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

APPLICATION OF ATOMIC ENERGY IN AGRICULTURE

(Annual Report 1966)

Association No. 003-61-4 BIAN

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SUMMARY

The research activities of the EURATOM-ITAL Association were directed in 1966 to the application of atomic energy to agriculture.

They were carried out at the Association's Institute in the Netherlands as well as in other Institutes in the Community.

In addition to classical plant breeding experiments, studies were undertaken to investigate the improvement of mutagenic efficiency and the analysis of irradiation effects, as well as to assess the possibilities offered by irradiation for food preservation. Related physical problems were also investigated. The behaviour of specific nuclides in plants and soils as well as the development and improvement of nuclear techniques for agricultural research have also formed part of the activities of the Association.

KEYWORDS

AGRICULTURE

PLANTS

RADIATION EFFECTS

PRESERVATION

FOOD

IRRADIATION

ENVIRONMENT

SOILS

RADIOISOTOPES

USES

BIOCHEMISTRY

BETA DETECTION

DOSEMETERS

Crops

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

The research activities of the Association EURATOM - ITAL have in 1966, as in previous years, dealt with problems concerned with the application of atomic energy in agriculture in an effort to improve crop production and food storage.
Investigations were carried out at the Association's Institute in the Netherlands as well as in a number of laboratories and institutes within the European Community. Although geographically distributed in Belgium, France, Germany, Italy and the Netherlands, scientists involved in the research programme have either carried out joint projects or pursued complementary individual research keeping in close touch with their colleagues interested in similar investigations.
Obviously, research topics and methods used have been chosen in relation to the scope of the Association and the facilities available. At its Institute, these include a neutron irradiation facility consisting of an environmental controlled plant growth room under the core of a 100 KW reactor, the 'BARN' (Biological and Agricultural Reactor Netherlands); two γ-irradiation facilities, a 300 Ci Cs^{137} greenhouse and a 5000 Ci Cs^{137} indoor γ-field placed at the center of 4 separate rooms totalling 140 m^2 controlled environment area for plant growth, as well as a 250 KeV X-ray machine and a 2 MeV 'Van de Graaff' electron generator. Twelve controlled environment rooms for plant growth and 4 controlled temperature rooms are also available.
This report of research activities in the past year has been subdivided for easy reference into the main aspects of investigation i.e. mutation breeding per se; investigations designed to improve the mutagenic efficiency of the treatments used; analysis of irradiation effects; preservation of food by means of irradiations, and, physical studies related to the above investigations. The influence of environmental factors on crop production has been considered from the soil and plant points of view. Finally improvements in nuclear techniques which could contribute to the accuracy or speed of determination of these studies with radioisotopes have been considered.
In 1966, meetings arranged at the Association's Institute have been attended by scientists from Belgium, France, Germany, Italy and the Netherlands involved in the Association's programme on mutation breeding and related genetical studies.
A meeting on plant-soil relations has dealt mainly with problems involved in foliar uptake and gathered, besides scientists from the Association and their colleagues from the

EURATOM Biology Division at Ispra, Italy, others from France, Germany, the Netherlands, the United Kingdom and the United States of America.

Members of the staff of the Association or its subcontracting partners have participated to a number of national or international meetings and working groups.

A list of subcontracts, of meetings attended and study visits made as well as the names and addresses of scientists are given as appendices to the present report.

The presentation and lay-out aims at allowing an easy reference of activities and recent results obtained by scientists involved in the research programme of the Association EURATOM - ITAL. Although grouped on subject and material used results of various scientists have been kept purposely separate, unless the project was carried out jointly, to allow direct exchanges with readers. When more than one name is mentioned in the margin the order does not infer any seniority and contacts could be made to any of them.

It is hoped that such reporting in a somewhat more detailed fashion than usually done will foster exchanges of ideas and results between scientific readers.

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

RADIATION EFFECTS ON PLANTS

The use of radiations to improve agricultural and horticultural plants as well as storage possibilities of raw or processed food have again been the aims of investigations carried out by the Association EURATOM - ITAL in this facet of its research activity.

Mutation breeding per se has considered desirable characters such as yield increase, disease resistance and earliness in agricultural crops while colour and flower shapes have been selected as positive criteria in ornamentals. The removal of cross barriers as well as the promotion of heterosis events between different species has also formed part of this aspect of increasing breeding potential.

The study of factors which may increase or alter irradiation effects thereby offering larger possibilities for mutation breeding form the second facet of studies in this chapter. A comparison of different mutagenic agents with the pertaining investigations of dose and dose rate relationship, analysis of plant stage sensitivity, assessment of physical, chemical and biological variables which may interact upon the genetic and physiological responses of irradiated plants are reported upon. Further investigations aimed at reducing the importance of the chimera problem as for example by irradiation of single cells; at decreasing the detrimental effect of irradiation upon fertility factors in M_1 and M_2 plants and modifying mutation spectra and frequencies.

A third facet has been the analysis of the irradiation effects, an approach to mutation breeding allowing to observe from different angles and at different levels physiological, histological, cytological or genetical factors in the mutation event per se.

The preservation of food by means of radiations has dealt mostly as far as investigations carried out at the Association's Institute are concerned to the applied aspects of such research to answer the question whether the process is or not applicable to a restricted number of food items.

Physical studies required for the proper development of investigations on radiation effects have also been carried out and are reported upon within this chapter. Special attention has been paid to a further investigation on the relation between radiation energy deposited in the biological specimen and its response.

MUTATION BREEDING

IRRADIATION AS A TOOL FOR INCREASING VARIABILITY AND PRODUCING DESIRABLE MUTANTS

Studies with both sexually and asexually propagated plants have been continued in 1966.
Unfavourable climatic conditions have caused some damage to field trials, yet, data available indicate some encouraging and positive results.

ASEXUALLY PROPAGATED PLANTS

Fruit trees

BODERGAT* DOMMERGUES*

Numerous morphological and leaf colour mutants were isolated and a plantation of 1950 trees was set up. A comparative experiment with X-rays and neutrons on pear and apple buds was not conclusive and will be repeated. Only the 4 kR treatment of the variety 'Reine des Reinettes' gave interesting material which will be screened for spur types in 1967.

Apples

VISSER**

The possibilities of pre-selection after one growing season are being investigated through the performance of shoots formed after irradiation of the grafts. No correlation could be found between growth measured in the first year (1965) and second year (1966) after treatment. Growth reduction in 1965 appear to have been due to a direct damaging effect of radiation rather than to mutation. At the end of the second growing season a number of possible 'spur' and 'dwarf' types were selected. The first are defined as having shoots with shorter and thicker internodes than normal while 'dwarfs' have shorter and thinner shoots than normal. Grafts made of these selections will indicate in subsequent growth whether the deviations are the result of mutation or are due to environmental causes.

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Pears

VISSER

Shoots of 'Doyenne du Comice' and 'Beurré Hardy' were irradiated with X-ray doses of up to 4 krad and then grafted. Differences between treatments were negligible indicating that even the highest dose was too low. New material will be treated with 5, 6 and 7 krad and grafted on seedling stocks.

Cherries

Two hundred grafts of F 12/1 on 'Limburgse Boskriek' were irradiated with X-rays. Survival after the first growing season was 74, 62, 53 and 19% respectively for the control, 2, 3 and 4 krad. Observations will be continued in the second season. The same doses were applied to 1500 scions of the sweet cherry variety 'Schneiders Späte Knorpelkirsche' which will be grafted on 'Limburgse Boskriek'.

Ornamentals

Dahlias

BROERTJES*

Tubers of a number of cultivars of the garden dahlia (Dahlia variabilis) were irradiated with various dosages of X-rays in an effort to obtain mutations in flower colour and shape. The optimal dose range was found to be 2 - 3 krad, as far as production of rooted cuttings, speed of rooting, development of young plants as well as mutation frequency are concerned.
A large number of flower colour and shape mutations were observed in the varieties 'Salmon Rays', 'Arthur Godfrey' and 'Eldorado'. Four mutants of the first named variety have already been commercialized.

Roses

BODERGAT
DOMMERGUES
PAYNOT**

After it had been established that mutagenesis in roses was promising, experiments were started to improve the methods of isolating mutations by making successive series of trimmings followed by individualizing each mutation by grafting.
A total of 203 mutations were found among 966 graftings of different varieties. White and yellow varieties were not found to mutate.

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BODERGAT
DOMMERGUES
PAYNOT

In 38 irradiated buds of 'Christian Dior' treated with 5 kR X, 35 were found to carry mutations at first observation. On second observations 17 appeared to come from the principal buds and 24 from stipular buds with 4 belonging to both categories.
A biochemical analysis of flower pigments of the different mutant types was carried out. The mutations involved either a dilution or an intensification of one or several of the pigments. The pigments were diglucosides of pelargonidol, of cyanidol, of peonidol and monoglucoside of cyanidol.

Carnations

LE COUVIOUR*
DOMMERGUES
GILLOT*
PAYNOT

It was possible by vegetative propagation to fix each phenotype of a chimeric M_1 plant of Chabaud carnation. It was found that the purple flowered part contained only cyanidol in its flowers while those of the control part had only pelargonidol. A genetical analysis of the mutation is hampered by the cross fertilizing nature of this species. The biochemically most interesting M_1 plants were propagated to obtain large number of cuttings of the normal and mutated parts. Six M_1 plants which yielded genetically pure clones have been irradiated. Results are not yet available. Rooting of cuttings subjected to 4 and 10 kR X was 81 and 88% respectively of the controls. The effects of radiation on growth of the shoot apex are evident.

SPARNAAY**

Several mutations of flower colour and flower shape were found in F_1 and F_2 hybrids of 'Sim' and garden carnations, after irradiation with doses ranging from 3.5 - 8 krad. Most of these were unfavourable but some mutants represented improvements in petal shape and flower size. Several hybrid carnation clones, together representing a full range of colours, were treated with 5 and 10 krad of X-rays to study which flower colours mutate most readily. So far, pink was found to mutate to red and yellow, whereas purple mutated to white. These trials show that the hybrid clones are able to support the same X-ray doses as do the commercial 'Sim' varieties. As the latter are much better known with regard to their variation in production pattern and quality factors, further tests aimed at improving productivity will be carried out with a number of 'Sim' varieties.

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Tulips

VAN EIJK*

Bulbs of the tulip varieties 'London', 'Lustige Witwe' and 'Preludium', irradiated with 250, 350 and 450 rad of X-rays immediately after lifting in 1965, will be screened for major mutations in 1967 when the new bulbs developed in 1966 will flower. Some minor effects could already be observed, such as blue stripes or sectors, or yellow edges in the white- and red variety 'Lustige Witwe', yellow stripes and one entirely yellow flower in the red-flowered variety 'London', and white or orange stripes in the pink variety 'Preludium'. The highest aberration frequency was observed at 350 rad or at 450 rad. Other varieties were subsequently subjected to doses of 300 - 500 rad to find valuable colour mutations and to establish which colours mutate most readily, and in which direction.

Potatoes

Screening of tuber-lines (sub-clones) for resistance to diseases and pests and for cooking quality

FERWERDA**

Studies carried out under this heading were continued in 1966. Large numbers of sub-clones derived from the 'Bintje' variety were submitted to a heavy infection by the following pathogens: wart disease (Synchytrium endobioticum); Phytophthora; leafroll and Y-virus and root eelworm (Heterodera rostochiensis). In all cases small numbers of clones (0.4 to 2%) remained free of disease symptoms. It should be remarked that some unaffected individuals were encountered also among the untreated (non-irradiated) sub-clones.
The Phytophthora infection experiments provided some encouraging indications. All the members of 5 sets of two closely related sub-clones derived from the same irradiated mother tuber, therefore largely identical genetically, and even one group of four related sub-clones remained free of disease symptoms. This similarity in reaction pattern may be indicative of resistance but repeated tests will be necessary to confirm this impression.

Thirty-eight 'Bintje' sub-clones classified as 'abnormal' in the standardized cooking test of 1965 were retested in 1966. Eleven of these showed again the abnormal cooking properties. These results suggest that this kind of physiological properties may also be permanently altered by mutagenic treatments.

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SEXUALLY PROPAGATED PLANTS

Cereals

Barley

DOMMERGUES TOUVIN*

Results have shown that a wild barley highly resistant to oïdium could not be used as a parent because resistance was linked with a particular roughness of the seed coat. To disrupt this unfavourable linkage, F_1 plants obtained by crossing this wild barley variety with a cultivated one were treated with 3 mutagens: EMS (0.25%, 24 hours at 25°C), γ-rays (20 kR) and fast neutrons (100 kW, NF 1 x 10^8 / cm^2sec for 20, 30 and 40 minutes). The M_3 of the first two treatments is tested for resistance to oïdium and thinness of the grain. The M_1 of the neutron treatment has been harvested.

GAUL**

In the variability experiment with 'Amsel' barley the 30% highest yielding and the 10% lowest yielding M_6 families were selected according to their ranking over two years, and grown in 1966. Records were taken of: number of segregating chlorophyll mutants, heading dates, number of (partially) sterile spikes and kernel yield. Results of these studies show a reduction of the average kernel-yield of the XN and XR families and an increased genotypic variance as compared to the control. However, the relative mean kernel-yield of the families derived from mutagenic treatment increased from generation to generation; for instance, in the experiment with 'Amsel' barley the relative yield of the XN families increased from 85.0% in M_3 to 90.7% in the M_5. This can be explained by the effect of natural selection within the families, which evidently takes a direction desired by the breeder. However, there is also a very pronounced response to artificial selection in both directions. This response to selection is in accordance with the high correlations between yields in successive generations found in these experiments (r ranging from 0.28 to 0.40). In evaluating these relatively high correlation coefficients one should consider that small plots were used. For heading date, the correlation coefficients ranged from 0.52 to 0.83.

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Diploidization of autotetraploid barley

GAUL

Eight hundred seventy six progenies were grown in 1966 in the pedigree breeding programme. From those more than 2000 plants were harvested and their fertility is being studied. Selection for fertility in the tetraploid material derived from crosses of mutagen-treated varieties proved to be successful in both directions. Fertility in 1966 was generally a few percent lower than in 1965. Heading date in the tetraploid material showed a remarkable variability, the extremes differing by 30 days. The heading date of the earliest tetraploid lines corresponds with that of the common diploid barleys grown commonly in Germany. One of the high-tillering lines (F_6) was tested in a drill-trial along with the hybrid bulk from which it was selected and with the original cross parents. In addition, two other tetraploid barleys used in the cross breeding programme were included in the drill-trial. One F_6 selection line proved superior in tillering and kernel-yield to both parent varieties, the hybrid bulk and two other tetraploid varieties. These results confirm earlier data showing a response to selection for tillering.
A comparison was made of the fertility of diploid and tetraploid varieties. In the diploid varieties the fertility varied between 54 and 98%, in the tetraploid barleys between 21 and 67%. On the basis of 26 barley varieties it could be shown that the fertility of autotetraploid barleys is dependent on that of the corresponding diploid ($r = 0.73$).

Wheat

DOMMERGUES
TOUVIN

Observations of treatments (EMS: 0.3% and γ-rays: 15 kR) have been pursued to isolate mutants of Triticum vulgare for agronomic qualities. Two selection methods have been compared on progenies of material treated. The first screens the M_2 to isolate mutants for tiller height, precocity or disease resistance. The second screens the M_3 without selection in M_2. One mutant was found resistant to Puccinia tritici. Some mutants found in M_2 are multiplied for micro-trials in 1967 and concern tiller height, precocity, number of spikes per linear meter and 1000 grain weights. Crossing of mutants will be used to combine desirable characteristics. Three of the most promising mutants were included in yield trials. The genetics of some M_2 mutants of crosses between different mutation types is being studied in the F_2. The F_3 will aim at elucidating in more details the heredity of yield characters and the possibility to associate lateness, tillering, spike fertility and resistance to

DOMMERGUES
TOUVIN

oïdium. Using the M_3 selection, 125 lines were kept. After a comparative study of characters concerned with productivity 36 families were planted in M_4 and will be evaluated in 1967.
Mutations of 'Cappelle Desprez' and 'Starke', having a springtype behaviour, are being studied in collaboration with the Wheat Laboratory of the 'Institut National de la Recherche Agronomique' in Versailles, France. The mutated character was located on chromosome 5A in 'Cappelle' and on 2D for 'Starke'. From physiological studies the 'Starke' mutant appeared to be a typical springtype whereas that of 'Cappelle' is a dual purpose type. Both mutations are related to a reduced cold resistance. One mutant from 'Cappelle' had two mutations, shortness and springtype. Since the M_1 have not been artificially self-pollinated but only isolated from other wheat varieties it is possible that this combination arose from an illegitimate cross between 2 different mutants.

An experiment designed to ascertain whether screening of mutants can be facilitated when plants are grown under abnormal environmental conditions was carried out in collaboration with the Wheat Laboratory in Montpellier, France. A Durum wheat line was treated with 0.3% EMS for 24 hours at 23°C. About 1700 self-pollinated M_1 plants have been harvested and sown in F_2. Screening will consider morphological characters particularly the alternative behaviour or 'dual purpose'.

GAUL

In the variability experiment with the winter wheat 'Heine Stamm 2806' all families were transferred without selection from the M_6^2 generation into M_7^2. In the selection experiment derived from the M_4^2 of the same material the second selection step was performed on the basis of ranking over two years. The 28% highest yielding and 28% lowest M_6^2 families were sown in a replicated trial (see also page 9).

Legumes

Peas

GOTTSCHALK*

In 1966, 3 mutants of direct interest for practical breeding were studied:

* Institut für Genetik der Universität Bonn, Bonn, Germany.

GOTTSCHALK

Mutant No. 1201 A is characterized by a forkening of the stem resulting in formation of two corresponding stems in the upper part of the shoot. The action of this gene consists in an alteration of the monopodial branching type of the species Pisum sativum to a dichotomic system. This alteration leads to an increase not only in the number of flowers but also in the number of pods per plant resulting in an increased yield. The yield capacity of this mutant type was analysed in 1966. The reduction of the degree of penetrance of the mutant gene could be observed again and there is no doubt that this behaviour must be regarded to be a specific peculiarity of this gene influencing negatively the yield capacity of this useful mutant strain. Nevertheless, considering yield analyses of 6 subsequent generations, seed production is comparable or slightly better than that of the original line due exclusively to an increase of the number of pods per plant.

Mutant No. 68 C is characterized by a considerable increase in the number of ovules per carpel resulting in an increase in the number of seeds per pod. The number of pods per plant is unfortunately somewhat reduced, but this trait could possibly be improved by cross breeding.

Mutant No. 489 C is a fasciated mutant type which seems to become a really useful one. In contrast to most other fasciated lines it has an excellent seed production considering two subsequent generations, the M_4 and M_5. The total yield of this strain reached mean values of 132% (1965) and even 191% (1966) of the corresponding values of the control line. This high yield is due to a large increase in the number of pods per plant as a consequence of the stem fasciation. Disadvantages are a somewhat reduced number of seed per pod, slightly longer internodes than in the initial line 'Dippes gelbe Viktoria' and a few days delay in flowering. An attempt will be made to further improve the capacity of this strain.

The three mutant lines have been crossed in all combinations to combine their main positive characteristics. Many F_1 and some F_2 plants will be grown in 1967. In the search for new mutations, an M_2 was grown from neutron treated material and 120 different mutant types were found. The M_3 will be grown in 1967.

Ornamentals

Petunias

CORNU*
DOMMERGUES

An attempt has been made to replace for genetical studies the highly heterozygous carnation by Petunia. Initial experiments were devoted to purifying the experimental stock, to carry out genetical and chromatographical analyses of the flower pigments and to test the effects of mutagenic treatments. Self-pollinating varieties were classified into 5 groups related to flower pigmentation, and subjected to controlled inbreeding. Crossings of white x coloured and of coloured x coloured varieties were made to determine the dominance relations of the different colour types. Pigment analysis has shown a rather complex system of complementary dominant factors. The F_1's have been backcrossed to both parents to determine more accurately the number and characteristics of the various dominant genes.
Treatment of dry seeds with 0.3 - 0.7% EMS for 24 hours, at 25°C was carried out on the variety 'Satin Rouge'. The optimal concentration appears to be 0.4%; above this dose sterility was too high. Flower mutations were obtained, at optimal treatment, in 4.6% of the plants. They concern pigment distribution (30%), colour types (30%), floral morphology (10%) and various physiological or pathological malformations. Many chimeric plants with chlorophyll aberrant sectors were noted.
Preliminary γ-irradiations of non-rooted cuttings of 5 varieties with doses of 2 - 12 kR (6 hours) indicate that the optimal dose compatible with satisfactory rooting was between 2 and 4 kR.

Other crops

Brussels sprouts

NIEUWHOF**

Certain mutations found in 1964 in the progeny of irradiated 'Roodnerf' may contribute to studies on Brussels sprouts hybrids. A glossy leaved mutant was selfed for two generations. It is hoped that sufficient material will be available in 1967 to use this mutated line as a parent in which the 'glossy leaves' character is used as marker gene to detect illegitimate selfing in hybridization work. A mutant characterized by purple discolouration of the stem is maintained and multiplied with difficulty due to sterility when selfed. Both these characters are monogenic recessive. Some M_3 lines were very heterogeneous, and it is not yet known whether this is due to mutation or to other causes.

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Tomatoes

Evaluation of Easy Peeling mutants

BERKHOLST*
CONTANT**
ROMBOUTS***
VERKERK****

Following investigations carried out under an Association subcontract a number of tomato mutants of variety 'Money Maker' were screened for their practical potential. Among these an easy peeling mutant line (ep) appeared very promising. It was further studied in 1966 with regard to its yield performance, pericarp anatomy, cellulase and pectinase activity at different depths of the fruit wall. No anatomical differences in fruit wall were found between the mutant and its mother variety. Pectinase and cellulase activity in the skin were much higher in the mutant than in 'Money Maker'. The F_1 and F_2 resulting from a cross of this mutant with an 'oblate fruit' (obl) mutant of 'Money Maker' showed that 'easy peeling' and 'oblate fruit' were monogenic recessive. A breeding programme has been started with selected lines in the Netherlands and in Italy. Genetic and biochemical studies will be continued.

Influence of rooting and spacing

CONTANT
VERKERK

Testing of tomato lines and mutants under different rooting or spacing conditions was continued. These studies were initiated to select varieties suitable by their growth characteristics for large scale experiments in growth controlled rooms. Their results may also be of value for greenhouse cultivation under commercial conditions. Results available show that a restriction of rooting volume reduced intervarietal differences in plant vigour and increased the ratio fruit weight: weight of vegetative parts. In 12 cm pots, all varieties produced two clusters with well-developed fruit containing from 75 to 133 seeds. 'Chanasyk Early' which proved to be a potential breeding stock for commercial cultivation at close spacing (20 x 20 cm) in the glasshouse in winter has been crossed with 'Money Maker'. Line 587-21-1958 proved to be very useful as a parent of multirecessive hybrids in spite of its very bad fruit and cluster shape and unfavourable growth habit.

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*** Afdeling Levensmiddelentechnologie, Landbouwhogeschool, Wageningen, the Netherlands.

**** Laboratorium voor Tuinbouwplantenteelt der Landbouwhogeschool, Wageningen, the Netherlands.

CONTANT
VERKERK

It is now used in the 'specific loci' radiobiological programme of the Association initiated at the 'Comitato Nazionale per l'Energia Nucleare' (see also page 27). MM 706-9, although of poor practical value, proved to be the most attractive alternatives to the mother variety for studies involving large plant populations in pots kept under controlled environment conditions or in greenhouse where close spacing is also a necessity.

Selection and evaluation of mutants

A new approach for the selection and breeding of tomatoes was attempted involving the alternating selection for major mutations and mutations of quantitative traits. M_2 tomato seedlings from material treated with fast neutrons, electrons and X-rays were screened for visible mutations which approximated normal vigour at seedling stage, and flowered and fruited satisfactorily. The M_3 progenies have been tested and 23 mutants were selected from the varieties 'Glorie' and 'Money Maker' three of these exceeded control fruit yield during the first month of harvesting. These data reflect an improved earliness of 5 - 6 days over the control varieties. The best mutants had also positive features of vigour, growth habit, and colour. Selection in successive generations for earliness and fruit yield under winter and summer conditions is in progress.

OTHER ASPECTS OF IRRADIATION AS A TOOL FOR INCREASING VARIABILITY IN HIGHER PLANTS

Removal of self-incompatibility and breaking down of cross barriers between plant species

Tomatoes

ECOCHARD[*]
HOGENBOOM[**]
DE NETTANCOURT[*]

At the end of 1965 a systematic study was initiated to determine the possible use of irradiation for inducing self-compatibility in L. peruvianum and for removing the cross barrier between L. peruvianum (0_+) and L. esculentum ($0\rightarrow$). This project is based upon the hypothesis that irradiation may mutate the incompatibility gene(s) or induce physiological changes in pollen tube-style relationships.

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Effect of irradiation upon self-incompatibility in a clonal population of L. peruvianum (acute irradiation of pollen, styles and flower buds and chronic irradiation of growing plants)

ECOCHARD
HOGENBOOM
DE NETTANCOURT

No significant increase in seed set was observed following acute irradiation (1 and 2 kR) of styles (250 flowers treated).
In the case of chronic irradiation, the highest dose rate tested (17.30 rad / hr) inhibited flowering and fruiting processes whereas intermediate exposure rates (4.70 and 7.50 rad / hr) significantly increased the number of fruit per cluster and the number of fruit per plant. A striking increase in the number of seeded fruit per fructifèrous cluster was observed at 7.50 rad / hr (figure 1, page 17). Consequently, and in spite of the fact that the actual number of viable seeds per fruit was not higher in the irradiated series, the total number of viable seeds per plant was greater than in the control individuals (figure 1). Germination tests proved that most seeds obtained were viable. Experiments are in progress to find out if the observed stimulation resulted from gene mutations in the gametes or from a physiological effect on pollen tube growth and fertilization processes. In the meanwhile, it is interesting to recall that chronic irradiation at similar dose rates is known to be very prejudicial to the seed setting capacity of self-compatible species of Lycopersicum. It can therefore be stated that self-compatible and self-incompatible species in this genus respond very differently to chronic exposure of ionizing rays, the detrimental effect of irradiation upon fertility being more than compensated in L. peruvianum by a radiation induced increase in self-compatibility. Whereas irradiation of styles and growing plants were restricted to a single clone (60080 - 6), in the case of pollen and flower bud irradiation 5 clones of 40 - 50 plants each were involved. Self-pollinations of untreated plants with irradiated pollen (3000 and 5000 R X, acute) as well as self-pollinations with pollen obtained from flowers treated at the bud stage (200 - 400 R X) have been carried out. To prevent flower drop, clusters of the plants were sprayed every 7 to 10 days with 25 ppm naphthaleneacetic acid solution. Preliminary results are summarized in the following table (page 18):

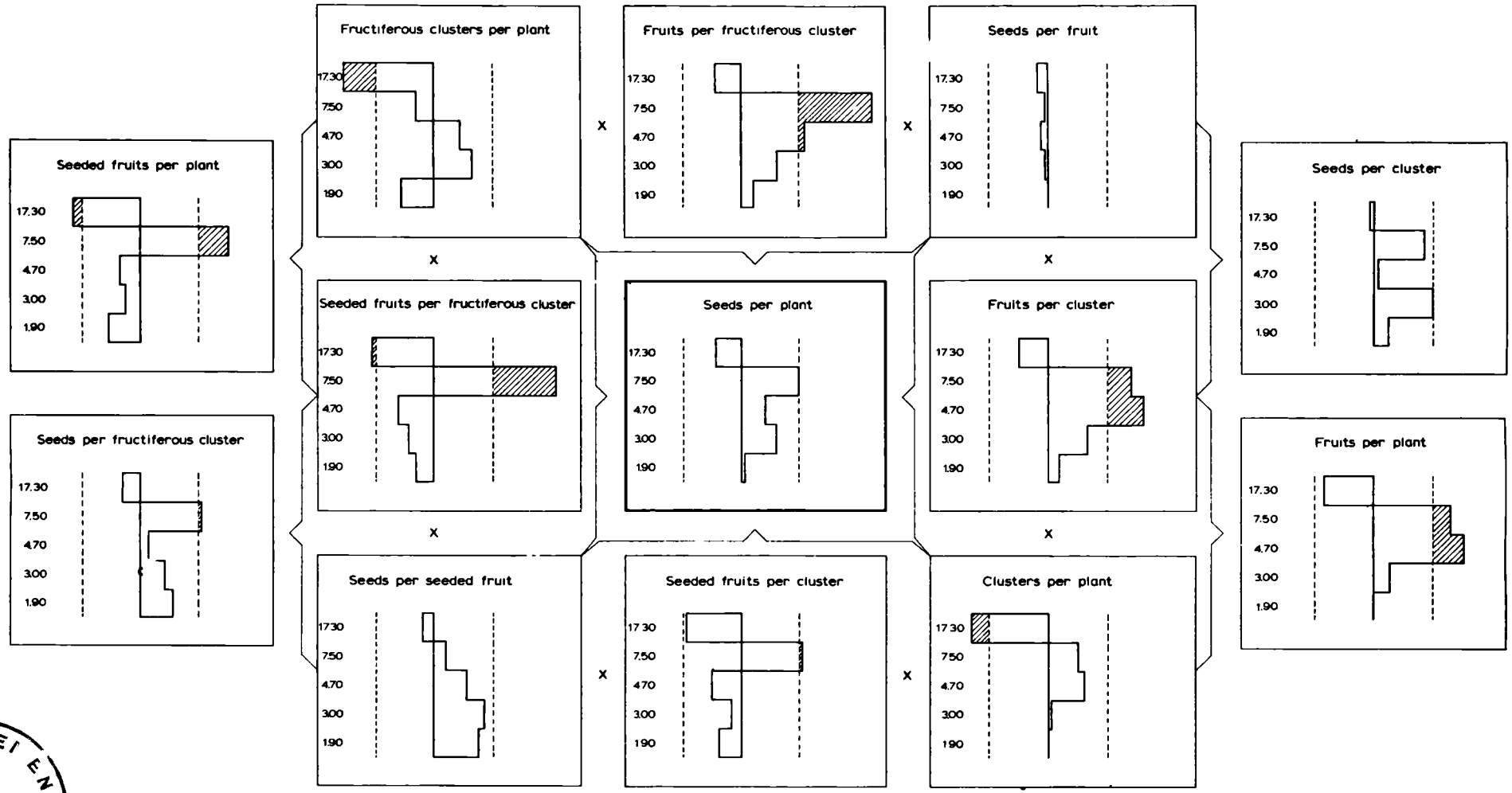

Figure 1 Deviations from control means and 95% confidence limits (dotted lines) for the 13 criteria examined in the clonal population of L Peruvianum after chronic exposure to 17.30, 7.50, 4.70, 3.00 and 1.90 rad/hour

For each criterium, negative deviations (depression) are on the left of the axis whereas positive differences (stimulation) appear on the right. The X signs and the accolades indicate the contribution of each factor to seed-set per plant.

HOGENBOOM

Respective numbers of flowers pollinated (f) and seeds obtained (s) which will be tested for self- and cross-compatibility behaviour:

Treatment	Clone number 65503-4 f	s	65503-9 f	s	65503-11 f	s	60080-3 f	s	60080-8 f	s
Control	528	209	202	-	660	-	585	7	520	1
200 R / buds	1519	361	472	1	828	2	978	1	1335	201
400 R / buds	1542	385	408	2	732	2	1247	2	1296	61
3000 R / ripe pollen	490	15	636	3	406	8	535	1	718	-
5000 R / ripe pollen	500	13	647	171	398	2	808	1	828	-

Effects of irradiation upon cross-barriers between L. peruvianum and L. esculentum

ECOCHARD
HOGENBOOM
DE NETTANCOURT

The chronically irradiated plants of L. peruvianum were also used in crosses with L. esculentum. No hybrid progeny was obtained from 972 pollinated flowers.
In the case of acute irradiation of the male partner (L. esculentum) at various stages of microsporogenesis a large number of seeds have been obtained which have not yet been tested for their hybrid constitution. A comparison of the results recorded after self-pollination (L. peruvianum x L. peruvianum) and cross-pollination (L. peruvianum x L. esculentum) suggests that foreign pollen actually stimulates pseudo-compatibility processes in L. peruvianum.

Beans

KOOISTRA*

Some progress was made towards obtaining sufficiently fertile material of the hybrid Phaseolus vulgaris x P. ritensis, though it is still inadequate for irradiation experiments. A second backcross of the amphidiploid F_1 to P. vulgaris yielded a few plants, probably resulting from successful crossing, some of which possess a deviating chromosome number and a fertility of approximately 50%.

* Instituut voor de Veredeling van Tuinbouwgewassen, Wageningen, the Netherlands.

IMPROVEMENT OF THE MUTAGENIC EFFICIENCY

EFFICIENCY OF DIFFERENT MUTAGENIC AGENTS

Effects of irradiation with fast neutrons, X-rays and electrons on seeds

CONTANT
VERKERK

Two year old dry seeds of tomato varieties 'Money Maker' and 'Glorie' were subjected to X-rays (0 - 80 krad), electrons (0 - 102 krad) and fast neutrons (0 - 9 hours exposure in the 'BARN' reactor at 100 KWatt and at 1.20 m height from the floor). Seeds soaked for 24 hours were irradiated with fast neutrons for periods of up to 4 hours.
Germination delay was found to increase linearly with dose but was much smaller after neutron irradiation than after X-rays or electrons. Final germination percentage (appearance of the radicle) was only moderately affected by lethal doses of radiation (Schwarz effect). The lowest neutron dose administered to dry seeds seemed to stimulate germination. No such stimulation was noted in experiments with soaked seeds but the lowest dose given may have been still too high.
For the other M_1 criteria observed, the Reduction Dose $(RD)_{50}$ values are shown in the following table (page 20). The M_2 data show that RBE for electrons is 0.83 whereas the dose reduction factor due to seed hydration prior to neutron irradiation is approximately 2.3.

Changes in neutron sensitivity in the course of seed germination

Seeds of Arabidopsis thaliana race Li.2 were irradiated with fast neutrons after 0, 1/3, 1, 3, 6, 12, 24, 48, 72 and 96 hours from the beginning of aerobic hydration. The criteria studied were essentially the same as in the seed irradiation studies on tomato (see page 20). The ultimate length of the longest leaf was used as a criterium of somatic damage, whereas fertility was expressed as the number of seeds per silique (overall fertility), the number of ovules per silique as a percentage of the control (female fertility) and the percentage of ovules fertilized (male fertility). Ripening seeds were classified into normal appearing, embryonic lethal and chlorophyll deficient categories.
Preliminary results available indicate that radiation sensitivity with regard to fertility reduction increased up to 3 hours of hydration. At longer hydration times there was a slightly reversed tendency. Male fertility was more strongly affected than female fertility.

Table : Relative biological effectiveness of different radiations

Criterium observed	Dose / exposure at which 50% reduction occurs							
	X-rays (krads)	Electrons (krad)	(RBE)	Fast neutrons				
				dry seeds (a)*	(b)**	soaked seeds (a)	(b)	DRF***
Length of cotyledons	28	33	0.85	6.9	4.0	2.6	10.8	2.65
Fresh weight (18 days)	23	30	0.77	5.2	4.4	1.5	15.3	3.47
% transplantable seedlings	47	49	0.96	13.0	3.6	3.6	13.0	3.60
% plants with more than 8 seeds per fruit	34	39	0.87	5.7	6.0	2.2	15.5	2.60
Number of seeds per fruit (sterile plants included)	24	33	0.72	3.5	6.8	1.4	17.1	2.50
Number of seeds per fruit (only plants with more than 8 seeds per fruit)	26	34	0.76	3.8	6.8	2.0	13.0	1.90

* (a) = hours of exposure

** (b) = 'X-rays equivalent' of 1 hour exposure (krad)

*** DRF = Dose reduction factor due to seed hydration

EMS, X-ray and neutron treatments of beet and pea seeds

HERINGA* Mature flowers of the dry pea variety 'Pauli' were irradiated with X-rays, at exposures ranging from 500 - 8000 R; the pollen from these flowers was used on emasculated flowers of the same variety. Pollinations with unirradiated pollen served as a control. Pod setting was estimated two weeks after pollination. Failure of pods to set was complete at 2500 R. Results indicate that storage of the treated pollen had a favourable effect on both the percentage of successful crosses and on the average number of seeds per pod.
The effects of different mutagens on sterility of M_1 plants were assessed in a comparative trial on seeds of 'Pauli', involving a range of thermal neutron doses, 12 kR X and 0.2 and 0.3% EMS (16 hours at 24°C followed by 1 hour washing in tap water at 20°C). The X-ray treatment reduced the germination capacity to 70%, compared with the control value of 87%, whereas neither of the EMS treatments affected germination. A neutron exposure of 70 hours was sufficient to inhibit germination almost completely in the field, although it still reached 69% in the greenhouse.

SPECKMANN* Experiments were made to find a suitable EMS concentration and X-ray dose for mutagenic treatment of beet (Beta vulgaris). Using the LD_{50} as a criterion it can be stated that ± 20 kR X and 2 - 3% EMS gave the best results. In previous experiments doses of up to 80 kR X gave a satisfactory survival and no explanation can be offered for this discrepancy. An M_1 of irradiated and EMS treated beet seed is sown in the field.

Comparison between mutagenic effects of EMS, γ- and X-rays and neutrons

WELLENSIEK** In 1966 an extensive M_3 of the experiment referred to in earlier reports was grown. It involved the offspring of five normal appearing individuals from each M_2 line which segregated for a visible mutation, lethals and chlorophyll aberrations excluded. The material was limited by poor seed set on the M_2 plants; for this reason, the X-ray treatment had to be omitted. Preliminary results are summarized in the following table:

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** Laboratorium voor Tuinbouwplantenteelt, Landbouwhogeschool, Wageningen, the Netherlands.

WELLENSIEK

	Number of lines tested	Mutated lines No.	%	% of mutated lines with n = 1	n = 2	n = 3
EMS 0.08%	50	35	70	77.1	17.1	5.8
EMS 0.10%	74	36	49	88.9	8.3	2.8
EMS 0.12%	11	10	91	40.0	50.0	10.0
γ 7 kR	10	3	30	100.0	0.0	0.0
Neutrons, 16 hours	5	3	60	100.0	0.0	0.0

The superiority of EMS for the induction of visible mutations is confirmed though no classification of mutant types has yet been made. After EMS treatment, new mutations often appear in the M_3; this is only rarely the case after irradiation. This, together with results on tomato (see also page 34) support the concept of delayed mutation after EMS treatment. Sterility was found to be much stronger after EMS treatment than following irradiation.

Comparison of neutron radiations in three reactors

To compare irradiation effects following treatment in three different reactors when only total fluxes values were requested an experiment was carried out using seeds of the 'very early' pea mutant, a pure line, irradiated with thermal neutrons. The total fluxes requested were: 0.0; 0.5; 1.0; 2.5; 7.5; 10.0; 20.0 and 30.0 x 10^{12} N_{th} / cm^2. The three reactors had the following characteristics, A: flux $\pm$ 5 x 10^7, γ-contamination: 6 rad / hr; B: flux $\pm$ 3 x 10^9, γ-contamination: 750 rad / hr and C: flux 1.25 or 2.5 10^{10}, γ-contamination: 315 or 630 rad / min. Preliminary M_1 results showed a fairly consistent increase of damage with dose. Very important differences in effectiveness of the radiations from the three reactors, with regard to germination percentage, survival percentage of fertile plants, and number of seeds per fertile plant were noted. Effects from the irradiation in A were somewhat less than from B, while those induced in C were by far the most severe. The same tendency was observed with young and developing plants, as well as with the observed average stem length and number of seeds per pod. Mutation frequency will be studied in 1967. Results of this experiment indicate the

WELLENSIEK

difficulty in comparing data obtained by different workers using different irradiation facilities. In the above mentioned case not only did the fluxes differ by a ratio of approximately 1 : 1000 but also the γ-contamination differed considerably. In two cases no temperature was recorded and in one case irradiation was carried out not in the thermal column but in an irradiation room where controlled environment conditions were preset.

DOSE AND DOSE RATE RELATIONSHIP

Radiological investigations on Saintpaulia

BROERTJES

Further studies using Saintpaulia leaves as research material indicated that the dose response curves for survival and production of plantlets from petioles after acute X-irradiation are both S shaped. For survival the LD_{50} was 5 krad and for plantlet production 4.5 krad. The frequency of mutated plants increased linearly with dose, reaching 35% at 6 krad.
Extensive dose rate studies have shown that the lethal dose increases with decreasing dose rates, ranging from 6 krad at a rate of 1000 rad / min to approximately 15 krad when delivered at a rate of 10 rad / min. At dose rates of 4 rad / min or lower the leaves could not be killed. The exact critical dose rate, below which no killing occurs even at extremely high doses appear to lie at about 5 rad / min. At this dose rate the production of young plants is not severely affected and maximum mutation rate is greater than 35%. Production of young plants reacted sharply to dose rates of 100 - 1000 rad / min. No effect was noted at rates below a total dose of 30 krad. Results available suggest a difference between dose rates with regards to optimal mutation frequency taking into account survival and plantlet production. Following selection of a number of clones, one has been chosen for its uniformity.
As Streptocarpus may offer advantages over Saintpaulia in producing a far greater number of vegetative offsprings arising also probably from single cells, exploratory trials are under way to determine its suitability for mutation induction studies.

Responses of tomato seeds and seedlings to chronic thermal neutron irradiation

CONTANT
VERKERK

Presoaked seeds, and seedlings at the 2-leaf stage were irradiated for periods of up to 20 days, at a thermal neutron flux density of 4.3×10^7 N_{th} / $cm^2 sec$, and at two temperatures (10° and $23^\circ C$). Large differences were found in the dose response curve for apical destruction. Seedlings were more sensitive at 10° than at $23^\circ C$ probably partly due to the dose received per cell cycle. With hydrated seeds, the position was reversed to the extent that the 10° treatment sustained no apical damage or even growth retardation after 15 days of exposure. This high resistance may be due to the fact that in hydrated seeds which have not yet germinated much stored energy is available for recovery or repair of primary damage. On the plants with normal apex the number of leaves below the first cluster increased with dose in the case of plants irradiated at the seedling stage. In the case of seeds treated at $23^\circ C$ the number of detectable leaves decreased with dose and gave rise at the sublethal dose to plants with an average of 6 visible leaves below the first cluster against 11 in the control. Some plants at this dose had only one terminal inflorescence before growth was stopped; fruit development was normal. The delay in flowering ranged from 9 - 13 days at the sublethal dose.

The relative sensitivities of the four treatments with regard to fertility reduction (average number of seeds per fruit) are in general agreement with those for destruction of the main shoot apex.

Evaluation of data on mutation frequency is still incomplete and the latter cannot yet be related to the M_1 results. The mutant deficit is greater in the progenies of irradiated seedlings than in those of irradiated soaked seeds, indicating a reduction in average sector size with seedling development. Mutation frequency in all treatments was very low compared to that observed after acute irradiation of seeds. This could be ascribed to a repair or recovery of damage produced during chronic irradiation. The practical consequences of these findings are that for radiogenetical and mutation breeding studies acute irradiation of dry seeds is probably more efficient than either acute or chronic irradiation of hydrated seeds or seedlings.

STAGE SENSITIVITY AND CHIMERISM

Effects of radiation on different ontogenetic stages

Cereals

Barley (Hordeum vulgare)

CONTANT
DEVREUX*
DONINI*
ECOCHARD
MONTI*
DE NETTANCOURT
SACCARDO*
SCARASCIA*

The effects of irradiation of the male gametophyte during its development, of the male and the female gametes at maturity, and of both gametophytes at different stages of their development, have been studied on M_1 plants. Results obtained on seed set, germinability, seedling abnormalities, survival and seedling stages and at plant maturity, and mutation frequency at the seedling stage are summarized in the following table (see page 26). The M_2 data available seem to show that, in a diploid species such as barley, after treatment of gametes, the mutations are induced in heterozygous situation.

Wheat (Triticum durum)

An experiment similar to that carried out with Hordeum has been initiated with Triticum durum irradiating with Co^{60} γ-rays (1,500 R, 93 r/h) male and female gametes, together or separately. The aims are to compare diploid and tetraploid species (both with trinucleate pollen grain) when irradiation is applied at different stages during gamete development; to study the sensitivity of male and female gametes; and to analyse whether mutations are induced in M_1 plants in a heterozygous or chimeric situation. Seeds obtained after the different treatments have been sown in the greenhouse, and the experimental material will be enlarged in 1967.

Peas (Pisum sativum)

Three hundred M_1 plants from seeds obtained after pollination with X-irradiated pollen (700 R) have been grown in a greenhouse and their seeds collected separately from the main stem and each branch. No difference was found in the number of seeds per plant between controls and M_1 plants.

Tobacco (Nicotiana tabacum)

Analyses of 21 progenies mutant plants selfed from R_2 through R_4 were carried out in the field. Ten plants of each progeny were examined for plant habit, height, leaf- and floral morphology, while two plants were self-

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Fertility data, survival and mutation frequency after gametophyte and gamete irradiation of barley

Irradiated stage	Seed setting (%)	Germinability (%)	Survival at seedling stage (%)	Survival at maturity (%)	Number of M_1 plants analysed	Seedling mutations Number	Seedling mutations %
♂ (i)-HP*	30.06	71.79	96.42	90.96	1015	28	2.76
♀ (i)-HP	34.31	94.08	99.58	98.74	470	12	2.55
♂ (i)♀ (i)NP**	66.81	62.51	93.92	88.26	***	***	***
Test HP	34.31	92.13	99.75	95.90	***	***	***
Test NP	95.50	91.64	98.89	99.62			

* HP = Hand Pollination

** NP = Natural Pollination

*** in progress

CONTANT
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ed and one crossed with the control. Flower buds were taken for the analysis of meiosis and mean weight of 100 seeds, percentage seed set and germinability were determined for each mutant line. Chromosome number determinations and anaphase analysis were carried out at the seedling stage. Most mutants show a reduction in height, narrow leaves and changes in several floral characters, namely: colour, calyx size, length of corolline tube, length of stamens and pistil, and corolla width. The cytogenetic analysis reveals in the apical meristems of several R_4 mutants the presence of double bridges, which can be explained by the transmission of dicentric chromosomes. Multivalents were also found in the pollen mother cells (PMC) of some mutant lines, accompanied by a reduced pollen fertility. These structural abnormalities can evidently be tolerated because of the polyploidy of Nicotiana tabacum. About 220 capsules were collected, resulting from artificial pollination after irradiation at all developmental stages of the male gametophyte to investigate the relationship between these stages and radiosensitivity.
Three successive developmental stages of the unirradiated ovary were collected for embryological investigations. Mean weight of 100 seeds, seed set and germinability have been determined. Furthermore, 4 successive developmental stages of the ovary were collected for the same purpose after 4,000 R γ-irradiation at 7 different exposure rates during the three-day-long zygote resting stage. For all these treatments, mean weight of 100 seeds, seed set and germinability have also been determined.
Experiments on 'in vitro culture' of tobacco ovules and ovaries, and on the artificial fertilization of gynaecia cultivated in tubes since two days before anthesis, using DULIEU's technique, gave very satisfactory results.

Tomatoes (Lycopersicum esculentum)

A joint programme carried out by scientists from the Association's Institute in Wageningen and from the 'Comitato Nazionale per l'Energia Nucleare' at Casaccia, aimed at ascertaining whether chimera formation can be avoided following PMC, pollen and gametes irradiation. It attempts to compare the efficiency of PMC, pollen, gametes and seed irradiation for mutation induction; to determine optimal conditions for seed irradiation as well as to compare various kinds of radiations with very different linear energy transfer value (LET). It also aims at a comparison of radiosensitivity for the characters studied, in commercial varieties; and an estimation of mutability per unit absorbed dose for 13 genes.

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By means of histological sections it was ascertained that the formation of the microsporocytes was complete in the flower buds of 3 - 3.5 mm, and that 24 hours after hand-pollination the pollen tubes are still growing in the style: this information is an essential preliminary to the irradiation of PMC and of both gametes after pollination but before fusion. Seeds from the first and second cluster of 100 M_1 plants which resulted from γ-treated (3,000 R) gametes of the variety 'Potentate' have been collected for M_2 analysis. In 1966 emphasis was shifted to 'Money Maker' as a test variety. It was irradiated at the PMC (200 and 400 R) and at the gametes stage (1,500 and 3,000 R). Data on seed setting after flower irradiation and consequent seed germinability, M_1 survival and mean number of M_1 plant per M_0 fruit harvested are summarized in the following table (see page 29).
For each treatment, including the control, 130 M_1 plants have been planted in the greenhouse. From these, M_2 seed will be harvested for tests of mutation frequency and chimerism.
To estimate directly in M_1 the mutability of specific genes, 382 M_1 plants coming from gametes irradiated with 2,000 R of γ-rays after cross pollination of the lines IA (containing the maker genes: d, r, y, p, s, o) and LA 780 (marked with ag, c, yv, H) were analysed. Due to the small size of the M_1 population no mutation of the above mentioned genes was found. A new cross using another male line containing the recessives br, c, f, j, n, sp, u is being made for the same purpose and applying the same dose. So far, 18,482 seeds were collected which will be tested for mutation frequency and chimerism in 1967. At the dose used seed set per fruit was reduced to less than half of the control values (Table page 29).
Pollen irradiation (γ-rays) was carried out at four different doses (3, 5, 8 and 11 kR) and crosses made with unirradiated plants as female partners. Seed set was reduced to 75% at the two lowest doses as compared to 50% at 8 kR and 10% at 11 kR. Seed germination was high in all cases and ranged from 90% at the lowest doses to 80% at 8 and 11 Kr. Two hundred M_1 plants from the 3 and 5 kR treatments are grown in a greenhouse and M_2 seeds will be harvested for tests of mutation frequency and chimerism.
Neutron irradiation of meiotic buds, pollen and gametes is scheduled for 1967.
Studies on the changes in radiation sensitivity during seed hydration and germination have been initiated. Tomato seeds were subjected to fast neutrons after 0, 0.5, 3, 6, 12, 24, 48, 72, 96 and 144 hours of aerobic hydration.

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Fertility data after irradiation of tomato PMC's and gametes

Material	Irradiated stage	Dose in R	Number of M_0 fruit harvested	Number of seeds per M_0 fruit harvested		Total number of M_1 seeds	Germinability (%)	M_1 survival (%)	Mean number of M_1 plant per M_0 fruit harvested
				Mean number	Coefficient of variation (%)				
Money Maker	Control	-	43	56.09±4.649	54.37	2412	90.80	86.00	48.24
Money Maker	Gametes	1,500	70	46.96±2.809	49.99	3288	83.20	79.20	27.20
Money Maker	Gametes	3,000	66	11.92±1.037	70.64	787	82.00	70.40	8.39
Money Maker	PMC	200	67	73.25±3.942	44.05	4908	89.20	93.60	68.56
Money Maker	PMC	400	52	62.63±3.906	44.97	3257	92.80	90.80	56.87
IA x Lyc 24	Control	-	81	75.64±4.009	47.70	6127	*	*	*
IA x Lyc 24	Gametes	2,000	574	32.20±0.826	61.46	18482	*	*	*

* Data not yet available

CONTANT
DEVREUX
DONINI
ECOCHARD
MONTI
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SACCARDO
SCARASCIA

Criteria observed were: germination, root growth, growth of cotyledons, occurrence of dominant foliage shape and colour aberrations in the treated generation, data of flowering and of first fruit ripening, number of seeds per fruit in the first and second cluster and frequency of recessive mutations and of mutated individuals in the M_2. Evaluation of results obtained and testing of the M_2 generation are in progress.

Micro-irradiations with α-particles and UV-rays

GILLES*

Micro-irradiations with Po^{210} microneedles have been continued using various doses on microsporocytes at different stages of meiosis: metaphase I, anaphase I, prophase II and metaphase II. Effects have been observed during the further development of the sporocytes. All usual types of aberrations were thus induced at the irradiated points: chromosome fragmentations, normal or abnormal recombinations, anaphase bridges, alterations of the nuclear spindle which is partly destroyed though recovery may occur after some delay. Effects on the structure of the cell wall have been observed, leading to its bursting at one point. Following this exploratory work, radiation effects on different stages were compared more accurately using a dose of 50,000 particles delivered in one minute. Radiosensitivity of the sporocytes was shown to vary considerably throughout meiosis. In spite of within-stage variation, interphase, prophase II and metaphase II seem to be more sensitive than the other stages investigated. Sporocytes were also irradiated with 2,750 Angström UV-rays in interphase and prophase II, which are stages with slow development and high radiosensitivity. Using a 1 micron spot, very marked responses were noted after irradiations ranging from 5 to 60 seconds. Such drastic effects are probably due to the scattering of the rays.

STUDIES DEVOTED SPECIFICALLY TO THE CHIMERA PROBLEM

Studies on chimeric structure in carnation

BODERGAT
DOMMERGUES
GILLOT

Two varieties are used the red flowered 'William Sim' and the white flowered periclinal chimera 'White Sim' arising from the former by spontaneous mutation. Irradiation of the latter gave several red clones originating from the deep layers. Among these, one was found to be genetically pure and undistinguishable from 'William Sim'. New irradiations of red clones have been carried out to test the histological structure of the various phenotypes. These

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BODERGAT
DOMMERGUES
GILLOT

are related to colour and shape of flowers and leaves. These experiments planned to disturb the chimeric structure for the benefit of the depper layers are simultaneous with experiments aiming at the opposite. Neo-formation of buds have been achieved by hormone (indoleacetic acid, kinetin, etc.) applications to mature plants. Meristematic tissues have been cultivated on modified Murashig-Skoog medium to obtain neo-formative buds. These are also propagated by cuttings and 400 plantlets will be irradiated with γ-rays after flowering.

Distribution of the mutated tissues in gametic layers of M_1 plants

DOMMERGUES
GILLOT

After EMS treatment of Papaver somniferum, the capsules of M_1 plants were selfed. Screening of the M_2 for mutations have taken into account the distribution of mutated tissues in the different capsules harvested from a plant and the growth rate of the M_1 plants. The percentage of mutated M_1 plants and mutated capsules are somewhat higher in plants with normal growth. Analysis of the difference between the two groups, one with normal growth and one slower, showed no difference. Ninetyfive percent of the mutated capsules carried one chlorophyll mutation while 5% carried two. Those containing one mutation showed a mutant deficit in approximately 91% of cases and a surplus in 2.5 - 3% of cases. The progenies containing two mutations had a lower percentage (75 - 80%) of cases with a mutant deficit and a higher percentage (10 - 13%) with a mutant surplus in comparison with an expected 3 : 1 ratio.

DOMMERGUES
GILLOT
TOUVIN

The study of the repartition of mutated tissues in the female gametes of maize was carried out either directly by endosperm mutations or indirectly by examining the genetic segregation of mutations. Both approaches were combined by emasculating EMS treated individuals of the V7 inbred line followed by hand-pollination with pollen containing the sh 2 allele. Only 2 out of the 486 ears harvested were fully fertile containing 50% mutated grains and indicating that the mutated sector had involved the whole inflorescence. The seeds from the normal ears will be sown in 1967 to study the segregation for chlorophyll in the M_2 mutations.

Use of chlorophyll mutations for ontogenetic labelling

DOMMERGUES
DULIEU*

Some chlorophyll somatic mutations regularly transmitted from 1 cell to their daughter cells can be used as labels for ontogenesis. A chlorophyll mutation occurring as a chimera in Nicotiana tabacum variety 'Sansum' of which the white constituent can be transmitted through the maternal gametes has been used for this purpose. It was multiplied vegetatively to obtain a wide range of possible combinations between the white and green constituents of the chimera. Variations in the part taken by each constituent were studied.

Sectors were found to arise by radial replacements (the periclinal axis becoming mericlinal) or by basipetal replacement (in the case of sectorial or mericlinal chimeras). It is thus possible to have a better insight of the growing apex at all its stages and observe the formation of the lateral organs, leaves and buds.

Results indicate that the genesis of leaf mesophyll does not contrary to Avery's conception, arise from one initial sub-epidermal cell but from an entire row of cells. Their filiations are first distributed linearly along the primordial axis and at a later stage disperse laterally by intercalary and diffused growth, the more active if the mother cells are located in the median zone of the future leaf.

In the observed chimera, radial replacements of one apical layer by another occurs frequently but only in an inward direction; thus the tunica layers can penetrate into the corpus but not the opposite. One must therefore resort to artificial techniques in order to recover phenotypes in internal layers.

Lateral variations of the sector size along the stem are inversely correlated with the size of the heteromorphic sector. The finer sectors often disappear when the corresponding meristematic initiating region has made two or three subsequent leaves. The largest sectors involving 1/3 - 1/2 of the stem disappear only in the inflorescence. These observations are in agreement with the assumption that regeneration of the initiating region in the meristem occurs very slowly from the polar cells.

The smallest lentiform sectors originate in the lateral organogenic region, while the large sectors arise from a region extending into the actual apical zone though rarely high enough to affect the terminal inflorescence.

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DOMMERGUES
DULIEU

Other chlorophyll deficiencies appearing after mutagenic treatment change their phenotype along the somatic filiations. Some mutations of this kind are probably plastid mutations. However histological and cytological observations of the areas with heteroplastidic cells suggest that interactions between normal and altered organelles or between the organelles and other particles does exist.

Development of methods to avoid or reduce chimerism after mutagenic treatment of potato

FERWERDA

An experiment was designed to ascertain whether very young tubers the eyes of which were still undifferentiated or in the very first stages of differentiation might provide a suitable starting material for mutagenesis. Approximately 7000 premature seed tubers varying from pea to walnut size were collected and divided into three lots, which were irradiated immediately after harvesting as well as 3.5 and 7 months later. The irradiation dose ranged from 3.5 to 9.5 krad. Before and after irradiation the tubercules were stored at 3 - 5°C. The material was planted and grown in a greenhouse. It appeared that these small prematurely harvested tubers were capable of withstanding dosages of up to 9.5 krad, which would have been lethal to buds of full grown seed tubers. Irradiation after 3.5 months of storage caused the least damage. Results obtained infer that irradiation followed by 1.5 - 2 months of cool storage prior to planting is the most favourable treatment. The incidence of sectorial chimeras in the aerial parts as well as in the tubers was evidently less in the plants grown from very small tubers (diameter 3 - 10 mm) than in those grown from slightly larger tubers (diameter 10 - 18 mm).
In the material irradiated 3.5 months after harvesting the incidence of yellow and yellow-splashed-red tubers was considerably lower, reaching only 50%o, compared to 75%o in the two other treatments.
It appears that chimerism cannot be avoided by starting from premature seed tubers. The chimerical pattern can at best be simplified. Microscopic examination showed that in very small tubers (diameter approximately 5 mm) the development of the 'eyes' has reached a stage where they consist of a homogeneous group of embryonic cells not yet distinguishable into tunica and corpus. It is therefore necessary to treat ontogenetically even younger stages and in this respect the initials of adventitious buds may offer possibilities. An attempt is being made to localize the parts of the plant where adventitious buds generate easily.

The chimeric structure of M_1 plants after EMS treatment of the seeds

HILDERING*
VERKERK

Continuing studies reported in 1965, the first two clusters of 200 M_1 plants grown from EMS-treated seed were harvested as individual fruit. Sets of axial shoot cuttings were grown and from all of them the first and second clusters were harvested on a cluster basis. A total of 82 M_1 plants were chosen for a complete M_2 analysis involving 30 - 40 seed lots (fruit or cluster progenies) per M_1 plant, each lot consisting of 49 seeds sown. The analysis of these 140,000 seedlings is not yet complete. Preliminary results indicate that the frequency of M_2 mutant individuals remains constant along the entire M_1 plant. This constancy is also found with respect to fertility. The number of leaves below the first cluster on the M_1 plant was found to be positively correlated with mutant frequency in the M_2; e.g. plants with respectively 8, 9 or 10 leaves yielded 9, 11 and 14% mutant M_2 individuals. Results also indicate that in the majority of cases the first cluster on the mother-plant was chimeric while the second was non-chimeric. From mutant analysis it appears that in upward direction along the main shoot the size of tissue sectors increases whereas the number of sectors decreases, indicating that extinction of cell lines along the stem is essentially at random. Approximately 40% of the mutations found in the axillaries of the cotyledons of a mutant line from 'Money Maker' occurred also higher up the plant. This was not found in 'Money Maker' itself. These differences may be correlated with the size of the apical zone between the cotyledons in the embryo. Histological studies on this point are in progress. These differences in degree of differentiation were probably due to ripening conditions and were not genetically determined. Results at hand show that a large number of initial cells may take part in the formation of the plant. For instance, one plant was found with not less than 14 different mutated sectors.

Induction of micromutations by Ethyl-Methane-Sulfonate (EMS)

HILDERING
VAN DER VEEN*

Out of the M_1 material used for the analysis of chimerism following EMS-treatment, 46 plants (mother plants or side shoots) were selected on the basis of good fertility and the absence of visible mutations in the M_2. Twenty five M_2 plants per progeny were grown individually alternated with control plants (which in this case consisted of a mutant of 'Money Maker', derived from EMS-treatment).

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HILDERING
VAN DER VEEN

The number of leaves below the first cluster was recorded and analysed. On the basis of this analysis 6 plants with a higher and 6 plants with a lower number of leaves below the first cluster, in comparison with their neighbouring control plants, were selected from these M_2 families. Subsequently seeds from these selected M_2 individuals were sown and the young M_3 plants planted out. Results are not yet available.

INFLUENCE OF BIOLOGICAL FACTORS ON IRRADIATION EFFECTS

Genetic, cytoplasmic and maternal influences upon differences in radiosensitivity between two subspecies of the genus Lycopersicum

CONTANT
DE NETTANCOURT

Two experiments with acute X-irradiation of L. esculentum, L. pimpinellifolium and their reciprocal hybrids, one on seeds and one on growing plants at the stage of bud initiation have been completed.
Results obtained so far indicate that the differences in radiosensitivity which were observed between the four genotypes tested cannot be explained on the basis of variations in volumes of interphase nuclei.
The greater tolerance of L. pimpinellifolium to chronic irradiation at low dose rates is ascribed to the fact that this fast-growing species accumulates a smaller dose per cell cycle than L. esculentum. This conclusion stems from the observation that the relative radiosensitivities of the two species are reversed when acute irradiation of the flowering plant is substituted to low dose rate exposure.
The greater sensitivity of L. pimpinellifolium to high dose rates and to acute irradiation of flowering plants can be related to the observed differences in numbers of meristematic cells between the two species, the tunica of L. esculentum containing more than twice as many cells as L. pimpinellifolium.
After acute irradiation of dry seeds, the reciprocal hybrids express approximately the same resistance as their most resistant parent (L. esculentum) with regard to the capacity of the main shoot apex to survive and to set fruit-bearing clusters. In the case of growth depression however, which is the consequence of all damages impinged upon all embryo parts, the hybrid plants tend to display the same degree of retardation as their pistillate parent. This maternal effect is considered to be related to the total number of cells in the embryo at the time of irradiation, L. pimpinellifolium and the hybrid L. pimpinellifolium x L. esculentum (small embryos with low cell numbers) being more

CONTANT
DE NETTANCOURT

depressed than L. esculentum and L. esculentum x L. pimpinellifolium (large embryos with high cell numbers). A correlation analysis of embryo size and growth depression following acute irradiation of seeds and seedlings will be performed with the 8 backcross lines to test these hypotheses further.

Significance of macro-mutations

GAUL

The material of this programme was split into a part analysed quantitatively and another analysed qualitatively. The quantitative part involves the study of hybrid plants derived from crosses of various varieties with short-awned mutants to investigate the variability of a mutant character in a new genetic background; erectoides mutants, to investigate the independent variability of several mutant characters in a new genetic background; and semi-naked mutants, to evaluate the increase of penetrance of a mutant character. In the first subject, the combination Mutant short-awned 5 x 'Bulchi Gofa' was transferred from the F_3 to the F_4 generation in the form of 110 single plant progenies. Variability of awn length increased strikingly from F_2 to F_4. There was a definite association between the awn length of individual parent plants and that of their progenies. However, progenies of specific F_3 awn length classes were not yet true-breeding in F_4. This is postulated to be the result of modification of the mutation effect by many genes of the new genetic background. The continuation of the programme will show whether or not it is possible to select true-breeding types with an anw length ranging from 0 to approximately 20.0 cm.

In the study of independent variability of two or more mutant characters in a new genetic background, 729 single plant progenies were grown in F_4. These are derived from 26 combinations. A few of the plant progenies were already in F_5 owing to winter multiplication in the greenhouse.

Three combinations have been analysed so far. Results of the combination of the Mutant erectoides 16 x 'Barin', show that it is possible to select F_4 plants which have a short culm similar to Mutant erectoides 16 but which, in contrast to the mutant, have significantly longer spike internodes. This confirms earlier results on the hybrid between Mutant erectoides 16 and variety 'Bulchi Gofa' which is very remotely related to 'Haisa II', the mother line of the mutant. The present example proves that the independent variability of mutant characters can also be achieved in crosses with closely related varieties like 'Barin'.

GAUL

Two (mutant x variety) crossing combinations have for the first time shown that the modification long culm plus dense or semi-lax spike is indeed possible. In the hybrid of Mutant erectoides 2 with 'Ubamer Baco' a long culm is combined with a semi-lax spike, the spike-internode length being intermediate. In the cross of Mutant erectoides with 'Oderbrucher' a long culm is combined with a dense spike; there is no significant difference between the spike-internode length of the hybrid and Mutant erectoides 16. The experiment concerning penetrance of mutant character, done with semi-naked mutants has been harvested and the analysis is in progress.
Practical aims are also involved in the material which is analysed qualitatively. Thirty-one families derived from 18 different hybrid combinations were selected. In 1966, 430 single plant progenies of mutant x variety combinations were selected in the field in F_3, F_4, F_5 or F_6. Furthermore 155 F_1 crossing combinations involving up to 8 parents were grown and all plants harvested to be grown in F_2.

Mutagenesis and genetics of peas

GOTTSCHALK

As in previous years studies on pleiotropic gene action received much attention in 1966. A second case of so-called pleiotropism could be identified as being due to the action of at least two adjacent closely linked genes which apparently mutated simultaneously. This concerned a cochleata type mutant with a strong chlorophyll deficiency and lethality. A consistent 3 : 1 segregation ratio was found for the entire complex of deviating characters of this last mentioned lethal mutant but crossing experiments yielded one case of crossing over in a total of over 2000 germ cells used for fertilization. This is an indication of the extremely close linkage of the 2 genes in question, but also evidence that the double mutation is not due to loss of a small chromosome piece containing a small group of genes. Another group of mutants recently under study is yielding data which indicate a corresponding situation. This means that one is entitled to generalize this result to a certain extent and that probably many cases described as pleiotropic gene action are actually due to a mutation of whole groups of adjacent genes.
From investigation of a whole series of leaf shape mutants it was possible to construct a model showing how the alterations in the shape of leaflets, tendrils and stipules in the leguminous family could have taken place in the course of evolution.

INFLUENCE OF ENVIRONMENTAL FACTORS ON IRRADIATION EFFECTS

Boron studies with Vicia faba

ECOCHARD
OOSTERHEERT*

The influence of B^{10} enrichment on thermal neutron sensitivity has been studied. It was found that plants grown on a medium receiving boron of which 91% was B^{10} (cross section of 3,500 barn) were significantly more susceptible to thermal neutrons than plants supplied with the same amount of boric acid prepared with boron containing 5% B^{10} (cross section of 192 barn). When this energy release process component is altered in an 18 : 1 proportion the overall effect of the irradiation was modified by 25%. Dose response relationship was linear for each treatment for exposure times of up to and including 2 hours. For periods longer than 2 hours the observed damages did not increase although higher amounts of tetrad micronuclei could be scored in previous reported X-ray experiments.

Since a plateau in the yield of induced micronuclei was noted in both treatments (boron containing 5 and 91% B^{10}) for exposure periods longer than 2 hours and performed at 23°C, the experiment was repeated at 12°C. It was found that not only did the yield of aberrations produced per unit exposure time increased for each treatment but that the dose response relationship stayed linear up to and including 2.5 hours. Measurements considering the geometric arrangement (room containing plants) of the experiments indicated a neutron flux of 5.2×10^7 N_{th} / cm^2sec and a γ-contamination of 14 rad / hr. Similar measurements made in the same plant growth room when empty gave lower values especially for the γ-rays.

The number of aberrations per cell / minute was 0.999×10^{-3} and 0.819×10^{-3} respectively for the two boron treatments. They amounted to 0.317×10^{-3} for γ-treatment at 14 rad / hr.

Considering the determined chemical composition of the irradiated tissues to be O_2 = 78.51; H = 10.26; C = 7.94; N = 1.37 and B = 0.00026% of the total fresh weight, the distribution of the absorbed dose in the neutron irradiation was calculated to be as follows: γ-contamination: 0.23300; H (n,γ) reaction: 0.00699; N (n,p) reaction: 0.03220; B (n,α) reaction: 0.063899 (for boron 91% B^{10}) and 0.003233 (for boron 5% B^{10}). The relative biological efficiency in comparison with γ was 11.02 and 2.18 respectively for protons and α-particles.

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ECOCHARD
OOSTERHEERT

Since these values do not agree with published result based on pure proton or α-irradiation it could be assumed that N and B are unevenly distributed in the tissues.

EMS-treatment of pea seeds

SPECKMANN

An experiment was made to determine the effect of washing pea seeds after EMS-treatment. The washing procedure had no measurable effect on germination capacity, number of chromosome-aberrations in mitosis, pollen fertility or seed setting. The survival of the M_1-plants and the number of pods per plant were however favourably influenced by the washing. After a storage period of 14 days the non-washed seeds did not germinate properly and soon died. During the storage period the percentage of chromosome aberrations was found to increase from 8 - 50%. The germination capacity and the chromosomal behaviour of the washed seeds were equal after storage, to the non-stored material. It thus appears that washing of seeds after EMS-treatment restores physiological damage. This resulted in a better plant development and thus in a better seed production; it did not however improve the seed setting.
Repetition of former experiments with EMS-treatment of pea seeds which differed in water content gave similar results. Differences in pollen fertility in the M_1 as found in the dun pea variety 'Aureool' were not found in the canning pea variety 'Pauli'. These experiments will be discontinued.

ANALYSIS OF THE IRRADIATION EFFECT

CYTOLOGICAL INVESTIGATIONS

Cereals

Cytogenetics of durum wheat

BOZZINI*
GIORGI*

Three main lines of research have been pursued in 1966, namely caryotype analysis, aneuploid isolation and study of mutations involving the physiology of meiosis. Caryotypes of Aegilops aquarrosa, of Triticum aestivum variety 'Chinese spring', of the F_1 hybrids (Aegilops speltoides x Triticum boeoticum) and (Triticum aestivum 'Chinese spring' x Triticum durum 'Cappelli') have been investigated and the corresponding idiograms drawn.

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BOZZINI
GIORGI

The chromosomes of the hexaploid 'Chinese spring' appeared to be practically identical to the chromosomes of tetraploid T. durum 'Cappelli' or T. dicoccoides (AA BB) plus the DD genome of Aegilops squarrosa.
The F_1 hybrid (Ae. speltoides x T. boeoticum) possesses, however, some chromosomes which differ in morphology from chromosomes of T. durum and T. dicoccoides. Morphological analysis of chromosomes allows sometimes to distinguish between the components of the three genomes involved in Triticum speciation.
The caryotype of two morphological mutants showing high agronomic performance have been investigated. No evident differences were noted from the mother line 'Cappelli'. Such data will be checked by an analysis of PMC meiosis of F_1 individuals resulting from crosses between mutant and mother line.
More than 700 individuals derived from aneuploids or from backcrosses of aneuploid lines to 'Cappelli', have been analysed for chromosome number. About 200 aneuploid individuals have again been backcrossed to 'Cappelli', to transfer the aneuploid condition into a 'Cappelli' background.
The analysis of more than 1000 seeds from the second backcross of Triticum durum aneuploids to the standard variety 'Cappelli' has been started. Aneuploid seedlings will be grown to maturity for the third backcross to 'Cappelli'.
Analysis of chromosome number of seedlings derived from desynaptic mutants of 'Aziziah' and 'Cappelli' are also being screened for chromosome number in an attempt to isolate additional aneuploids.
Among the mutations involving the physiology of meiosis, seven desynaptic lines have been studied both cytologically and genetically. An accurate analysis of the behaviour of chromosomes at PMC meiosis has been made. Certain desynaptic lines are giving a limited progeny.
Pachytene analysis of PMC of 'Cappelli' has been started. Investigations are in progress although the high number of chromosomes and their clumping make this type of study very difficult.

Embryo-endosperm relations in irradiated seeds

FLORIS*
MELETTI*

Seeds of Durum wheat 'Cappelli' were exposed to 1.5, 2.0 and 2.5 kR of X-rays (180 kV, 6 mA, 200 R / min) after pre-soaking for 24 hours at 20°C. The seeds were then subjected to embryo-transplantation operations, in all combinations, EM(i)/EN(i); EM(i)/EN(u); EM(u)/EN(i) and EM(u)/EN(u), in which EN symbolizes endosperm, EM = embryo, i = irradiated and u = unirradiated.

* Istituto Botanico della Università, Pisa, Italy.

FLORIS
MELETTI

The material thus obtained was germinated and grown for about two months in the greenhouse; at this stage, plants which then had 3 - 4 leaves, were transferred to the field to reach maturity (summer 1966). Growth and development were very satisfactory. Observations were made on survival, ear formation and flowering. The seeds from all plants were harvested for genetic studies. The offspring has been screened at the seedling stage for chlorophyll mutations. Results are shown in the following table (see page 42).
The response of the material to these treatments appeared to be very heterogeneous, as shown for instance by the irregular data for EM(u)/EN(i) which yielded mutant spikes after 1.5 and 2.5 kR but not after 2.0 kR although the number of spikes analysed was equally high. It is however, highly significant that in EM(u)/EN(i) mutant spikes were up to 7 times more frequent than in the control homotransplant EM(u)/EN(u). The chlorophyll mutation types scored were: albina, chlorina, viridis and tigrina.
Cytological analyses have been carried out on root apices of EM(u)/EN(i) transplants in comparison with the control homotransplants EM(u)/EN(u). For the first time in this type of study, X-rays of low energy (180 kV, 6 mA) were used, at exposures of 500, 1000, 2000 and 4000 R. The aim was, to investigate whether irradiation of the endosperm with low-energy X-rays could induce, in the embryo, radiomimetic effects similar to those already obtained in 1965 with high-energy X-rays. The results obtained have confirmed this point. Data summarized in the following table (see page 43) show that 2 kR was the only effective dose.

Peas

Cytogenetics of peas

GOTTSCHALK

The three groups of mutants referred to in the annual report of 1965 were studied more intensively and a very good insight was obtained in the action of groups of genes causing either a reduction of chiasma frequency or a complete breakdown of meiosis. The first hybrids between different desynaptic lines were grown during the summer. They were all fully fertile indicating that the differences in the degree of effect on chiasma formation are not due to different alleles of a multiple series, but to different non-allelic genes of the genome. A 9 : 7 segregation is expected in the F_2 to be grown in 1967.
The study of the behaviour and the consequences of the presence of one and two translocations and their transmission to successive generations was continued. It is

Screening of M_2 seedlings from embryo-transplantation in seeds of Triticum durum variety 'Cappelli' treated with X-rays (180 kV, 6 mA, 200 R / min) after pregermination (24 hours at 20°C)

Material	Dose kR	No. seeds or transplants	Plants analysed	Spikes analysed	Mutant No.	Mutant spikes %	Seedlings analysed	Mutant seedlings No.	Mutant seedlings %
Control	1.5	500	147	348	23	6.60	9.329	132	1.41
EM(i)/EN(i)	1.5	500	170	428	8	1.86	12.684	50	0.39
EM(i)/EN(u)	1.5	500	146	333	8	2.40	8.371	24	0.28
EM(u)/EN(i)	1.5	500	356	996	8	0.80	36.469	12	0.032
Control	2.0	500	34	75	3	4.00	2.186	27	1.23
EM(i)/EN(i)	2.0	500	50	110	1	0.90	2.818	3	0.10
EM(i)/EN(u)	2.0	500	29	49	1	2.04	1.496	5	0.33
EM(u)/EN(i)	2.0	500	344	940	-	-	33.438	-	-
Control	2.5	500	1	3	-	-	92	-	-
EM(i)/EN(i)	2.5	500	63	146	-	-	3.618	-	-
EM(i)/EN(u)	2.5	500	11	19	-	-	370	-	-
EM(u)/EN(i)	2.5	500	356	962	5	0.51	35.980	7	0.019
EM(u)/EN(u)	-	250	200	730	1	0.13	26.412	1	0.0037
Control	-	250	75	217	-	-	8.547	-	-

FLORIS
MELETTI

Cytological analysis of embryo-transplants on irradiated and unirradiated endosperm

Transplant	Exposure kR	Number of anaphases analysed	Percent aberrant analysed	Chromatid break per 100 anaphases	Chromosome break per 100 anaphases
EM(u)/EN(u)		716	7.6	13.5	0.6
EM(u)/EN(i)	0.5	692	8.3	13.4	1.5
EM(u)/EN(i)	1.0	1140	9.5	13.2	1.5
EM(u)/EN(u)		690	7.8	12.6	0.4
EM(u)/EN(i)	2.0	757	16.1	23.3	3.0
EM(u)/EN(i)	4.0	797	7.7	10.9	2.9

GOTTSCHALK

surprising that plants heterozygous for two translocations which result in a ring of six chromosomes during first meiotic prophase, still show a fertility of approximately 50% when compared with the original line.

SPECKMANN

Two semi-sterile mutants of peas have been analysed cytologically. One is a desynaptic mutant, the other is characterized by spontaneous chromosome breakage and suppression of the homotypical phase of meiosis. Trisomics were found in the progeny of the mutants. Crosses were made to ascertain the genetical background. Crosses were also made with mutants having characters of possible agricultural value (short straw, erect growth, etc.).

Tomatoes

Cytogenetic studies on tomato

RAMANNA*

Techniques have been developed for the study of pachytene chromosomes and of somatic metaphase chromosomes. After identification of the mitotic and of the meiotic chromosomes their morphology has been compared and both series have been homologised.
The differentiation of the pachytene chromosomes into chromatic and achromatic parts corresponds with the same differentiation, though somewhat less sharp, of the somatic metaphase chromosomes. The length-relation between chromatic and achromatic parts however, differs greatly between the pachytene chromosomes and the very much shorter somatic metaphase chromosomes. The length ratio of pachytene to somatic chromosomes is about 5 for the chromatic parts and about 30 for the achromatic parts. Achromatic parts which in the pachytene chromosomes are 9 μ or shorter cannot be recognized in the somatic metaphase chromosomes.
To study the cytological effects of EMS on pachytene chromosomes of tomato healthy shoots from control plants, variety 'Money Maker', containing flower buds with their anthers at young pachytene stages, were immersed with their cut stem in 0.3% EMS solution (24 hours at approximately 23°C). After treatment the shoots were placed in water for 12 hours. The anthers were fixed in propionic alcohol, mordanted with iron acetate for 48 hours, and squashed in 1% aceto-carmine. Pollen mother cells were screened for chromosome abnormalities. It has not been possible so far to establish whether the EMS solution reaches the flower buds and produces cytologically detectable effects on pachytene chromosomes. A low frequency of aberrations involving centromeres has been recorded in EMS-treated buds giving the impression of two chromosomes fused at their centromeres. Results do not allow to conclude whether this fusion is a matter

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RAMANNA

of 'stickiness' or a real 'break-and-exchange' process. The phenomenon appears certainly not due to simple crossing or interlocking of chromosomes.

Allopolyploidization of autotetraploid tomato

Plants were obtained by rooting shoots grown from callus formed on cut stems of unirradiated tomato plants. Three out of a total of fifty six were tetraploid; two of these died probably due to a highly chimeric constitution, but the third survived and its offspring is being studied.
The first shoot above the second cluster of 200 M_1 plants irradiated with thermal neutrons at the seedling stage (see page 19) was grown. Among these 200 cuttings, four were chromosomatically aberrant; two of these possessed 2n = 23 chromosomes, one possessed 2n = 23 + 1 centric fragment, whereas one probably contains at least five translocations. Shoots have been produced in the callus of all four individuals (clones) which are being screened for their chromosome number. The meiotic behaviour, especially chromosome pairing, of any tetraploid shoots will be studied and compared with the meiosis of normal callus-induced autotetraploid plants already available.

GENETICAL INVESTIGATIONS

Maize

DOMMERGUES
TOUVIN

Twenty-eight endosperm mutations have been classified in floury, shrunken and sugary groups. They were crossed amongst each other and with 16 USA lines with marker genes to localize the mutated ones. Similarly a liguleless mutant line was crossed with a liguleless marker line. Results obtained indicate that in the 12 starchy lines 3 mutations could be found. In the 7 half-starchy lines, 4 different mutations were noted, the other being identical. Three lines with shriveled endosperm had the same mutation, identical to the factor for etched endosperm of the marker SP.37 on chromosome III. Two of the three tested mutants with shrunken endosperm were identified with the gene sh2 of chromosome III. The 3 sugary mutants tested were related to the gene su1 on chromosome IV. The character liguleless corresponds with the gene lg1. Since each mutation group included sometimes several origins the phenomenon cannot be related to mutagenesis of the different genes concerned but correlated with the cultivation technique of the M_1 plants. These were isolated from other corn varieties

DOMMERGUES
TOUVIN

to avoid out-crosses. The arising of a similar mutation in several lines can be explained by spontaneous crossings between M_1 plants and not necessarily by an increase in mutation frequency.

Genetical analysis of potato mutants

Ivy leaf type

FERWERDA

Observation of 28 F_2 progenies (originating from ivy individuals randomly taken from a segregating F_1-progeny) confirmed that 'ivy leaf' is a monofactorial heterozygote. The average segregation ratio ivy-leaf: normal was again approximately 2 : 1. Progenies consisting exclusively of ivy individuals were not encountered. This lends support to the view expressed in previous reports that homozygous ivy is non-viable.

Periclinal chimeric structure of skin colour deviations

For some tuber skin anomalies (notably the yellow-splashed-red type) the periclinal chimeric structure has already been demonstrated by eye excision experiments. Genetic evidence confirming this view was obtained from a series of test crosses showing that yellow-splashed-red and the uniformly red skinned normal type are genetically identical as far as their sub-epidermal tissue is concerned. Consequently, the two types differ only in the genetic composition of the epidermis. Similarly, a periclinal structure was made evident for a deep red skinned anomaly (B 167) derived from the 'Burmania' variety. This fact had not yet been revealed by eye excision experiments. Some yellow skinned mutants obtained from normally red skinned varieties were found to be genetically homogeneous throughout the tuber.

Allelic tests on 'potato leaf' mutations in L. esculentum

HILDERING

A complete 6 x 6 diallel cross was grown, including 6 potato leaf mutations. All F_1 showed the perfect potato leaf phenotype while all F_1 from female potato leaf x male normal showed normal leaves. The six mutant genes of independent origin appeared to be alleles of the same locus.

Genetic analysis of pea mutants

WELLENSIEK

Linkage and translocation in cochleata mutant: in a backcross of (F_1 r tl coch gp x RTl Coch Gp) x r tl coch gp clear linkages between all four genes were found and the order of the genes is as above. This implies that a translocation between chromosomes V and VII has taken place. Whether this translocation has been brought about by mutation or already occurs in the material of origin could not yet be ascertained.

WELLENSIEK

Genetics of earliness: In former years the behaviour of mutants for time of flowering led to the hypothesis that gene N_o^m (medium stem node number for first flower = early flowering) acts as mutagenic gene towards N_o^h (high stem node number = late flowering in original line), resulting in a mutation from N_o^h into n_o (low node number = very early flowering).
This hypothesis has already been tested and confirmed in F_2 of the cross 'late' x 'early mutant'. In 1966 a large F_3 was studied. The usual 2 : 1 and 3 : 1 ratios were found, but in 3 F_3 lines from late F_2's, 5 very early flowering plants, out of a total of 467, were found. This not only confirms the above hypothesis, but the percentage of very early flowering plants approaches very closely that in F_2.
Linkages of the mutant 'golden' and 'stipules attached to petioles': The factor 'golden' appears to be located on chromosome I, whereas the gene for 'stipules attached to petioles' is located on chromosome V. These results will be verified with a tester line containing known recessive genes on all chromosomes. Crosses with stringless mutant: The character 'stringless' could not yet be localised and even its inheritance is unclear, since under field conditions it fails to breed true. This apparent instability is probably due to modifiers. The combination of 'stringless' with the character 'sugar pea' has been recovered so that breeding for incorporation of these traits into a commercial type can continue. The mutant collection now contains 55 fertile and 5 sterile mutants.

PHYSIOLOGICAL INVESTIGATIONS

Factors governing plant radiosensitivity

DNA content in the cell nucleus

AVANZI*
BRUNORI*
CERVIGNI*
GIACOMELLI*
MODUGNO*
SCARASCIA

The cytophotometric determination of the amount of DNA per nucleus of 4 mutants of T. durum 'Cappelli', namely 'chlorina', 'solid stem', 'brachytic' and 'short straw', of which the radiosensitivity has been investigated in the γ-field, has been completed. Three mutants, 'short straw', 'brachytic' and 'solid stem' have a very similar DNA content, respectively 95.47, 96.32 and 96.32% of the 'Cappelli' control. The mutant line 'chlorina' showed the highest decrease of nuclear DNA content reaching only 92.57% of the control.

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BRUNORI
CERVIGNI
GIACOMELLI
MODUGNO
SCARASCIA

The cytophotometric determination of nuclear DNA content of 23 species of Nicotiana has been undertaken. Preliminary data show a good relationship between the basic number of chromosomes and the nuclear DNA content. Species with 2n = 48 chromosomes have an amount of nuclear DNA similar to that in N. rustica 'Brasilia' (2n = 48). Species having 2n = 24 contained about half as much DNA per nucleus as N. rustica. The only exception found concerns N. langsdorfii, which with only 2n = 18 chromosomes contains about 80% of the nuclear DNA of N. rustica 'Brasilia'.

The effect of radiations on peroxidase isozymes

The separation of peroxidase isozymes in leaves of 8 days old barley seedlings, variety 'Esau' from irradiated (60 kR X) and unirradiated seeds has been carried out by electrophoresis on starch gel. The zymograms, developed by benzidine saturated water containing 0.3% hydrogen peroxide, show for irradiated leaves a separation of 5 isozymes at the cathode and 3 at the anode, while for unirradiated leaves only 3 isozymes at the cathode and 2 at the anode can be separated. The staining intensity of all the bands in irradiated material is higher than in the controls indicating a greater enzymatic activity. The deep radiation-induced alteration of the systems regulating the biosynthetic pattern leads to a differentiation of new forms of the peroxidase isozymes. Peroxidase analyses were carried out on germinating barley seeds, 1, 2, 6, 8, 10 and 14 days after irradiation to study the appearance of new isozymes. The first two separations were done on the embryos whereas the other four were on the first leaves of the seedlings. The zymograms made 24 and 48 hours after irradiation are identical with three bands at the anode and two at the cathode. The zymograms of peroxidase from the first leaves after 6 days of germination show the appearance of further bands, one at the cathode for unirradiated leaves and one at the anode and three at the cathode for irradiated leaves. No further changes were noted in the electrophoresis carried out 8, 10 and 14 days after irradiation. The separation of peroxidase isozymes has also been carried out in pollen of irradiated and unirradiated Lilium flowers. Irradiation was done with 1,000 R of X-rays (250 kV, 25 mA, 25 R / min) at different stages of development of the male gametophyte. The zymograms of peroxidase isozymes from pollen of flowers irradiated at different stages of development show three bands at the anode and one at the cathode, whereas the zymograms of pollen from unirradiated flowers show only two bands at the anode and one at the cathode. The staining intensity of all the bands in irradiated material is higher than in the controls.

Effect of IAA on growth of irradiated pea internodes

AVANZI
BRUNORI
CERVIGNI
GIACOMELLI
MODUGNO
SCARASCIA

Studies on growth response in etiolated pea internodes irradiated with 30 and 50 kR X-rays and then treated with 10^{-5} M indoleacetic acid (IAA) for a period of 3, 6, 12 and 24 hours, showed that after 12 hours of treatment growth was reduced to approximately 85% of the control at the lower exposure and 80% after the higher exposure. Glutathione content decreased rapidly in the first 3 hours of treatment in both control and irradiated internodes reaching after 12 hours an equilibrium corresponding to approximately 50% of the initial value. The equilibrium value was slightly higher in irradiated internodes. Experiments have also been done with pea seedlings to investigate the effect of radiation on growth and glutathione content in the shoot apices and in the third internode of these seedlings. For this purpose 8 days old seedlings irradiated with 30 kR X (250 kV, 25 mA) were placed in a greenhouse. Nineteen hours after treatment growth was measured as fresh weight of apices and of the third internode or as the length of the third internode. In irradiated seedlings the increase in weight, compared to the controls, was reduced to 34% for the apices and to 14% for the internodes. The length of irradiated internodes was reduced to 31% of the controls. Conversely, the content of reduced glutathione, whether related to fresh weight or to 100 mg of total proteins, increased in the apices and internodes of irradiated seedlings, relative to the controls. This phenomenon was more evident in apices.

Formation of adventitious buds on potato tuber slices

MIEDEMA*

Striking varietal differences in adventitious bud formation have been noted on tuber slices. No correlation could be detected between their formation and other characters.
In a series of experiments with growth substances, tuber slices of the variety 'Bintje' have been treated with different concentrations of auxins, anti-auxins, gibberellin and kinins. Indoleacetic acid, indolebutyric acid, naphthalene-acetic acid and 2,4-dichlorophenoxyacetic acid induced formation of roots but not of buds. No organogenesis took place in trials with gibberellic acid or with the kinins kinetin, adenine, benzyladenine and coumarin nor with the anti-auxins triiodobenzoic acid, maleic hydrazide and trans-cinnamic acid.

* Stichting voor Plantenveredeling, Wageningen, the Netherlands.

MIEDEMA

A method has been developed for the induction of adventitious buds on rooted potato slices. Tuber slices treated with auxins will form roots on the heel directed cut surfaces. When the roots are exposed to light, buds will form on the roots or on tuber tissue near a root. In a greenhouse experiment 60 - 70% of the rooted slices formed one or more buds. Starting from mutagenically treated tubers or from mutagenically treated roots it may be possible to grow mutants via these adventitious buds. Physiological investigations have been started to determine the most suitable conditions required. A histogenetical study to elucidate the organogenesis is in progress.
Concerning the mutagenic treatment technique it appears that X-irradiation inhibited the formation of roots. Treatments with SH-compounds before irradiation did not prevent this inhibition. After EMS-treatment at 0.13 and 0.65% normal root formation occurred and the roots formed adventitious buds.

CULTURE TECHNIQUES

Culturing microsporocytes, microspores and ovaries

GILLES

Although culturing of microsporocytes is possible on a routine scale since the end of 1965, the methods used have been further improved, especially for those cases which involve irradiations with α-particles. For these applications the medium consists of 3% agar and 7% sugar. This relatively hard medium allows the sporocytes to be kept in place between the coverslip and the slide on the upper face of the agar block. Moreover, with such a medium the images produced by phase contrast are much clearer. Culturing of microspores has been much improved by the addition of nucleosides and amino-acids to a standard medium such as BRAUN's or STERN's; using these additions complete microspore development is possible up to the final stage of the binucleate pollen grain. This new technique enables the irradiation of any stage of the sporocytes followed by their culture until pollen maturity, which can then be used for artificial fertilization. Three suitable culture media for ovaries on which artificial fertilization can be carried out were developed.

Tissue culture as a tool for radiation research on plant cells

SAUER[*]

Growth rate of callus tissue culture of Parthenocissus tricuspidata was studied as a function of naphthaleneacetic acid (NAA) and kinetin levels present in the

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SAUER

culture medium. Optimal growth resulted from a combination of kinetin (10^{-9} - 10^{-7} M) and NAA (10^{-5} - 10^{-6} M). At 10^{-5} M, kinetin in combination with very low concentrations of NAA, gave good growth of tissues. This was not the case at higher NAA levels. No improvement in growth could be noted by the addition of a number of nucleic acids to the medium. The addition of L glutamine however stimulated chlorophyll production in tissues of P. tricuspidata. Two media differing from Heller's in the ratio of chemical constituents gave significantly better growth of cell suspension cultures. Irradiated saccharose (2 Mrad in 4 hours) added to the medium used reduced growth rate of P. tricuspidata by 10 - 20%. This may be due to the low pH of 3.3 in the irradiated sugar solution resulting from the formation of acids in contrast to a pH of 6.2 in the unirradiated culture medium. It may also be due to the formation of new compounds similar to those of the metabolized sugars which may replace them in the enzyme substrate coupling. Under the experimental conditions used, when a complete synthetic growth medium was adopted no toxic effect was found on P. tricuspidata. These results lead to believe that some substances present in the natural constituents of certain culture media may be responsible for the toxicity reported by some authors. Investigations are in progress to determine whether irradiated sugar may react with these substances causing the toxicity noted.

PRESERVATION OF FOOD BY MEANS OF RADIATION

The evaluation of electron- (shallow treatment) and γ- (penetrating treatment) irradiation as a food preservation method has been continued in a number of projects investigated either by the scientists of the Association, by those of associated subcontractors or by scientists from other institutes working in close collaboration with those of the Association.

The facilities available, a 2 MeV electron generator allows studies with electrons or Bremsstrahlung. Investigations have considered in particular the effect of electron irradiation since most food products do not need to be totally irradiated for an increase in shelf life. Parallel to these studies related to the applied aspect of food preservation research, studies have been carried out to assess the effect of irradiation on the ripening and the structure of tomato fruit.

Fruit and vegetables

Apples

STADEN*

Much attention was devoted in 1966 to the irradiation of several apple varieties with electrons and Bremsstrahlung. The doses used were 40, 80, 120 and 180 krad. Damage was noticeable on fruit treated with doses higher than 80 krad. This was more evident following electron than penetrating rays treatment. Scald was rather successfully controlled at 40 krad. Lenticel spot on 'Jonathan' fruit from one origin was strongly reduced by irradiation at 80 krad. Results obtained for the control of spot were not clear cut. The effect of an irradiation dose below 80 krad on firmness and flavour was also not conclusive.

Pears

Treatments with electrons and Bremsstrahlung showed that the influence of 80 and 180 krad irradiation on rot percentage of the variety 'Beurré Hardy' is small. Irradiated fruit were however found by a taste panel to be similar or better than controls.

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Tomatoes

LANGERAK*

Previous experiments have proved the value of irradiation for the extension of shelf life of tomatoes. Results however have been relatively variable because of the heterogeneity of the samples used. In experiments carried out in 1966 an effort has been made to obtain homogeneous samples. They were restricted to the variety 'Money Maker', and fruit treated with 1.7 MeV electrons or Bremsstrahlung (maximum 1.7 MeV) were compared for hardness, colour, weight and 'Schnittfestigkeit'. Only 'A' size tomatoes (diameter 47 - 57 mm) were used. Treatments were 50, 200, 250, 350 and 500 krad. Before treatment tomatoes were tested for hardness with a non-destructive hardness meter and only those of hardness 6 - 8 were used. Following treatment a visual sorting and a second selection with a remission meter were carried out. Tomatoes were measured in the red field of the 620 mm wavelength and in the green field with the 550 mm wavelength. They were classified at λ = 620 mm at an indication of 32 - 34% reflection and at λ = 550 mm at an indication of 6 - 8% reflection. Loss of weight, as well as hardness and 'Schnittfestigkeit' were determined. The last-mentioned was measured by exposing tomato slices to a horizontal centrifugal force (695 rpm) for 2 minutes, and the loss in weight was considered a measure of the 'Schnittfestigkeit', that is the ability for the fruit wall and loculus to hold the gel substance within the cavities of cut fruit. Results available indicate an obvious delay in fruit softening following application of ionizing radiation. This delay was found to be proportional to the dose applied. Although immediately after treatment tomatoes are softer than controls, 2 or 3 days later some recovery occurs.

Increase of softness in tomatoes irradiated with ionizing rays and stored at 20°C (values are percent of initial softness)

	1,7 MeV electrons			
Storage	0 krad	50 krad	250 krad	500 krad
1 hour	2.3	4.2	7.9	16.9
4 days	71.5	63.2	38.1	14.0
11 days	139.2	116.0	63.5	32.2
	Bremsstrahlung (max. 1,7 MeV)			
1 hour	2.3	7.9	13.7	27.9
4 days	71.5	62.1	42.3	23.0
11 days	139.2	124.4	83.9	38.9

* Association EURATOM - ITAL, Wageningen, the Netherlands.

LANGERAK

Colour was also influenced by ionizing rays, reflection at higher dose being larger than at lower dose. Preliminary results showed that the 'Schnittfestigkeit' is unfavourably influenced by increasing doses of Bremsstrahlung. During storage, loss of weight was found to be very low and hardly influenced by irradiation.

Soft fruit

Previous experiments have indicated the advantages of using a combination of packing and radiation to extend shelf life of various soft fruit. Experiments were carried out in 1966 to further ascertain this point. Strawberries, raspberries, cherries, red currants and plums were irradiated unwrapped or wrapped in different plastic foils. Products were stored at 20°C and approximately 75% RH.

Strawberries picked in 200 gram standard commercial boxes were wrapped in polystyrene, perforated polyvinylchloride, cellophane PT and cellophane DSAT. They were then irradiated with Bremsstrahlung (maximum 1,7 MeV, 120, 160 and 200 krad). Part of the experiment was combined with a heat pre-treatment to increase the radiosensitivity of moulds. Mould infection increased following 4 days storage particularly on the non-irradiated product. Irradiation was found to decrease mould infection in direct relation with dose applied. Heat pre-treatment did not give any better results than irradiation alone. Packing reduced dessication of the strawberries, and, during the first 3 days, mould infection. When storage was extended, the percentage of mouldy strawberries increased in the wrapped more than in the unwrapped fruit due to the higher humidity present. Cellophane PT was an exception. Very encouraging results were obtained when the product was wrapped in this foil before irradiation.

STADEN

Extensive experiments carried out on 9 varieties, showed that 200 krad irradiation was the highest dose possible without loss of flavour. Fruit sprayed while growing with a fungicide ('Eupareen') had a better keeping quality than untreated ones. A combination of this fungicidal spray application and irradiation treatment after harvesting proved to be the best.

Cucumbers

LANGERAK

Irradiation with penetrating rays at doses of 25 - 250 krad of cucumbers unpacked or prepacked in shrinking polythene followed by storage at 10, 15 and 20°C indicated that a 100% extension of shelf life could be obtained in the prepacked samples. Radiation even at the lowest dose used produced some discolouration, the best

LANGERAK

storage temperature was found to be 15°C. At 10°C low temperature decay was noted while discolouration appeared at 20°C.

Potatoes

VAN KOOY*

Practically all studies on sprout inhibition by irradiation is carried out with penetrating rays. If internal sprouting is no problem, surface irradiation may be sufficient. The effect of a 1.7 MeV electron treatment compared with γ-irradiation indicated that following 2, 3, 5 and 7 months storage at 9°C no sprouting was noted on the 10 krad γ-treated lots while with 15 krad electrons the sprouting was respectively 0, 6, 18 and 30% of the control. Controls averaged 1.7 and 3.8% sprouts per potato after 2 and 3 months storage respectively. These experiments were carried out with an early sprouting variety 'Eigenheimer'. The sprouts appearing on the 15 krad treated potatoes 3 and 5 months after treatment were very short. Taste panel evaluations indicated that at the end of the storage period the electron irradiated potatoes were preferred. Non-irradiated potatoes were not judged edible after 3 months storage.

Cut vegetables

STADEN

Cut onion, Swedish turnips, carrots, leeks and red cabbage were irradiated with Bremsstrahlung. Results indicate that prepacked onions, Swedish turnips and leeks had a considerably longer shelf life following irradiation with doses of 50 and 100 krad.

Dried vegetables

METTIVIER MEYER**

Following encouraging results reported in 1965 with dry kale, experiments were carried out on 11 important vegetables used mainly for soups. Cooking time was shortened in all cases following irradiation at 1000 and 3000 krad. Treatment was carried out with 1.7 MeV electrons on the dried vegetables. Cooking times in minutes are summarized in the following table:

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** Stichting Sprenger Instituut, Wageningen, the Netherlands.

METTIVIER MEYER

Vegetable	0 krad	1000 krad	3000 krad
Potato	15	5	-
Carrot	14	7	-
Spinach	10	5	-
Leek	16	-	8
Kale	30	-	15
Cauliflower	15	10	-
Butter-bean	35	-	20
Onion	25	-	10
Celery	25	-	12

Taste, flavour and structure were not adversely affected by the doses used. The mechanism of the change of structure was investigated and no influence was found on rehydration. Vitamin C was not influenced to any great extent by the irradiation treatment.

Peas and beans

STEINBUCH*

Experiments with peas, field peas and kidney beans have been carried out to ascertain whether irradiation caused a decrease in firmness of these pulses. Results indicate that this was the case for the two first mentioned crops, and the more so following irradiation with Bremsstrahlung than with electrons. Kidney beans however, increased in firmness following treatment. At 300 krad quality and colour of all material showed a clear deterioration.

Meat

CAMPI**

A general survey of microorganisms present on meat (beef) surface before irradiation was determined by the swab technique and agar plate counts. It was found to vary from 5,000 to 20,000 per cm^2. Pre-storage was not known and may have differed causing this variable contamination. Irradiation of cellophane packed meat at 250 krad electrons decreased the initial micro-organism population to less than 100 per cm^2. Irradiation at 500 krad decreased it to average values of below 1. The post irradiation development of micro-organisms was followed during storage at 0 to 2°C for periods of up to 27 days. Results show that the initial levels were reached after periods of approximately 14 days. Gamma-irradiation with 311 krad, being

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CAMPI — equivalent to 250 krad electrons when considered at the surface of the meat, retarded development of microorganisms to the pre-treatment level for a period of 4 weeks.

VAN KOOY — An attempt has been made to control Cysticercus bovis larvae in meat. Gamma-irradiation with doses of 50 - 300 krad have been used. Results indicate that cysts are very resistant to irradiation and this degree of resistance is particular for that form of life. Cysts survived 200 krad; while in meat irradiated with 300 krad and stored for 7 days, no viable cysts could be found.

Spices

VAN KOOY — The possibilities of replacing use of ethylene-oxide by irradiation for the sterilization of spices on an industrial level has been investigated. The total number of microbes for black pepper and ginger could be reduced, following treatment with a dose of 500 krad, by a factor of 1000. A taste panel could not detect any significant difference between the irradiation product and the controls.

Structure analysis of tomatoes

BERKHOLST — An attempt has been made to change the locular tissue of tomatoes and determine the part played by cellulose in the fruit constitution. Experimental results indicated that Bremsstrahlung at doses above 80 krad caused a decrease in the strength of the locular tissues. Results obtained following irradiation of fruit from different origins showed no clear relation between percentage of locular crude fiber (mostly cellulose) nor between cellulase activity and 'Schnittfestigkeit'.

Respiration measurements by gas chromatography

VAN KOOY — The technique for carbondioxide determination on fruit material has been further developed. Sensitivity was increased by improving the conditioning of the column on which separation between air and carbondioxide takes place, the gas flow control through the column, the constancy of temperature of the respired air to be analysed, the accuracy of the detector, and, finally using Helium as a carrier gas. An important advantage of carbondioxide detection by katharometer over flame detector is that carbondioxide and oxygen can be analysed in the same sample. The temperature of the sample to be analysed must be constant within 0.01°C to obtain an accuracy of 0.01%.

VAN KOOY

Using the technique perfected it is possible to determine in a very short lapse of time the respired air of fruit, to measure the immediate effects of irradiation upon the physiology of the fruit as well as changes occurring during a long period of storage.

RELATED PHYSICAL STUDIES

The physical studies related to the radiation research programme of the Association have continued in 1966 and as in the preceeding year may be subdivided into three separate sections: dosimetry, microbeam irradiation and instrument development.
The dosimetry programme was mainly concerned with the setting up of routine dosimetry systems for the different facilities available at the Association's Institute as well as to optimise the irradiation conditions in these facilities At present, most work is directed towards neutron dosimetry and spectrometry in the reactor facilities. Work has been started on three new detectors, two for neutron spectrometry and the third, a special ionisation chamber for neutron dosimetry.
The physical aspects of the microbeam irradiation of cells, which is the subject of a subcontract between the Association EURATOM - ITAL and the 'Université Catholique de Louvain' in Belgium has been concerned with the improvements in the production of microneedles for α-irradiation and the definition of the UV microbeam.
The instrument development work described here has been concentrated on the reduction of background noise in a four channel semiconductor system for the measurement of β-activity in plants.

D O S I M E T R Y

X- and γ-radiation

CHADWICK[*]
OOSTERHEERT[*]
PUITE[*]

A high dose rate facility was constructed for the irradiation of pollen and seeds in the large Cs^{137} γ-source. The facility has 14 turntables designed to accept 3 different diameter specimen tubes which rotate at 1 revolution / second. The dose rates obtainable vary between 214 krad / hr and 22 krad / hr.

A routine dosimeter system covering the range 5 krad to 5 Mrad is required for food irradiation studies. The Fricke-Copper dosimeter was found to be completely unstable and was abandoned in favour of the Cerric-Cerrous dosimeter system which has proved to be more stable and reliable.
An inexpensive photocell is now being used for the routine dose mapping of the X-ray fields in the electron generator.

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Electrons

CHADWICK
OOSTERHEERT
PUITE

A thin plate ionisation chamber has been constructed for work with the 1.7 MeV 'Van de Graaff' at the Association's Institute. The chamber has 1.5 mgm / cm^2 melinex foil plates and a 1 mm air gap; an aluminium diaphragm is used to define the sensitive volume of the chamber. Considerable corrosion of the aluminium frame of this chamber has occurred due to the ozone produced by the intense electron beam.
A thin plate photocell has been used to measure electrons, further work is necessary to optimise the system.
A large scale experiment on irradiation of oranges was carried out in collaboration with the Israel Atomic Energy Commission. The dosimetry of the experiment was done with the cooperation of High Voltage Engineering Co., USA. Different dosimetry systems were used: blue cellophane, CaF_2-teflon and LiF-teflon thermoluminescent discs. Good agreement was obtained between these systems. It was found that when fruit is rotated under an electron beam the energy distribution in the surface layers is somewhat altered from the normal Bragg distribution. This effect was measured using thin strips of LiF-teflon thermoluminescent tape pushed into the orange skin. Calculated and measured distributions gave good agreement.

Thermal neutrons

The proton response arising from the $N^{14}(n,p)C^{14}$ reaction occurring in different liquids used with Li^7F thermoluminescent powder was thought to be masked by the $Li^6(n,\alpha)T^3$ reaction occurring in the Li^7F. Another thermoluminescent material CaF_2:Mn was therefore used instead of the Li^7F. The proton response versus nitrogen content per cm^3 was approximately linear. Efforts to increase the reproducibility of the results and the sensitivity have concentrated on etching the crystals, but have so far not been successful.
The effect of different materials, such as Hoagland culture solutions, plastic containers, etc. normally used in plant growth, on the thermal neutron flux and γ-contamination in the irradiation room of the Association's Institute Reactor 'BARN' have been studied. Briefly, the water in the nutrient solution on which the plants are grown doubles the thermal neutron flux to the plant but also raises the γ-dose rate by a factor of 2.6. The thermal neutron flux, cadmium ratio for gold and γ-contamination were also measured as the D_2O level in the diffuser was lowered; results indicated that the full tank, normally used, may not give the most favourable irradiation conditions. The shape of the epithermal neutron flux was also measured using boron and gold. The shape is normally assumed to be 1/E but in fact this assumption is too general and it is more often of the form $1/E^k$ where k may vary. The measurements in 'BARN' showed that $k \sim 2$ in the irradiation room.

CHADWICK
OOSTERHEERT
PUITE

Construction of a special ionisation chamber has been started, its outer wall is made to rotate and a thin film of tissue equivalent liquid forms the electrode. The gas in the chamber is also tissue equivalent. The chamber will be used to measure neutron doses for different plant materials having different compositions.

Fast neutrons

The He^3 filled semiconductor sandwich spectrometer referred to in last year's report has been mounted in a shield of lead to reduce the γ-interference level in the neutron spectrum; in spite of this the lower energy end of the spectrum could still not be resolved. When the spectrum measured with the He^3 detector is normalised at the 5 MeV level to the spectrum measured with the Li^6 detector a good agreement is obtained down to 1.5 MeV where the γ-interference in the He^3 detector begins.
Work on two new types of neutron detectors using the Li^6 reaction has been started: the prototype of one detector has been constructed and will be evaluated during the coming year. A theoretical appraisal of the other detector was encouraging but a number of practical problems still have to be overcome.

M I C R O - B E A M I R R A D I A T I O N

α-particle irradiation

GILLES*

A new technique has been developed for the preparation of more uniform Po^{210} microneedles. Originally the Po^{210} deposition was carried out by electrolysis using dry batteries to give 6 volt potential, and it was almost impossible to predict the amount of Po^{210} deposited; the fixation of the Po^{210} was also poor. A d.c. supply giving a current of 100 μA gave a very regular deposit of Po^{210} and an acceptable fixation.

UV-irradiation

The UV microbeam equipment referred to in the 1965 report is normally used with a 1 μ diameter spot of UV having a wavelength of 2750 Å. A 10 μ diameter spot is used to control the focus and as a criterium of efficiency, since a 10 second irradiation with a 10 μ spot destroys the nucleus completely. Recent developments have indicated that the sharpness of the microbeam can be further improved,

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GILLES

by avoiding scattering and spreading of the beam. Improvements involve the use of a new quartz lens and the use of a uranium or aluminium mirror with the dichroic filter.

β - MEASUREMENT SYSTEM WITH SEMICONDUCTOR DETECTORS

DE SWART*

A new β-measurement system with semiconductor detectors for use in biological research has been developed. In this system, background noise is eliminated electronically using an anticoincidence method. Noise pulses coming from the mains are collected with a special filter, fed through a separate amplifier and pulse shaper, and together with the output pulses of the detector, into an anticoincidence circuit. Initial noise reduction was ± 5% of the original value. Further improvements were made by applying adjustable pulse rise and fall times in the pulse shaping circuit and the noise was further reduced to less than 0.5% of the original value. The system expanded to accept four detectors has been successfully tested for use in biological experiments.

* Association EURATOM - ITAL, Wageningen, the Netherlands.

THE BEHAVIOUR OF SPECIFIC NUCLIDES IN PLANTS AND SOILS

As in previous years, studies in this facet of the research activities of the Association have considered the uptake of various elements through leaves and roots of beans and oats. The distribution of Sr and Cs in grass and soils from 8 locations in the Netherlands, as well as laboratory studies on the distribution of these elements in soil columns have been ascertained. The joint project with the EURATOM Biology Section at Ispra (Italy) and the 'Centre d'Etudes Atomiques' (CEA) in Paris has developed further and samples from the 6 locations in France, Germany, Italy and the Netherlands are included in the current research programme.

Physical studies related to these plant and soil investigations with radioactive isotopes have considered factors influencing the optimal use of autoradiography at the light or electron microscope level, improvements in the assembly and use of semiconductor detectors as well as the development of required equipment.

STUDIES ON FOLIAR AND ROOT UPTAKE IN BEAN PLANTS (PHASEOLUS VULGARIS)

LEVI[*]

Investigations on the penetration, transport and distribution of various chemical elements applied to primary leaves of whole plants grown in water culture under optimal environmental conditions have been continued in 1966.

Foliar uptake of monovalent cations

The pathway and distribution pattern of K, Rb and Cs applied to single primary developing leaves has been followed in periods of up to 7 days after treatment. Samplings made in the 1 - 48 hour period following treatment indicate a relative similarity of penetration although Cs appeared to penetrate slightly more than either K or Rb. Quantities accumulated at the point of application were about equal for K and Rb and in both cases smaller than for Cs. Movement of all three elements appeared to be at first downwards through the midrib of the treated leaf to fraction of the root systems and later upwards to the same treated leaf before a slightly generalized distribution occurred. While no significant loss of K to the solution could be registered up to 96 hours after treatment losses of Rb and Cs

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LEVI

were significant 24 hours after start of the experiment, reaching up to 6.5 and 8.1% respectively after 7 days. Preliminary results available indicate that simultaneous application of K with Cs or Na did not influence the penetration or distribution of the other ion. However the quantity of K circulating in the plant was higher for K applied alone or with Na than when applied with Cs.

Na penetration and downward transport was found to be similar to that of K, Rb or Cs. Its upward transport however was much less important and slower. Losses to the solution were very much greater than for Rb or Cs, reaching in one case 47% of the total activity applied to the plant, 7 days after treatment. Accumulation at the point of application decreased steadily with time. This was not the case following K, Rb or Cs application. The total quantities of Na transported within the plant were slightly higher than for the three other ions.

Foliar uptake of divalent ions

Besides a normal penetration and accumulation at the treatment area, no significant movement of Ca, Sr, S and Zn could be detected in bean plants under the experimental conditions used (23°C and 50% relative humidity). Obviously no loss could be noted from roots since the radioactive elements never moved out of the treated leaf tip where it was applied.

Averages of the non-retained fractions of the Sr^{85} applied to primary leaves and which could be removed by washing with 10 ml of water, as well as the quantity retained in the treated area are shown in the following table:

Hours	Washing solution (% of total applied)		Treated leaf tip (% of total in plant)	
	Sr^{85}	Cs^{134}	Sr^{85}	Cs^{134}
1	95.4	83.1	92.2	94.5
2	94.8	86.8	96.6	95.9
3	89.5	70.9	97.7	93.9
4	83.1	52.4	99.8	94.8
5	92.4	43.2	94.1	95.3
6	84.5	49.2	96.3	94.8
9	77.5	-	97.3	-
24	75.5	-	94.5	-

LEVI

Influence of environmental factors on leaf uptake

Studies with Cs^{134} have been continued and considered the influence of temperature, humidity and air movement. Uptake and distribution at 15, 23 and 30°C and relative humidities of 40 - 60 and 80% have been studied. Results indicate that not one single factor but a combination of the three factors considered may be responsible for an increase or decrease in foliar uptake. The very marked effect obtained at different air circulations was striking, yet the Cs held in the treated area did not vary appreciably in proportion to the total in the plant. The same increased uptake in the absence of air movement was noted in the case of studies with Na.

Leaf-root relation studies

To determine leaf-root relations existing in bean plants and further confirm results previously reported the root system of normal plants was divided into 5 fractions i.e. mother root and 4 lateral groups, kept separate, individually aerated yet all in the same complete nutrient medium. Four days after application of Cs^{134} to one primary leaf, it was found that accumulation was greater in one group of lateral roots than in any treated leaf not considering the area of application. The total in the root system was found higher than that in the rest of the plant again not considering the treated leaf tip. Measurement of the activity in the solution of each root fraction showed a definite range with maximums significantly higher than background.
Similar studies were carried out with K and Na, and results indicate a similar pattern of distribution. With Na, leakage followed closely the distribution in the various root fractions, showing in one experiment losses from one group of lateral roots of almost 60% of the total Na excreted, while the Na accumulated in that group of roots had reached 50% of the total Na in the system.

Root-leaf relations

Autoradiographic studies have been continued considering the distribution of P^{32}, S^{35} and Ca^{45} in leaves of bean plants deficient in the element applied. Very localized distribution of P and S was noted in leaf segments similar to the ones mentioned in previous reports. Ca however was found again to move first in the whole vascular system.

STUDIES ON CALCIUM UPTAKE BY LEAVES AND ROOTS OF OAT PLANTS

RINGOET*

The penetration and transport of Ca^{45} following its application to a young oat leaf was studied. The uptake was found to increase linearly with the amounts of Ca applied within a range of 100 - 240 µg / application. No basipetal transport was observed when amounts smaller than 50 µg were applied. For amounts of up to 100 µg, the rate of accumulation in the tissue underneath the application site was relatively higher. At applications of more than 240 µg translocation to the leaf base was more than proportional to the amounts applied.
The influence of the transpiration rate of the leaf on the uptake of the foliarly applied Ca was determined by varying light intensities, covering various leaf parts or increasing the osmotic pressure of the culture solution surrounding the roots by addition of mannitol. Direct absorption at the application site was found to increase at higher light intensity while transport to the leaf tip was enhanced at higher transpiration rate. When the leaf tip was covered or the treated plant was kept at a higher osmotic pressure of the culture solution, translocation to the leaf base was found to increase.
Further experiments considered: the effect of the following calcium salts $CaCl_2$, $Ca(NO_3)_2$, $CaSO_4$, $Ca(OH)_2$, $CaCO_3$ and $Ca(H_2PO_4)_2$, their concentration, the volume of the drops, the influence of rewetting the site of application during and after drying out of the applied drop and finally the influence of K^+, Na^+, Ca^{++}, Mg^{++}, Sr^{++} ions when present with the calcium in the treating solution.
Results available so far indicate that 24 hours after treatment, only 1 - 2% of the total Ca applied (0.03 - 0.05 M $CaCl_2$) remained adsorbed to the leaf surface. The major part of this adsorbed Ca was found to be water soluble and a small fraction was exchangeable. Uptake of the Ca from solutions of different salts could be directly related to their water solubility. The amounts of Ca^{45} adsorbed to the leaf surface were small when other cations were present in the solution applied. This finding was also true for the transport to the leaf tip above the point of application. Differences between the effects of the various cations on Ca^{45} uptake and transport to the tip are small. Basipetal transport was noted below the point of application only when calcium or strontium ions were present at high enough concentra-

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tion in the foliar applied solution. Calcium uptake from drops applied to young oat leaves appears to start at a very fast rate (isotopic exchange) while absorption during a second phase of the uptake process is slow and directly proportional to the concentration of the applied solution. In the experimental conditions used, the duration of this phase was found to be 4 to 6 hours depending on the drying-out time of the drop by evaporation. Rewetting during this stage delayed the start of a third phase of rapid calcium uptake. This third phase appeared to start when the applied drops were almost dry. After 8 to 10 hours the uptake of the foliarly applied calcium was completed. This final uptake did not appear to be due to a damage to the cuticle or the epidermal cells by the concentrated solution present on the leaf surface at the end of the drying-out process of the applied drops. No such damages or plasmolysis of cells could be microscopically observed at concentrations of up to 0.05 M $CaCl_2$ (100 µg).
The uptake process showed the same general picture at very low concentrations of the foliarly applied solutions (0.5 to 10 µg Ca).

GIELINK[*]
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The movement of foliarly applied Ca in oat leaves has also been followed by histoautoradiographic studies of the various leaf parts at different periods (2, 4, 8, 12, 24, 30 hours) after treatment. Calcium was first observed in the epidermis and mesophyll cells under the application site. Its transport to and presence in the xylem vessels of the leaf tip was ascertained. There, at first, its movement out of the xylem vessels into the second ring of thin walled bundle sheath cells surrounding large differentiated vascular bundles was noted. Lateron, calcium moved into the mesophyll and epidermal cells of the leaf tip where it accumulated in particular groups of cells probably as complex organic phosphates or pectates.

Uptake of Ca by roots of oat plants

A histoautoradiographic study of the location of Ca^{45} in the root tip and zone of secondary root formation of oat plants, 2 hours after uptake had started from an aerated complete mineral solution and from a petridish receiving the isotope in locally applied drops was carried out. Results indicated that Ca was mainly accumulated at the surface of the root caps while none could be detected in the meristematic region.

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RINGOET

It was found at the root surface in the epidermal cells and in the xylem vessels in the zone of differentiation of secondary roots (see also page 70).

In vivo localization of β-emitting isotopes by energy absorption

MELLONI*
RECHENMANN*
RINGOET

The energy absorption of the electrons of a continuous β-spectrum by living tissues results in the change of the shape of the spectrum and in a shift of the maximum energy to the lower energy region. The shift of the maximum energy has been chosen as a criterium for the energy loss which is related to the average tissue thickness crossed by the electrons. The maximum energies of the 2 spectra (with and without absorber) are given by the intersections of the quasi-linear parts of the Kurie plottings with the energy axis.
The semiconductor spectrometric assembly described in the previous reports allows with this method to measure tissue thicknesses in the range of 5 - 35 mg / $_2$cm^2, with an error obviously smaller than 5 mg / cm^2, when Ca^{45} is used as a β-emitter.
An attempt has been made to localize Ca^{45} following its uptake from oat leaves. Results were in agreement with the picture described in the autoradiographic studies.

Distribution and accumulation of Sr^{90} and Cs^{137} in soils and grass under normal agricultural field conditions

FRISSEL*
POELSTRA*

The study of the fate of these major fallout nuclides in grasslands started in 1959 has been continued and expanded to include now 8 locations within the Netherlands and 6 within other countries of the European Community. The adsorption isotherms of Sr and Cs have been determined to ascertain their linearity. The amounts adsorbed at equilibrium concentrations of 0.03 and 0.3 μC Sr^{85} / ml were measured and all isotherms appeared linear. The present Sr^{90} concentration being 3 x 10^{-6} μC / ml it is apparent that the results obtained can be extrapolated to field conditions, excluding extra selective adsorption at very low concentrations. Measurements of Sr^{85} 1, 9 and 31 days after treatment indicated a constant adsorption in a reclaimed peat soil (Schonebeek), a podsol (Hannover) and a Graulehm (Ahrweiler) while in a terra rossa (Bari), a claysoil (Porto Tolle), a löss (Amiens) and a calcareous soil (Amiens) which contained $CaCO_3$ the

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adsorption decreased considerably during the first 10 days. The difference may be ascribed to the $CaCO_3$ going in solution during the measurements, increasing the Ca concentration and thus decreasing the distribution ratio of Ca between solid and liquid phases. This results eventually in a desorption of the adsorbed Sr^{85}.
The adsorption of Cs is much stronger than that of Sr. Differences between the 1, 9 and 31 days measurements were not significant. From the adsorption curves obtained it is apparent that Cs in soils with a high organic matter content is much less adsorbed than in other soils, resulting in a much higher uptake factor. Because the rate of leaching is very low an accumulation of Cs may be expected in all soils. So far, it has been assumed, generally, that Cs was fixed so strongly by all soils that its presence could hardly create a dangerous situation; peaty soils may be an exception to this rule.

Studies with undisturbed soil columns

The purpose of these experiments is an attempt to describe the transport phenomena in undisturbed soils. A method has been developed to bring into the laboratory for experimentation under controlled conditions profiles of 11 cm diameter and 100 cm length. A rain simulating installation allows to vary the rate and quantity of water delivered and a specially designed scanner is used to determine the location and accumulation of the isotope considered (see also page 74).

Determination of the labyrinth factor by diffusion measurements

FRISSEL
POELSTRA
VAIDYANATHAN*

Methods of measurement of diffusion coefficients of ions in moist soil blocks were standardized. Diffusion coefficients of traces of Sr diffusing into moisture saturated pastes of Ca-saturated Dowex 50 mixed with sand and Ca clay mixed with sand were determined for use in the calculation of Sr traces distribution in columns subjected to leaching. Satisfactory agreement was found for the resin - sand columns between calculated and experimentally found values. The labyrinth factor γ, calculated from the column experiments was 0.69, the experimentally obtained value was 0.72 ± 0.05.

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Void-volume determination with Tritium

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POELSTRA
VAIDYANATHAN

Further experiments were carried out to determine the void-volume from the breakthrough curve of tritium (as H_2^3O). The calculated and determined void-volumes were found to fit well. This method will therefore be also used for the unsaturated soil columns. It is also possible to calculate the dispersion which occurred in the solution flowing through the resin bed. Dispersion has the dimension of a diffusion constant and is most times combined with it, according to:

$$D_{\text{apparent in column}} = D_{\text{apparent}} + \lambda.d.u_w$$

where λ = the packing or dispersion parameter,
d = the particle diameter and
u_w = the interstitial velocity.

An analysis of the breakthrough curve of tritiated water resulted in a λ of 1.5. The mean value found for Sr was 2.1.

NUCLEAR TECHNIQUES IN AGRICULTURAL RESEARCH

Sample preparation for histoautoradiography

GIELINK

The freeze-drying technique described in last year's report was further developed. The sample container of the micro freeze-dryer was rebuilt for metacrylate embedding. During freeze-drying the metacrylate is solidified and has a temperature of approximately -180°C. On the moment of embedding it is warmed up to liquid state at -80°C, while the vacuum is maintained.
The main advantage of using metacrylate lies in its ability to penetrate plant tissues. The embedded material can then be used immediately for slicing with an ultramicrotome. Losses of Ca^{45} and C^{14} were found to be very low when the method was properly used.

Measure of double labelled proteins

KERKHOF*
DE SWART

To measure the interaction between some milk proteins K- and β-caseine were labelled with respectively I^{125} and I^{131}. Both proteins were mixed, separated by paper-electrophoresis, and the radioactivity of the proteins determined. A technique to measure selectively the radioactivity of the 2 iodine isotopes was developed using a scanning system with 2 special types of scintillation detectors and a γ-spectrometer. It is possible to measure selectively the 2 labelled components with a neglecting cross influence effect of less than 2%.

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Electron microscopic autoradiography

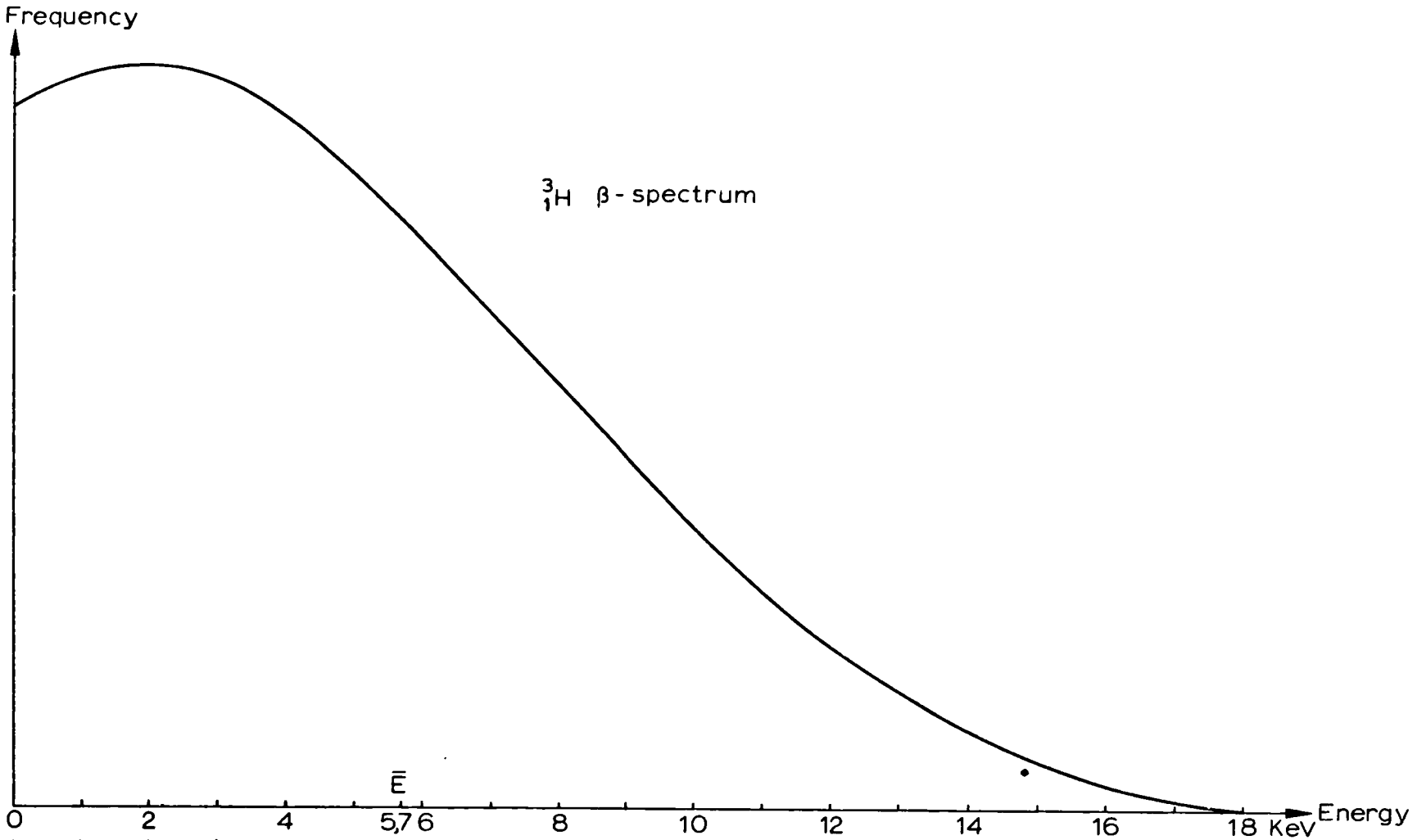
RECHENMANN

Theoretical and practical studies on resolution in electron microscopic autoradiography have been undertaken. In the first part of this study, the average number of grains touched by a β-particle of a given energy has been determined following a semitheoretical procedure suggested by LONGCHAMPS and GEGAUFF (1956). The probable range has been calculated by the formula of FELDMANN and BARKAS (1965) $R = 2 \times 10^{-2} W^{1.8}$ μm (W = electron kinetic energy) and compared with the values of the range-energy tables. Results (figure I) indicate that the probability of 2 or more grains in the emulsion to be touched by the electrons emitted by tritium was high. This may explain the so-called blobs reported by some authors as 'contamination' during development. In fact they can be due to the development of adjacent grains crossed by the same electron. They also confirm that the ranges of the H_1^3 electrons in emulsions are an important factor to be considered in the determination of the resolution in electron microscopic autoradiography.

A technique of embedding processed photographic emulsions in Araldite has been developed. It permits to include developed nuclear emulsions preliminary exposed to a tritium source and to cut them with an ultramicrotome for light- and electron microscopical observations. Preliminary measurements have shown that the average range of the electrons emitted by tritium is of the order of 0.5 μ, the maximum range being superior to 2 μ.

Emulsion thickness measurement

In electron microscopic autoradiography the thickness of the unprocessed emulsion has to be accurately known, particularly when more than one layer of grains are applied. An apparatus has therefore been developed to determine with fair accuracy the thickness of unprocessed emulsion. Monocinetic α-particles crossing an emulsion layer loose a fraction of their energy which can be measured by a seminconductor detector connected to a spectrometric assembly. The energy response of the detector being linear, the fraction lost is given directly by the displacement of an α-peak. The crossed thickness can be directly obtained from a computed range-energy curve with an accuracy of 0.03 μm. The instrument allows to make successive measurements automatically, following a preset programme.



10,72	10,95	10,95	10,6	9,9	9	8	6,97	5,88	4,88	3,79	2,8	2,16	1,55	1	0,55	0,25	0,05	% of emitted β's in 1 KeV interval
0,02	0,07	0,145	0,24	0,365	0,5	0,68	0,85	1	1,3	Range	$R = 2 \times 10^{-2} W^{1,8}$ µm (*)							
0,155	0,46	0,91	1,45	2	2,8	3,63	4,45	5,52	6,5	L 4	Theoretical number of crossed grains (**)							
0,2	0,67	1,4	2,5	3,2	4,3	5,9	7,7	9,3	11	Nuc 3.07								

Figure I: Spectrum of e^- emitted by tritium and corresponding theoretical ranges in nuclear emulsions or number of crossed grains.

Semiconductor detectors

RECHENMANN

To facilitate biological experiments with semiconductor detectors, a miniaturization of the related electronic equipment is advisable. Using a design suggested by SMITH and CLINE, the construction of small solid state preamplifiers (90 x 25 mm) with an equivalent noise of 3 KeV at 0pF input capacity has been initiated. A highly stabilized semiconductor assembly with pulse generator reference has been developed for spectrometric measurements. This assembly will permit thickness measurement by α-absorption and localization of β-emitting isotopes by energy absorption. A long-term stability of 0.2% could be reached.

DE SWART

β-measurement system with semiconductor detectors

(see page 62).

Milk monitor

STOUTJESDIJK*

In cooperation with the Service Institute for Applied Mechanics and Technical Physics in Agriculture (FTDL) in the Netherlands, a milk monitor was developed for routine measurements of I^{131} and Sr^{89} in milk, for emergency situations. The first design consisted of a lead castle with two GM dip counters, a β-counter for Sr^{89} measurement and a β-γ-counter provided with an aluminium absorber for I^{131}. Under normal conditions solutions with 0.01 μC / l Sr^{89} and 0.05 μC / l I^{131} gave countrates equal to background. In tests with higher backgrounds, under the core of the 'BARN' reactor and with a Ra^{226} source, giving an environment with a dose rate of 50 mr / h Sr^{89} could still be detected with sufficient sensitivity. This however was not possible with I^{131}. Even slight increases in background reduced appreciably the sensitivity for this isotope. The replacement of the β-γ dip counter by a scintillation counter with a 1.75 x 2 inches NaI(Tl) crystal gave an increase in sensitivity for I^{131} by a factor of 50. In an environment with a dose rate of 50 mr / h obtained with a Ra^{226} source an I^{131} solution with 0.07 μC / l gave a countrate equal to background.

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Rain-simulator

DE SWART

A rain simulation system has been developed to supply accurately controlled amounts of liquids to soil columns. Volumes of 35 to 560 ml / 24 hours had to be supplied on 25 spots within a 14 cm diameter area with an accuracy of better than 10%. Using a pulsed system, controlled by an electronic timing circuit 25 small pots are filled with liquid and unloaded with compressed air through capillaries. The supply per cycle approximates 6.3 ml while the time interval between cycles can be adjusted from 5 - 10^6 min in steps of 1 minute. Accuracy measured on the prototype was better than 1% (see also page 69).

Temperature measurement

A six channel temperature measurement system having an accuracy of 0.1 ^{o}C has been developed for measurements in controlled plant environment rooms and constant temperature rooms. The need for a fast response coupled with the required accuracy determined the use of miniaturized thermistors mounted in an AC bridge circuit. The thermistors have normally different calibration curves and a special correction circuit has been made to normalize the six channels to within 0.1 ^{o}C.

OTHER ACTIVITIES

The organization and coordination of mutation breeding has led to a number of projects carried out in cooperation with plant breeding institutes or private plant breeders. The primary role of the Association is to provide advise and treatment of the plant material supplied by the other party. The number of actual practical mutation breeding projects on January 1st, 1967 amounted to 68 in which 46 different crops were involved.

Food preservation by means of irradiation has increased in momentum as far as commercial interest is concerned. In 1966, requests for advise on possibilities offered by this method have been received from a number of industries. A pilot plant of the 'Proefbedrijf Voedselbestraling' is in construction near the site of the Institute, and its work will be connected to the food irradiation programme of the Association. Besides these advisory functions, the Association has made available its facilities and know-how to the Israel Atomic Energy Commission (oranges), the Institute for Fishery Products (fish) and the Central Institute for Food Research (meat). Other investigations using the facilities of the Association have concerned the effect of irradiation on cooking time of dried vegetables (Wilco N.V.), on insect desinfestation of groats (Koninklijke Lassie N.V.) and on sprout inhibition of different potato varieties as well as growth inhibition of potatoes after planting. Long term toxicity studies concerning wholesomeness of irradiated mushrooms and elimination of aflotoxins and aflotoxin producing moulds by γ- and electron-irradiation using the Association's facilities are carried out by the National Institute of Health in Utrecht.

In cooperation with the European Development Fund (FED) of the European Community the Association has carried out preliminary investigations on the suitability of ionizing rays for the preservation of meat and fish. These investigations as well as the technical advise given by the Association aim at allowing the construction of pilot plants in the Tchad and Ivory Coast Republics of Africa.

A course on the handling and possibilities offered by radioisotopes for plant and soil research was given at the Association's Institute and attended by scientific workers in the Netherlands.

The activities of the Health Physics Group have covered mainly the implementation of regulations concerning radiation protection and disposal of radioactive waste at the Association's Institute as well as in other laboratories in Wageningen, the Netherlands.

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APPENDIX I

MEETINGS, SYMPOSIA AND STUDY VISITS

Scientists of the Association have participated to the following meetings:

Collogue sur les mutations en horticulture, Gembloux, Belgium.

Erwin-Baur Memorial Lectures IV. Induced mutations and their utilization, Gatersleben, Germany.

Third International Congress of Radiation Research, Cortina d'Ampezzo, Italy.

Mutation breeding contact group, Wageningen, the Netherlands.

Fifth Yugoslav symposium on research in wheat, Novi Sad, Yugoslavia.

FAO/IAEA panel on preservation of fruit and vegetables, Vienna, Austria.

FAO/IAEA international symposium on food irradiation, Karlsruhe, Germany.

Second international congress of food science and technology, Warsaw, Poland.

FAO/IAEA study group meeting on the use of neutrons in seed irradiation, Vienna, Austria.

FAO/IAEA working group meeting on recommendations for the neutron seed irradiation programme, Vienna, Austria.

IAEA symposium on solid state and chemical radiation dosimetry in medicine and biology, Vienna, Austria.

Conference on radiation measurements in nuclear power, Berkeley Nuclear Laboratories, Glouster, United Kingdom.

Ninth international congress for microbiology, Moscow, USSR.

International EURATOM working group on soil-plant relations, Wageningen, the Netherlands.

FAO/IAEA seminar on the use of radioisotopes and radiation in dairy science and technology, Vienna, Austria.

Sixth international conference on corpuscular photography, Florence, Italy.

During 1966, a number of study visits were made by scientists of the Association. They include discussions held at the

'Laboratoire de Génétique' and the 'Station d'Amélioration des Plantes' of the 'Institut National de la Recherche Agronomique' in Paris, Dijon and Montpellier in France;

'Commissariat à l'Energie Atomique', 'Centre de Recherches Nucléaires', Saclay, France;

'Max-Planck-Institut für Züchtungsforschung', Köln, Germany;

'Plant Breeding Institute of Weilbullsholm', Landskrona, Sweden;

'Research Laboratories of the Pyrethrum Marketing Board', Nakuru and the 'Pyrethrum Research Station', Molo in Kenya.

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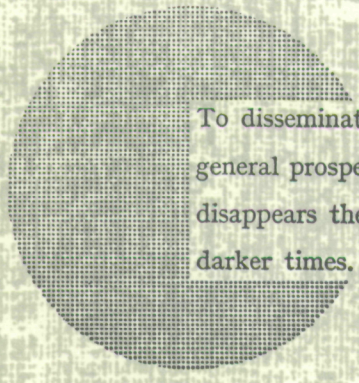

To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel

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