants (EM(u)/EN(u)). It was found that the irradiated endosperm decreased the mitotic index in the radicle meristems of the unirradiated embryo; the effect was greater with 1000 Kr than with 500 Kr. These results indicate that the growth depression induced by endosperms irradiated with high doses of sparsely ionizing radiations, according to MELETTI and D'AMATO, is in some way related to a reduction in mitotic frequency.

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 O F
R A D I A T I O N

The physiology of living organisms and food products can be influenced in such a way that among other things:

1. Ripening (senescence) is retarded, as a result of which the formation of substrates necessary for the growth and increase of micro-organisms causing deterioration is postponed or even prevented (for instance in fruit);
2. Cell elongation can no longer take place or only with difficulty, thereby inhibiting, for example, the sprouting of tuberous plants, root crops and bulbous plants;
3. Reproductive organs in harmful insects for instance are no longer able to produce living cells.

Furthermore some facts must be considered to judge the real value of this method or at least to make a reasonable prognosis as to its usefulness.

1. Over-influencing the physiology of the product can cause unacceptable deviations. This is particularly the case if radiation doses over 500.000 rads are applied.
2. The γ - and electron-radiations considered for food preservation are not selective in their effect. Micro-organisms are generally no more sensitive to radiation than the product to be preserved, or even much less sensitive. Therefore, the possibility of selection on the basis of sensitivity is practically non-existent. Radiation of that part of the product which is the seat of the infection or which contributes considerably to the desired physiological deviations has considerable attraction (surface irradiation or irradiation of certain layers in the product). This localized irradiation finally increases the efficiency because irradiation of the entire product, if not necessary, means dissipation of energy and induces unnecessary chemical deviations.
3. The method will be expensive, at least for the coming years, so that preferably high quality products should be considered.

The above-mentioned considerations led therefore to drawing a research program which was restricted in 1963 to the irradiation of soft fruit with electrons (energy 0.5 - 1.0 MeV).

PHYSIOLOGICAL INFLUENCES OF IRRADIATION ON SOFT FRUIT AND VEGETABLES

The influence of radiation on the physiology of soft fruit has been investigated for a number of years. The most outstanding metabolic process related to ripening and senescence of different kinds of fruit is the climacterium. The respiratory activity of these products falls to a very low level during the so-called pre-climacterium; it subsequently rises quickly at the beginning of the ripening processes and falls again afterwards at the beginning of senescence.

Van Kooy^{*}

Preliminary experiments on the influence of radiation on the physiology of tomato fruit showed a retardation of the entire ripening process. Further experiments revealed that: With pre-climacteric tomatoes an increased CO₂ production was always observed on the day following the irradiation treatment. The CO₂ production rate of post climacteric tomatoes apparently did not change by irradiation as evidenced by measurement on the day after the treatment. The question arose whether the metabolic activity of mature tomatoes does not change at all, or only during a short period of time, less than 12 hours after the irradiation treatment. To be able to measure the CO₂ exchange of fruit at short intervals of time after irradiation the Claypool-Keefer method was used. This method of measurement has been thoroughly studied and technical problems solved. Environmental conditions during transport are considered to be an important factor. Handling of the fruit seems to have much less effect on the respiration rate. A rise in temperature influences the CO₂ production rate in the same manner as irradiation does. It was found that the effect of a temporary increased temperature of 2°C was still noticeable six hours later.

It can be concluded that the study of immediate effects of ionizing irradiation on physiological responses of fruit is only significant under precise conditions of environment. More adequate methods for the determinations of slight changes in CO₂ and O₂ content of air passed over the fruit have been investigated. Gas chromatography is a suitable method for CO₂ and ethylene, but not sensitive enough for O₂. The 'Lloyd' gas analysis apparatus was modified in such a way that the volume of gas to be analysed could be increased from 10 ml to 50 ml, which improved the accuracy of this method. It can only be used for CO₂ and O₂.

Teijema^{*}

The softening which may occur in irradiated fruit limits the usefulness of irradiation as a means of preservation. A better understanding of the causes of the change in texture might eventually lead to the prevention of this unfavourable side-effect of irradiation. Relatively little is known about the biochemical background of the softening processes in ripening fruit and even much less of the softening processes which may occur in irradiated fruit.

Tomatoes were chosen as test material to study this radiation effects. In 1963 much attention was paid to cell wall analysis. Due to the very high activity of pectin methylesterase in tomatoes (and presumably in strawberries), bruising or cutting of the fruit at room temperature would result in a quick change in cell wall composition. Therefore, the intact fruit is frozen in liquid nitrogen and kept below melting point (ca -3°C) throughout the procedure of cell wall preparation.

^{*} Association EURATOM - ITAL, Wageningen, the Netherlands.

Teijema

After peeling, dissecting and grinding of the fruit material in the frozen state, the juice is pressed out in a Carver laboratory press at a low temperature (ca -5°C , pressure up to 1680 kg/cm^2). The cold press residue is quickly added to boiling ethanol, which inactivates the enzymes. Subsequent washings with hot ethanol remove a large part of the contaminating substances (sugars, organic acids, pigments, inorganic ions, etc.).

Further Ca-determination in micro-amounts (2 - 20 μg) and the determination of the (poly-) galacturonic acid content of dissolved or extracted pectin in micro-amounts (4 - 40 μg) and the (poly-) uronic acid content and methoxyl content in unextracted cell wall preparations in an amount of 50 - 200 mg (viz. about 10 - 50 mg pectin) were carried out.

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

Staden*

Besides these physiological and biochemical studies much attention has been paid to a technical screening program on surface pasteurization of fruit and vegetables with 1 - 3 MeV electrons. The doses applied varied from 50 - 300 Krads, and results recorded were:

Hard fruit:

Apples:

Tests with a great number of apple varieties showed that 'scald' can be almost completely eliminated; 'spot' may be distinctly increased; 'breakdown' of 'Cox Orange Pippin' can be decreased; no effect on 'bitterpit' could be observed. Other influences: the normal colour change could be promoted (at lower dosages) or retained (at higher levels). Taste was influenced by the dose level applied. The higher dosage may cause deterioration of both taste and texture. It is concluded that the best method of radiation of hard fruit is to roll the products, giving dosages of about 100 Krad, which are nearest to optimal conditions.

Pears:

The most suitable dose level may be found at about 100 Krad probably also for pears. Here, however, the fruit retains longer its green colour, especially at the higher dosages.

Soft fruit:

For soft fruit, the most promising results were obtained with strawberries, blackberries and raspberries. Better results were found with slightly unripe berries. With the other fruit belonging to this group no advantages were found (cherries, gooseberries, plums and grapes). Peaches were very sensitive to radiation.

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Staden

Vegetables:

A great number of vegetables are more or less susceptible to radiations. Damage was noted for instance on cucumbers and melons following 100 and 50 Krad irradiation respectively. Lettuce and cauliflowers on the other hand showed no damage at doses of up to 200 Krad.

Extension of the shelf-life was possible for tomatoes. The ripening process is slowed down as indicated by a slower decrease of the firmness and turning process from green to red.

Brussels sprouts treated with 200 Krad showed some brown discolourations at cutting area and an increased transpiration. The most spectacular results have been obtained with mushrooms; four advantages were found; a prolongation of the shelf-life, a longer closure of the cap, the best score for taste of the cooked product and no discolouration. It appears however that this favourable effect is obtained by applications of 3 MeV.

Depending on storage temperature a consistent postponing of deterioration can be realized.

Cut vegetables:

A start has been made to examine whether preservation of the quality is possible also with cut vegetables. The major troubles met with this kind of products are: drying-up, quick deterioration of taste, discolouration, mold attack, flavour changes.

Studies have been made with carrots, celery, swede, leek, red cabbages and onion. In many cases also the taste after cooking has been compared to the control. Before radiation, part of the material underwent special pre-treatments in order to control e.g. discolourations. Results available indicate that the following vegetables offer possibilities for radiation treatments: carrots (with pre-treatment); leeks (only fresh product); onion (only fresh product).

No advantage was found with irradiation of: celery (lower dose levels may give better results); swede (hardness increased); red cabbage (discolourations and loss of flavour).

GENETICAL HAZARDS OF IRRADIATED FOOD

Moutschen*

The study of the genetic effects of irradiated food was started using γ -irradiated glucose. It was intended as a model for future testing of effects on other substances. The first part of the research carried out, aimed at eliminating the possible interferences of external factors such as temperature, pH, etc. Afterwards, the radiomimetic effects of irradiated glucose was tested in the following plant material: Allium cepa (roots); Hordeum sativum (roots); Vicia faba (seeds and roots).

Treatment of the root meristem of 3 vegetal species tested with irradiated glucose (48 - \pm 70 Mrads) showed that distinct radiomimetic effects existed, although their genetic consequences could not be clearly seen.

* Université de Liège - Centre Interfacultaire des Sciences Nucléaires - Laboratoire de Génétique - Liège, Belgium.

Moutschen

Hordeum sativum was found to be the most interesting testing material because of its stability, its being highly selective and its great sensitivity. Irradiated glucose fed to mice produced nucleotoxic effects during spermatogenesis. The irradiated dosages were 5 Mrad γ -rays. It can be said of the experiments relating to the lethal dominants that no significant difference between the numbers of induced deciduomata was shown between animals fed with irradiated diet and those fed with non-irradiated diet. The experimental conditions should however, be varied, and in particular the females should be fed with irradiated diet at the same time as the males. The latter experiment was carried out in recent months.

When induced at frequencies higher than those of the control animals, the early dominant lethality would mean that under these experimental conditions the irradiated materials are capable of producing non-viable chromosomal aberrations. The spermatogenesis was studied in animals fed on an irradiated diet during this period. This study proved that chromosomal aberrations exist during meiosis, especially during the I anaphase. These lesions are of the radiomimetic type and consist of bridges and fragments. The latter are often very large and may consist of entire chromosomes. Hitherto it has not been positively shown that this effect resulted in lethal dominants. It would be necessary to extend the range of the experiments to show that a significant increase in the early dominant lethality is directly related to the production of chromosomal aberrations during the preceding generation.

No evidence of the induction of sex linked lethal recessive mutants was found in *Drosophila*.

THE BEHAVIOUR OF SPECIFIC NUCLIDES IN THE SOIL, CROPS AND ANIMALS

The introduction of radio-activity into the biosphere as a result of the testing of nuclear weapons and the possibility of disasters in industrial and experimental power plants as well as the disposal of radio-active waste has become a reality. There are situations in which foodstuffs may become contaminated to an impermissible extent and it becomes necessary to examine what steps can be taken for the prevention or reduction of contamination of food and its entire or partial decontamination.

In this connection it can first of all be stated that neither the amount nor the geographical distribution of the contaminants can be influenced. Hence a situation exists in which, in practice it is impossible to prevent direct contamination of crops and soils. The possibility of reducing the contamination level of food must be sought in both prevention of accumulation of dangerous nuclides in soils, important crops intended for human consumption or animal feeding stuffs and animal products, and in the elimination of the contamination in important food.

Comprehensive surveys, carefully planned and carried out have already allowed to gather a general picture of the distribution and accumulation of certain nuclides in the atmosphere, and on the earth's flora and fauna. While their value is obvious, they must be followed and completed by more exact knowledge obtained under precise conditions to allow a safe extrapolation when circumstances require it.

This aspect of what we call preventive research, will allow to introduce cultural and other practices capable of reducing or preventing an accumulation of dangerous nuclides in crops intended for human and animal consumption and in animal products intended for consumption. During periods of fall-out and in the case of crops intended for immediate consumption following isolation of the source of contamination, normal or special treatment of fresh food e.g. washing and boiling will wholly or partly remove the contaminant. Preservation (through evaporation, pasteurization, etc.) can also reduce or entirely remove the radio-activity.

In the case of crops consumed directly, foliar contamination is during periods of fall-out quantitatively the most important form.

The indirect contamination of these crops via the soil becomes quantitatively important when dangerous nuclides are able to accumulate in the soil in an absorbable form. In principle the soil / soil-solution link and therefore soil-solution / plant link can be influenced by appropriate cultural practices.

For effective prevention it is necessary to obtain an insight and knowledge of the penetration and subsequent distribution and redistribution of certain nuclides in the main food crops. If at the same time information can be obtained on the effect of climatic conditions on these phenomena, a forecast of the degree of accumulation in these crops or parts thereof is within the bounds of possibility.

Levi^{*}

A project concerning caesium penetration and retention by bean leaves and its subsequent distribution in the plant gave the following results: 3 hours after treatment approximately 80% of the isotope applied (1.0 μc in 0.01 ml) was washed out from primary leaves with 5 ml of water: Approximately 70% of all activity held 3 hours after application was retained by the treated leaf (primary, second or fourth trifoliate) up to maturity of the fruit.

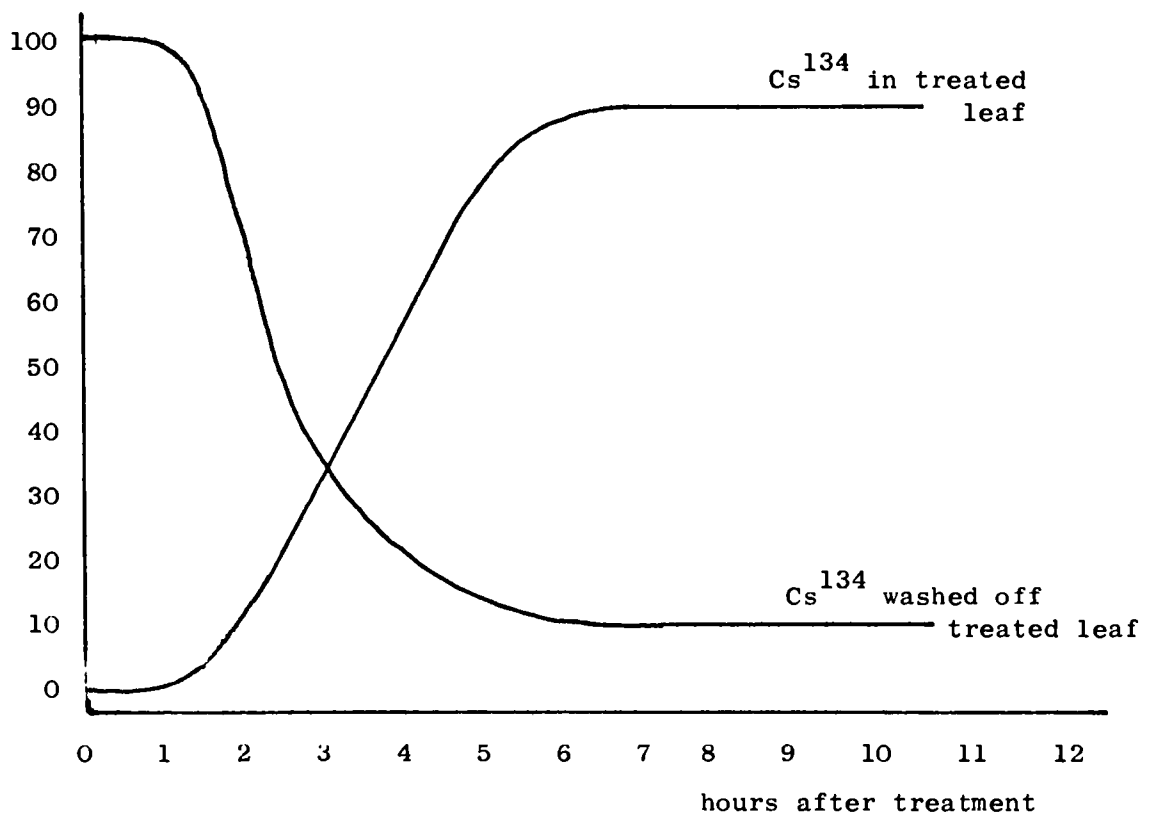
Cs-concentration in tissues was found related to age and size of leaf considered, immaterial of that treated. Accumulation of Cs¹³⁴ in the fruit was variable, but also related to age of plant at time of treatment and of the fruit at time of harvest. Single treatments of 1.0 μc per plant or double treatment of 2.0 μc per plant or triple treatment of 3.0 μc per plant showed somewhat proportional accumulations of Cs when 100 grams fresh weight of fruit was considered. A repeat experiment essentially confirmed the above findings. Statistical analysis is still pending.

^{*} Association EURATOM - ITAL, Wageningen, the Netherlands.

Levi

A number of leaf absorption tests were carried out to determine amounts of Cs^{134} adsorbed on the treated leaf of bean plants, and those distributed in the rest of the plant over a 1 - 72 hours period following treatment. Although treatment conditions were identical shifts in the curves were noted with time. The S shape remained for all experiments up to 12 hours following treatment. The slope of the curve was variable. From 24 - 72 hours the curves flattened. The washing solutions followed reciprocal S curves and indicated that practically no Cs was held by the leaf before 1 hour after treatment. The amount washed in subsequent periods varied but in general was not found to increase after six hours. From 6 - 12 hours following treatments an increased accumulation in the treated leaf and a decrease in the rest of the plant was noted. A test using Cs^{134} applied to primary leaves of beans throughout their developing period indicated that absorption was fastest at the younger stage but was about equal after 4 - 6 hours.

Percent
of con-
trol



Typical curves obtained in above-mentioned experiments showing trends in adsorption of Cs^{134} by primary leaves of beans following foliar application under controlled conditions.

Levi

Results from autoradiograms of leaf uptake experiments have indicated a constant leaf-root distribution of P^{32} , Rb^{86} and Cs^{134} . Further tests in which the root system or part of it only was fed radioactive isotope have shown repeatedly when only one set of lateral roots was treated, accumulation in leaves of beans grown under controlled conditions, in N/6 fractions of the leaves.

As regards the soil / soil-solution system, it has been established that strontium and calcium on the one hand and potassium and caesium on the other act more or less in the same way. Chlorine is a less suitable analogy for iodine. Soil science and physical chemistry together provide sufficient data to enable the behaviour of Ca, K and Cl to be predicted. If maximum and minimum estimates are then made for the differences between Ca, K and Cl on the one hand and Sr, Cs and I on the other, the behaviour of these nuclides can also be roughly delimited.

Frissel*

A theoretical study of the movement of strontium through soils was carried out. The following parameters were included in the calculation: concentration of the active element, adsorption capacity of the soil, total salt concentration, relationship between monovalent and bivalent ions, salts added (in the form of fertiliser), ion-exchange constants, soil moisture content and rainfall.

An attempt was made to find semi-empirical parameters to correlate theoretical assumptions with data obtained on four experimental farms in the Netherlands. The possibility of using the developed theories for soils outside the Netherlands is considered.

Poelstra*

Parallel to this theoretical work experimental research on the movement of long-lived fission products in different types of soils by means of soil columns was started in order to determine experimentally the above-mentioned limiting values.

For the investigation of the distribution patterns of specific nuclides in soil columns and the factors which influence these patterns, an experimental set up was designed which allows:

- a. Very low flow rates, which can be maintained on the same level during several months;
- b. A quantitative survey of the distribution pattern in the column, for 1 cm layers without disturbing the pattern itself.

With regard to the soil solution / plant system it is obvious that the necessary investigation will have to be so planned as to enable the various factors involved in the absorption of certain nuclides to be properly evaluated. Although a good deal of general information is available on the soil / plant relationship, the correlations are still very unclear between the state of nutrition of the soil and the rainfall, and the accumulation of fall-out products in the various agricultural crops. Field observations of the Sr^{90} content in soil and grass have therefore been elaborated and summarized.

* Association EURATOM - ITAL, Wageningen, the Netherlands.

Ringoet^x

Little is known about the effect of the environment, type of plant and physiological status of the plant on the uptake of certain ions from the soil. It is therefore necessary to study root ion uptake under strictly controlled conditions. Most environmental factors such as light, temperature, etc. cannot be controlled in the field but the mineral and water reserves in the soil form an exception. Therefore the project related to the uptake of Ca and Sr by plants or parts of plants under various conditions of moisture tension was continued.

The effect of solutions of increasing osmotic pressure on Calcium- and Strontium-uptake^{xx} by oat plants has been measured. No direct relation exists between the uptake of both nuclides and the water uptake as measured by transpiration. Passive uptake by mass-flow in the transpiration stream, is eliminated as a possible uptake-mechanism. An active uptake-mechanism, depending on metabolism, has to be accepted somewhere in the root-system. Preferential uptake by young and metabolic active parts of the plant confirms this relation between metabolism and uptake of both elements.

Unexpectedly, almost all calcium and an important part of the strontium taken up by different plant parts, were found still water-soluble and directly exchangeable after several days. Relatively more strontium was fixed in organic complexes or cell tissues (probably walls).

Van den Hoek^x

A study of the influence of extra Ca on Sr⁹⁰-excretion in milk of cows was carried out within the framework of Sr-elimination. It appeared that an amount of 500 kg Ca per ha, given to grass, caused a decrease of the Sr⁹⁰ content in milk of cows of about 33%. Furthermore the experiments revealed that this decrease was already apparent 8 - 9 hours after the intake by the cow of the first amounts of grass. This cannot be explained by the resorption of Ca and Sr occurring only in the walls of the intestines. There must be definitely also a resorption in the paunch and or in the net-stomach. Furthermore, these results indicate that equilibrium between milk and diet is reached long before the 5 days mentioned by Comar et al (Health Physics, Vol. 8 (1962) No. 2).

Deijs,
Wieringa^{xxx}

To study the possibilities of decontaminating food while it is being treated or processed, a joint project with the Institute for Biological and Chemical Research on Field Crops and Herbage (IBS) has been initiated. Preliminary experiments were set up to see whether any selectivity in the distribution of cations in pressed juice of grass could be obtained upon addition of different chemical substances.

^x Association EURATOM - ITAL, Wageningen, the Netherlands.

^{xx} Uptake means here the total amount of Ca⁴⁵ and Sr⁸⁵ measured in the different parts of the plant as a result of accumulation and translocation during the experimental period.

^{xxx} Instituut voor Biologisch en Scheikundig Onderzoek van Landbouwgewassen (IBS) - Institute for Biological and Chemical Research on Field Crops and Herbage - Wageningen, the Netherlands.

Deijs,
Wieringa

Quantities of minerals extracted from samples were very small. Differences in K, Na, Ca and Mg content were not large enough to allow conclusions to be drawn as to whether Ca and Sr could be extracted selectively. Addition of hydrochloric acid, sodium chloride or AIVirtanen acid gave somewhat higher extraction contents of minerals than controls.

IMPROVEMENT OF RADIO-ISOTOPE METHODOLOGY

The increasing interest in radio-isotopes as tools in agricultural research necessitates a constant awareness of instrumental development and of search for and improvement of methods in which these radio-isotopes can be utilised in an optimum way.

The GM-counter is still due to its low investment cost one of the most important tools for assaying isotopes in biological research. The applications of this counting technique are however limited because of unsatisfactory reproducibility due mostly to difficulties met in sample preparations.

In an effort to improve this situation a joint program was set up in collaboration with Prof. Dr. G.H. BOLT^{*}.

Various factors were considered as to their effects on results. They were: shape of planchette, method of drying, carrier concentrations, use of additives and method of counting. Results indicated that one of the most important factors was the shape of the planchette: a slightly concave one allowing no accumulation on the rim and a fairly uniform drying pattern was found best. The position of the sample appears also to be very important. Better results were obtained when three countings were made with turns of 120° each time than when the sample was counted three times in the same position. This could probably be ascribed both to irregularities in the window and in drying pattern.

Results obtained indicate that a single determination for γ - and hard β -emitters gave statistical accuracy of 0.8%, provided: background variations could be neglected, the fraction due to random desintegration process is kept below 0.4%, the amount of substrate is kept below the region in which self absorption becomes apparent (i.e. 0.2 mg/cm² for S³⁵; 0.5 mg/cm² for Ca⁴⁵) and the supporting salt solution contains mainly the non radio-active isotope as carrier. By multiple countings, the statistical accuracy can be brought to 0.4%. If not all conditions are favourable, accuracy levels will reach 1 or 2% very rapidly. It is therefore better to use liquid scintillation counting for isotopes of energy equal or lower to that of Ca⁴⁵ (0.25 MeV).

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Frissel

The problems with the liquid scintillation method concern also mainly sample preparation: one of the greatest difficulties is that water is usually insoluble or very poorly soluble in scintillation mixtures, whereas the isotopes to be measured are either dissolved in water or only soluble in water. A number of mixture tests were carried out in order to try to introduce more salt and water into the measuring system. Comparing the normal system: toluene + alcohol + water, with the best system found: dioxane + naphthalene + cello-solve + water, it appeared that in the latter system 7 times more water and 12 times more CaCl_2 can be absorbed than in the normal one. Furthermore this dioxane system allows work at -5°C , a temperature at which the toluene system demixes. Under optimum conditions (low salt and water content, low background) for a single determination an accuracy of 0.5 - 0.6% was obtained.

Possibilities of simultaneous counting of Ca and Sr are being investigated. It appears already that simultaneous counting of Sr^{85} and Ca^{45} by liquid scintillation is impossible, but that a combination of this technique and γ -spectrometry seems to be promising.

Sauer

An effort to improve the micro-autoradiography technique by depositing the radiation-sensitive emulsion into the cell thus providing a better geometry has failed because of the toxicity of the Ag^+ to the plant cells.

Rechenmann*

Preliminary experiments which aim at quantitative interpretation of micro-autoradiograms by track counting in liquid emulsions have been carried out. No definite conclusions can yet be drawn.

A methodical study of the possibilities offered by solid state detectors for their potential value in biological research has been carried out. It was prompted by the following properties which make their use very attractive for plant physiological studies:

1. Small dimension, which may eventually allow insertion into tissues for in vivo measurements;
2. The very low γ -efficiency of surface barriers allows for high efficiency counting when using $\beta+\gamma$ -emitters;
3. The proportionality between the energy of the incident particles and the diode response allows for simultaneous detection of two β -emitters of different energies.

Compared with GM-counters it appeared that for C^{14} the solid state detector gave a counting rate which was respectively 120% and 70% higher than Philips 18504 (window 2.5 mg/cm^2) and Anton 228 (1.4 mg/cm^2). These values were for Ca^{45} respectively 80% and 30%. For P^{32} and Tl^{204} the three detectors gave identical results. All these measurements were carried out at temperatures between 20°C and 25°C without cooling of the diode. Furthermore, in translocation experiments with Cs^{137} and Na^{22} it was clearly shown that the possible disturbing effect of γ -radiation was negligible in the case of the semi-conductor.

* Association EURATOM - ITAL, Wageningen, the Netherlands.

De Swart^{*}

An automatic micro-freeze dryer has been developed to dry completely thin plant material in 1 - 2 hours time.

An anti-coincidence counter for low level β -measurements has been successfully developed (Background approximately 0.8 cpm; counter diameter: 28 mm). A number of these instruments have been assembled during the year.

The development of a β - γ coincidence counter for neutron flux measurement through irradiation of gold foils has been initiated. This instrument is particularly required for accurate dose measurements in the BARN.

The development of a multi-scaling unit for measurements of half lives of isotopes, and measurements of long duration (e.g. isotope movement in soils) has been studied and construction has been started.

O T H E R A C T I V I T I E S

Oosterheert^{*}

On April 9 at 22.42 hours first criticality was achieved of the BARN (Biological Agricultural Reactor Netherlands), with 3121 grams U^{235} and the design core with an excess-reactivity of 1.5% was built-up and tested. Power calibration followed, resulting in an average thermal flux of 8.1×10^{11} n/cm²sec with core at a power level of 100 KW. After installation of an extra Bi-shield and polyethylene reflector in the irradiation room the slow neutron flux was measured to be 6×10^6 n/cm²sec. With a contamination of fast neutrons and γ -rays totalling 15% of the thermal neutron dose.

After a successful continuous run of 3 days the reactor was turned over to ITAL by RCN on November 13. From that date on the operating crew has been trained in practical operation of the reactor and its secondary systems.

In December a preliminary test run was performed to measure the fast neutron flux in the irradiation room with different D_2O -level.

Hekman^{*}

The activities of the health-physics group covered mainly the drafting and implementation of regulations of the radiation protection service and of the reactor. The supervision of the working areas, ventilation system of the radiochemical laboratories, film-badge service and the disposal of solid waste have been carried out on a routine-basis. The installation of a 300 C Cs^{137} source in the greenhouse and the preparation of an instruction book have also been completed.

^{*} Association EURATOM - ITAL, Wageningen, the Netherlands.

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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:

International Conference on Radiation Research, Natick, Mass. U.S.A. (D. de ZEEUW).

Symposium on Food Preservation, Wageningen, the Netherlands (J.G. van KOOY).

Planning Meeting on the Use of Isotopes and Radiation in Studies on Plant Nutrient Supply and Movement in Soil Systems, Vienna, Austria (M.J. FRISSEL).

Ist International Barley Genetics Symposium, Wageningen, the Netherlands (C. BROERTJES and R.M. ECOCHARD).

Symposium on Wheat Genetics, Sweden (C. FLORIS and P. MELETTI).

Journées d'Etudes sur l'Utilisation des Détecteurs à Jonction en Physique Nucléaire, Liège, Belgium (R.V. RECHENMANN).

XI International Congress of Genetics, The Hague, the Netherlands (S. AVANZI, A. BOZZINI, C. BROERTJES, M. DEVREUX, R.M. ECOCHARD, C. FLORIS, P. MELETTI, L.M. MONTI, K.R. NARAYANAN, G. SAUER, G.T. SCARASCIA, K. VERKERK and D. de ZEEUW).

VII Session of O.E.C.D. Study Group on Food Irradiation, Paris, France (D. de ZEEUW).

In 1963, the following meetings were held at the Association's Institute:

Wageningen Mutation Breeding Contact Group on March 5 - 6. 22 participants from France, West-Germany and the Netherlands attended.

International Food Irradiation Study Group, on June 19th with 13 participants from West-Germany, the Netherlands and the United Kingdom.

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

Association's scientists have paid study visits to the following centres during 1963:

Phytotron of Centre National de la Recherche Scientifique, Gif-sur-Yvette, France (C. BROERTJES and E.C. LEVI).

Institute of Genetics at Lund, Institute of Radiobiology at Stockholm and the Swedish Seed Association at Svalöf in Sweden (R.M. ECOCHARD, C. FLORIS and P. MELETTI).

Louvain University, Belgium (R.M. ECOCHARD).

EURATOM Biological Section, Ispra, Italy (A. RINGOET).

A P P E N D I X I I

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DIRECTOR: D. de ZEEUW

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E U R A T O M - I T A L:

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R.B. CONTANT
R.M. ECOCHARD
M.J. FRISSEL
H. HEKMAN
J. van den HOEK
J.G. van KOOY
E.C. LEVI
W.F. OOSTERHEERT
P. POELSTRA
R.V. RECHENMANN
A. RINGOET
G. SAUER
J.F. STOUTJESDIJK
J.G. de SWART
H.L. TEIJEMA (Miss)

ADMINISTRATIVE STAFF : H. DIRKSE (Technical)
P.H. van NIEROP (Finance and Personnel)
H.J. SONIES (Library and Documentation)

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A S S O C I A T E D W I T H E U R A T O M - I T A L B Y
S U B C O N T R A C T:

BELGIUM : Université de Liège - Centre Interfacultaire des
Sciences Nucléaires - Laboratoire de Génétique -
Liège: J. MOUTSCHEN, M. MOUTSCHEN-DAHMEN.

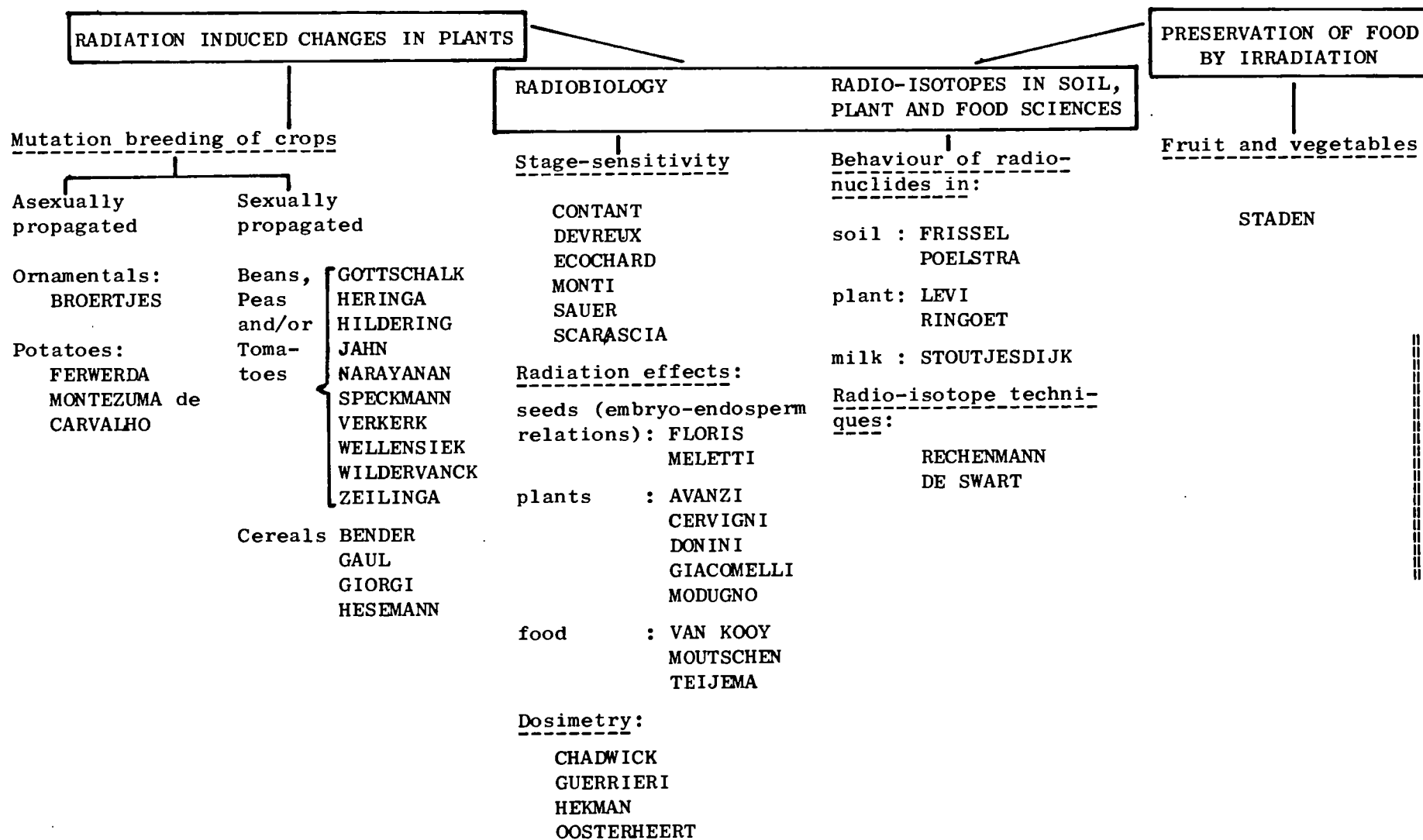
ITALY : Comitato Nazionale per l'Energia Nucleare - Centro
di Studi Nucleari della Casaccia - Laboratorio per
le Applicazioni in Agricoltura - Rome: S. AVANZI,
A. BOZZINI, T. CERVIGNI, M. DEVREUX, B. DONINI,
M. GIACOMELLI, G. GUERRIERI, G. MODUGNO, L.M. MONTI,
G.T. SCARASCIA.

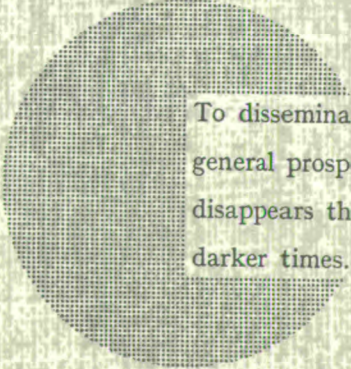
Istituto Botanico della Università - Cagliari
(Sardinia): C. FLORIS, P. MELETTI.

A P P E N D I X I I I (Cont.)
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- NETHERLANDS : Instituut voor Bewaring en Verwerking van Tuinbouw-
producten (IBVT) - Institute for Research on Storage
and Processing of Horticultural Produce - Wageningen;
J.J. DOESBURG, 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.
- Stichting voor Plantenveredeling (SVP) - Foundation for
Agricultural Plant Breeding - Wageningen: R.J. HERINGA,
G.J. SPECKMANN.
- Laboratorium voor Erfelijkheidsleer - 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).
- WEST-GERMANY: Institut für landwirtschaftliche Botanik der Universi-
tät Bonn - Bonn: W. GOTTSCHALK, A. JAHN.
- Max-Planck-Institut für Züchtungsforschung - Köln:
K. BENDER, H. GAUL, C.U. HESEMANN.

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 (1 9 6 3)





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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