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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 1962)

1964

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

Association No. 003-61-5 BIAN

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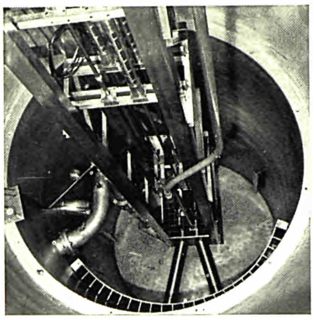

ANNUAL REPORT 1962

ASSOCIATION EURATOM-ITAL

ANNUAL-REPORT
1962

ASSOCIATION EURATOM-ITAL

Wageningen september / 1963

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INTRODUCTION

The first laboratory facility, viz. a part of the radiochemical laboratory at B-level, equipped with specialized measuring equipment for β-, γ- and stable isotopes, was brought into use last year. The first radiation source, an X-ray apparatus, was also installed. Our temporary accomodation at the Laboratory for Agricultural Chemistry was now no longer required. We owe the Board of Management of the State Agricultural University and Prof. Dr. A. C. Schuffelen and his assistants in particular a great debt of gratitude for the hospitality we have enjoyed for nearly five years. The construction of the reactor presented no difficulties and tenders were invited in the spring for the radiobiological laboratory. Provided there are no set-backs the reactor will be brought into operation in the spring of 1964. The last building stage, viz. the second part of the radiochemical laboratory, will most probably be concluded mid-1965.
To ensure proper and efficient collaboration at both national and international levels in the field of mutation breeding and food preservation by means of irradiation, sub-contracts were entered into with the following institutions:

Mutation breeding:

Afdeling Erfelijkheidsleer der Landbouwhogeschool, Wageningen
(Department of Genetics of the State Agricultural University, Wageningen)

Instituut voor Veredeling van Landbouwgewassen der Landbouwhogeschool, Wageningen
(Institute of Agricultural Plant Breeding of the State Agricultural University, Wageningen)

Afdeling Tuinbouwplantenteelt der Landbouwhogeschool, Wageningen
(Horticultural Department of the State Agricultural University, Wageningen)

Stichting voor Plantenveredeling, Wageningen
(Foundation for Agricultural Plant Breeding, Wageningen)

Instituut voor de Veredeling van Tuinbouwgewassen, Wageningen
(Institute of Horticultural Plant Breeding, Wageningen)

Max-Planck-Institut für Züchtungsforschung, Köln-Vogelsang, W. Germany
(Max-Planck Institute for Breeding Research, Cologne-Vogelsang)

Institut für Landwirtschaftliche Botanik der Universität Bonn, W. Germany
(Institute for Agricultural Botany of the University of Bonn)

Istituto Botanico della Università, Cagliari, Italy
(Botanical Institute of the University, Cagliari)

Food irradiation:
Instituut voor Bewaring en Verwerking van Tuinbouwproducten, Wageningen
(Institute for Research on Storage and Processing of Horticultural Produce, Wageningen)

Centre Interfacultaire des Sciences Nucléaires, Laboratoire de Génétique, de l'Université de Liège, Belgium
(Genetics Laboratory of the University of Liège, Belgium)

The necessary work contacts were ensured by setting up contact and project groups.
The international status of the Association was also consolidated by the engagement of two French biologists, thereby increasing the Euratom staff to four.
The present annual report includes a brief account of research and other activities and symposia and meetings visited by staff members and an article by Dr. Shapiro, a well-known expert in the radiobiological field and founder of mutation breeding work in the U.S.A. The inclusion of these kind of articles in our annual report is to be regarded as an attempt to give the reader a broader view of developments in a particular branch of science which is closely bound up with the research work of the Association. This article places particular emphasis on the status of radiation research in botany and agriculture.
Readers' comments on the annual report are welcome.

D. DE ZEEUW,
Director Euratom – Ital Association

Rosa 'Better Times' partly pink, after gamma irradiation by Dr. S. Shapiro at the Brookhaven National Laboratory.

Some comments on the status of Radiation Research in Botany and Agriculture

SEYMOUR SHAPIRO*)

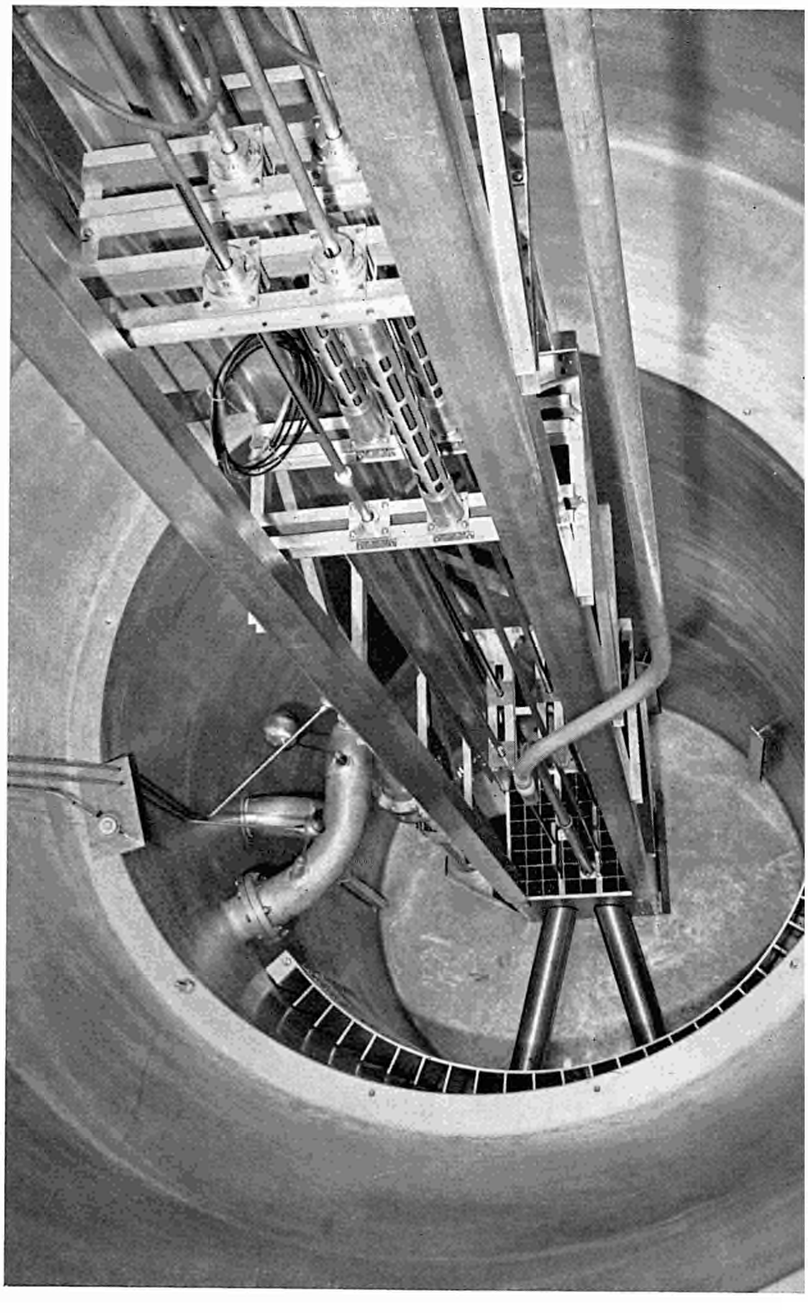
As the Institute for Atomic Sciences in Agriculture (ITAL) nears completion, with its chemical laboratories in operation, the reactor having been critical for the first time and construction of the botanical facilities well along under way, the time is perhaps appropriate to examine some facets of the field to which this new laboratory is soon to add its contribution.

The many ways in which atomic energy serves all of the other sciences have been enumerated and discussed repeatedly over the past few years. In modern agriculture we find the influence of atomic energy at every level of integration at which the plant (or animal) is considered.

At the level of the individual plant, our knowledge of physiology and metabolism has been enormously expanded since radio-isotopes of biologically important elements have been available on a large scale. Much information has been accumulated on a theoretical level and this has rapidly found its way into improved agricultural practices. To cite but one example, improvements in fertilizer composition and methods and timing of fertilizer application have been developed as a direct consequence of our better understanding of how various ions are absorbed and distributed throughout the plant. Use of the radioactive isotope is now commonplace and its position of importance among the tools of the agricultural scientist is thoroughly established.

When we direct our attention not at the individual plant but at plants as crop populations, one of the important areas of consideration is plant breeding. Radiation, because of its capacity to alter and rearrange the genetic material and thus produce mutations, has attracted considerable interest as a means of extending the limits of genetic variability. Radiations have provided geneticists, for the first time, with mutations in sufficiently large numbers to

*) Department of Biology, University of Oregon, Eugene Oregon U.S.A.



Interior of the aluminium pool; core construction with the two beam tubes.

enable them to systematically focus their attention at the basic nature of mutations. Plant breeders were quick to recognize the potential uses of radiation-induced mutations for crop improvement. A number of new radiation derived varieties of crop plants that are now being grown by farmers, in various countries, attest to the success of their efforts. Here again the interaction of theory and practice is evident.

At the level of the final agricultural product, the marketable commodity, we again find that radiation may play an important role to protect the crop from the serious ravages of insect pests and contaminating micro-organisms. Appropriate doses of radiation can disrupt the normal functioning of these destructive organisms and result in sterility or death. The transportation of some agricultural products may be simplified, and the range of distribution may be expanded, because of the findings that bulbs and tubers (e.g. onions and potatoes) intended as foods can be prevented from sprouting and that the ripening of fruits can be retarded by moderate doses of radiation.

These benefits and accomplishments of atomic energy, briefly mentioned above, have been realized despite a far from complete understanding of the interactions of living things and radiations. At all of the levels discussed our knowledge is still fragmentary and the practical applications have pressed close upon the limits of fundamental knowledge. In some instances the search for practical applications has passed the limits of available theoretical information. Trial and error experimentation has turned up responses to radiation exposure which are useful but about which there is little basic understanding. Under such circumstances efficiency is low and progress in utilization of these new phenomena is very slow.

Most of our information about radiation effects on living things comes from experiments with x-rays and gamma-rays, which are quite similar in their biological effects. Both are electromagnetic radiations, both produce relatively small amounts of ionization as they travel through tissue, and both respond in much the same fashion to factors which modify radiation sensitivity.

Neutrons are one of the particulate radiations and are unusual in their mode of action because, being uncharged particles, they may interact not only with the orbital electrons of the atoms they encounter as they traverse matter (as happens with x-rays and gamma-rays), but with the nuclei of these atoms. In this sense they

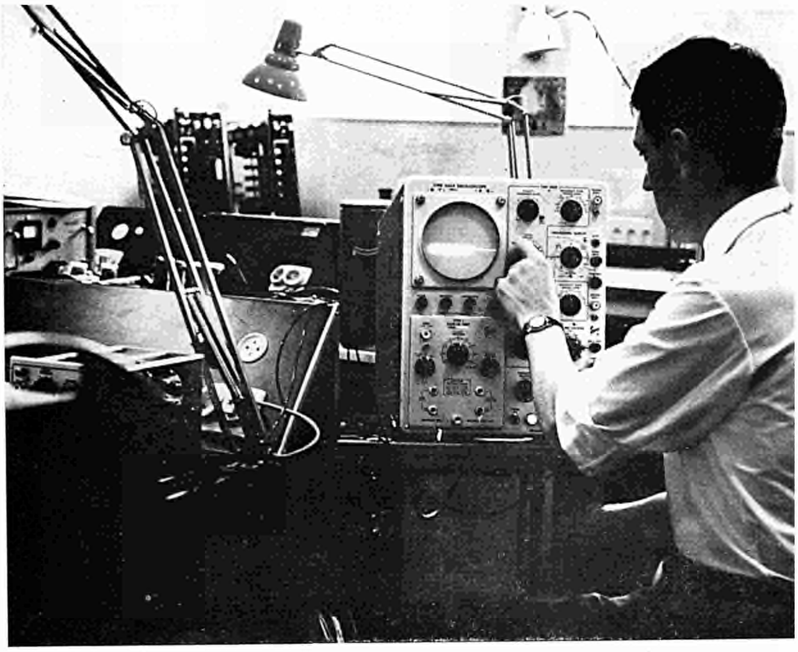
Reactor bridge driving mechanism and control plates.

are a unique form of radiation. In the interactions of neutrons with atomic nuclei other, charged particles and/or electromagnetic radiations are produced. These are emitted from the nucleus with which the neutron has collided and produce ionizations in the surrounding material. Thus ionizations do finally result from neutron irradiations but these ionizations are far more numerous and more closely spaced than is the case with x-rays or gamma-rays. In addition, as the neutron bombarded atom emits these secondary radiations, it becomes transformed (transmutated) into another kind of element. For example when the nucleus of a carbon atom is hit by a neutron a beta particle is emitted and the carbon becomes transformed into an isotope of nitrogen; similarly a phosphorus atom becomes a sulfur atom upon the emission of a beta particle. The latter moves through the irradiated material and effects ionizations until all of its energy has been dissipated.

By contrast with x-rays and gamma-rays, our knowledge of the biological responses to neutrons is very meagre. In a recent extremely thorough review of the biochemical, physiological, and morphological effects of ionizing radiations on plants (Gunckel, J. G. and Sparrow, A. H., Encyclopedia of Plant Physiology, 16 : 555-611, Springer-Verlag, Berlin, 1961), the authors prepared several tables in which the effects of various radiations are summarized. One of these lists the effects of various radiations upon the activity of 26 different enzymes. Of the 21 different scientific papers cited, only one was concerned with the effects of neutrons upon enzyme activity. Another table provides an extensive compilation of the morphological effects of irradiation of higher plants and contains some 538 citations. Only 12 of these, coming from but 10 papers, are from experiments performed with neutrons. The greatest bulk of the remainder come from studies with x-rays and gamma-rays.

The reason x-rays and gamma-rays have been used to extensively whereas neutrons have been the subject of only few experiments is related to the relative availability of these different radiations. X-rays are produced by relatively small and simple generators and gamma-rays are emitted by any of a number of radio-active elements (e.g. cobalt-60 and cesium-137) which can be safely handled by modest shielding.

Neutrons, on the other hand, are produced principally in atomic reactors which because of their size, complexity, potential hazard



Instrumentation and development department.

Control room reactor.
Instrument panels. In front the control desk.

and expense, have until very recently been restricted to a relatively few large atomic energy research centres. The *Biological and Agricultural Reactor of the Netherlands* (BARN), one of the principal facilities at ITAL can be expected to make an important contribution to our knowledge of neutron effects. BARN is a particularly exciting reactor to botanists and agriculturists because of its unique neutron irradiation chamber which is large enough to permit neutron irradiation of growing plants under controlled growth conditions for prolonged periods of time at relatively low dosages. Work of this sort has not been possible previously anywhere in the world. The significance of these chronic neutron irradiations will be enhanced by the possibility for having simultaneous chronic experiments with gamma-rays for comparative purposes and also facilities to compare such chronic irradiation experiments with others of an acute, or short term nature.

What are some of the gaps in our knowledge and what are some of the more serious questions which must be answered before we can expect to have a fuller utilization of atomic energy in biology and agriculture?

The use of radiations in mutation breeding was briefly mentioned above. Although, as stated, mutations of practical value have come from radiation treatment, the ultimate long-range value of this approach to plant breeding cannot yet be assessed. Those working in this field are unhappy about the largely random nature of radiation-induced mutations – the inability to predict or to direct the production of mutations in characters of the plant that are of major concern.

At present success is often dependent upon the ability to develop efficient selection techniques to sift the occasional beneficial mutation from among the large number of unaffected plants and from those with neutral or undesirable mutations. Attempts to increase the efficiency of mutation production (and thus to increase the likelihood that desirable mutations will be detected by the screening procedures) by altering the physiological conditions at the time of irradiation have not been as successful as originally anticipated. However, only a few of the possible means of accomplishing this have been explored and more research along these lines is necessary.

Although there are reports that the mutation spectrum of sparsely ionizing radiations, x-rays and gamma-rays, differs from that of

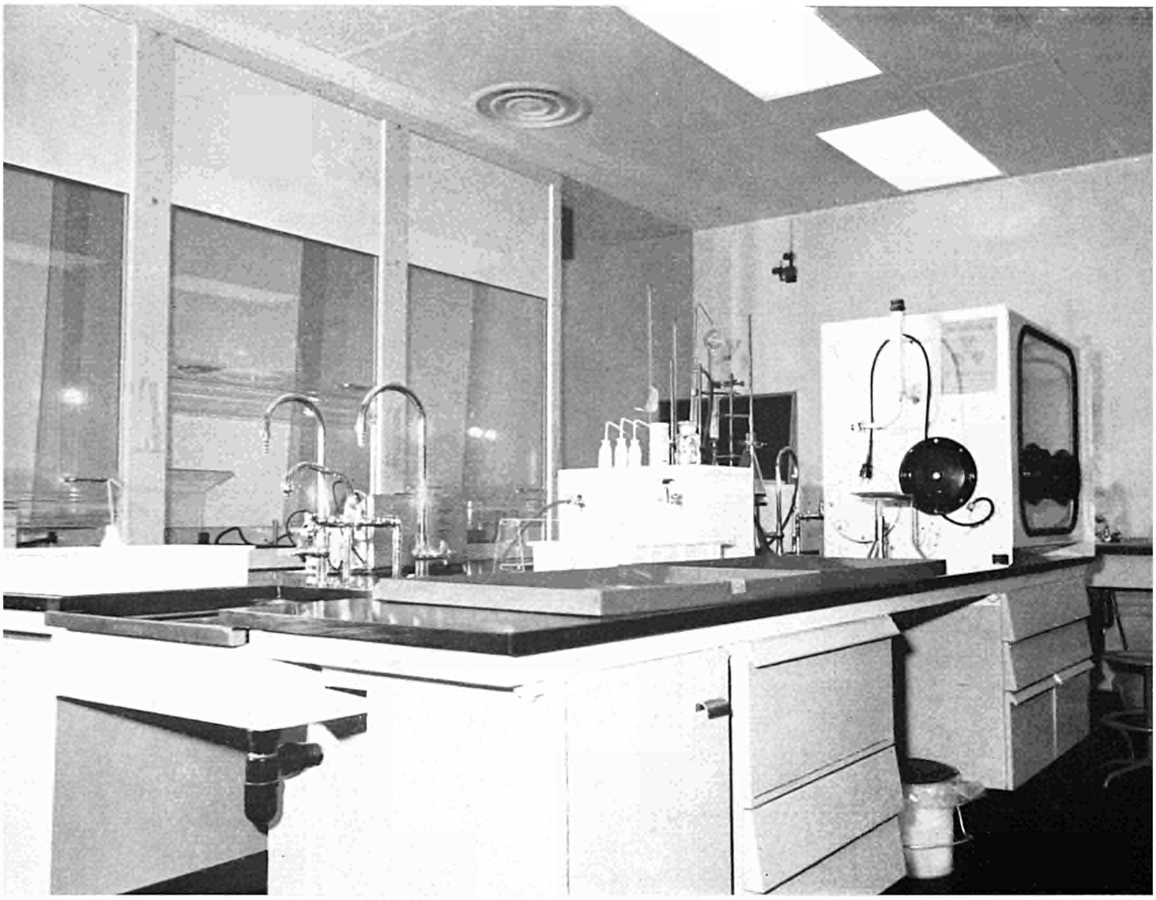
Bean plants in the growth chamber on a turning table.

the more densely ionizing radiations, neutrons, others fail to confirm this difference and the point is still under controversy. Further research conducted under more rigidly controlled conditions, such as will be available at ITAL, is required to resolve this important question. However, there appears to be agreement that the number of mutations that can be recovered from plants that survive treatment well enough to reach maturity is greater following neutron irradiations than from x-irradiation. This would impart a strong advantage to neutron irradiations. Should it finally be demonstrated that the kinds of mutations from both of these types of irradiation differ in important ways, then each would have its own advantages depending upon the objectives of the experiment.
One by-product of the interest in experimentally produced mutations, originally engendered by the radiation studies, has been a search for other mutagenic agents. A number of chemical mutagens have been discovered recently which appear to be far more efficient in their mutagenic action than are any of the ionizing radiations. When compared with ionizing radiations they not only produce more mutations but the spectrum of mutation types is quite different. It is thus possible that some degree of direction and specificity may be possible by the use of chemical mutagens. Thus far, to the best of my knowledge, the chemical mutagens have been used solely for basic studies and little is known of their effects on traits of economic importance.
More attention will undoubtedly be paid to these compounds by the practical plant breeder. Any final evaluation of mutation breeding will have to take into account chemical mutagens as well as ionizing radiations and compare both of these with the more conventional methods of plant breeding.
Present varieties of many of the major crop plants are the result of intensive genetic manipulation over a long period of time. The pool of variability has been exploited quite thoroughly through repeated recombination and selection, and natural mutation. The limits of genetic expression of some of these crops may already have been reached, or nearly reached for all practical purposes, and catalogued by the breeder. Under these circumstances major rearrangement of the genetic material through gross chromosomal aberrations (as can be accomplished by radiation treatment) followed by a return to a period of hybridizations may, in the long run, be a more profitable means of extending the range of variability

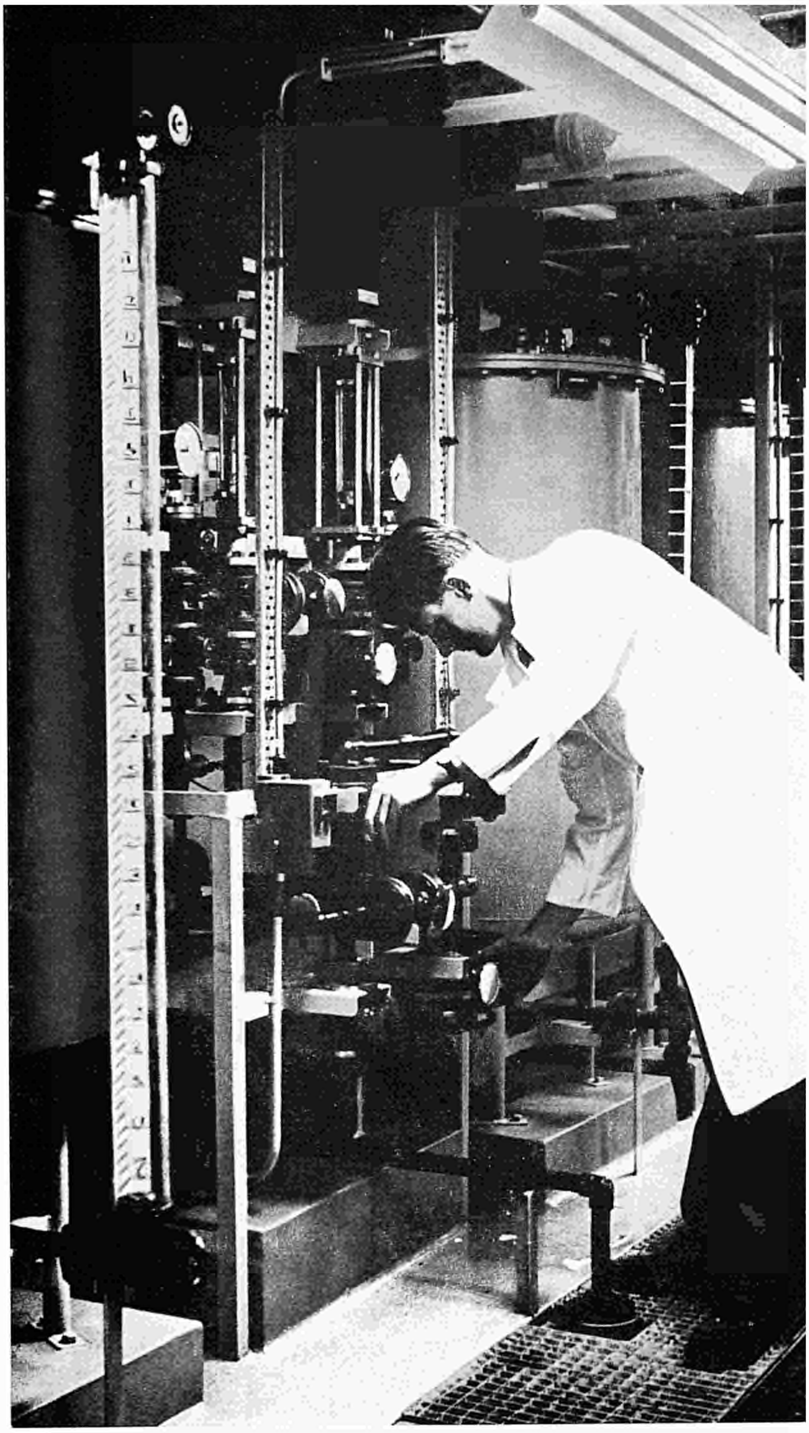
Laboratory in the radio-chemical department.

than by attempting to develop new varieties quickly by screening for particular single mutations soon after radiation treatment.
Moreover, it is interesting to speculate on the effect that radiations might have had on the development of plant breeding methods had they been available earlier, at the time when genetic theory was just beginning to be applied to the problems of crop improvement. If this speculation has any validity, an extension of it would indicate that it may be fruitful to utilize mutation breeding in those crops and in those countries where genetics is only now beginning to be seriously applied.
One of the vexing problems faced by those attempting to increase the number of mutations induced by radiations is that of the upper limit of radiation which can be tolerated by the plant without producing sterility or death. It is clear, from many studies, that mutations increase linearly with dose and therefore that the mutation yield could be raised very considerably if means could be devised to protect against the physiological damage which is believed to result in death before the full genetic effect of the treatment is realized.
It has been found that the sensitivity, particularly of dry seeds, is very much influenced by such things as moisture content and the oxygen tension of the tissues. These factors are important both during the time of irradiation and also in the period immediately following exposure. However, although manipulation of these parameters does alter the sensitivity of the material very considerably, it does not result in any appreciable separation of the physiological and genetic components of radiation damage. It should also be pointed out that the modification of radiation sensitivity by such factors as water content and oxygen tension apply to x- and gamma-irradiations but not to neutron irradiations.
Aside from studies of this type our knowledge of the physiological effects of radiation is quite meagre, particularly so with neutrons. The plant hormones, auxins, play an important role in controlling growth and differentiation yet virtually all that we know about how the auxin levels in the plant are altered following radiation treatment comes from the work of a single investigator. In these studies conducted with x- and gamma-rays, it was shown that the auxin, indoleacetic acid, was relatively unaffected by moderately high doses but that one of the enzymes active in the synthesis of indoleacetic acid was inactivated by low doses of radiation. Thus inter-

Laboratory with glove-box and fume-hoods.



ference with the continued production of auxin should be a consequence of exposure and irradiated plants should show symptoms of auxin deprivation. In some instances this is found but other radiation responses seem best interpreted on the basis of enhanced auxin production. Clearly many more studies of changes in auxin levels are required before we will have any degree of real comprehension of the role of this factor in the total radiation syndrome.
Researches on the effects of radiation upon respiration indicate that such changes do occur but that the direction and magnitude of these changes are not predictable. Some plant and isolated organs respond by an increase in respiratory rate while others exhibit an inhibition.
Therefore changes in the rate of respiration cannot be invoked to explain any given observed radiation effect without direct experimental evidence. Again we find a pressing need for re-examination and extension of the existing data, particularly with experiments performed with neutrons.
Radiation biology, as a relatively new area of study, offers many roads for research that are intimately bound to all other phases of biology and agriculture. One of the bonuses of work in any such new discipline is the totally unexpected response – the discovery which at first appears as an ancillary observation in an experiment designed for quite another objective, but which leads to fresh insights and developments. As additional research programs increase the total volume of effort in radiation biology we can look forward, not only to answers to the questions that are now being asked, but also to entirely new and unanticipated phenomena which will produce both theoretical and applied benefits.



Waste disposal; acid- and hydroxide storage tanks for regeneration of ion exchange column.

Activities

I. RESEARCH

In 1962, the EURATOM-ITAL Association research program dealt in particular with problems concerned with radiation induced changes in plants, food preservation by means of ionizing radiations, the behaviour of specific radionuclides in soil, plants and animals forming the 'food chain', and the use of radio-active substances in biological research.

RADIATION INDUCED CHANGES IN PLANTS

Mutation breeding of bean, pea, tomato and potato

This project, carried out in conjunction with a number of plant breeding and genetics institutes in Wageningen, concerns the study of the mutagenic effects of neutrons, X-rays, γ-rays and various chemicals on the crops mentioned above. It aims in particular to examine the influence of environmental conditions during irradiation on the nature and number of mutations resulting from irradiation.
The program has been extended by means of Association subcontracts to laboratories in other countries of the European Community. Special problems in the field of genetics and mutation breeding will be studied on peas and barley. This work will be carried out by GAUL (Mutation Laboratory of the 'Max-Planck-Institut für Züchtungsforschung', Köln-Vogelsang, W.-Germany) and by GOTTSCHALK ('Institut für Landwirtschaftliche Botanik der Universität Bonn', W.-Germany).
The working group on mutation breeding organized a meeting during which recent results of the joined mutation breeding project were discussed.

In 1962 the following observations were made in the research in progress:

BEAN:

The LD 50 dose of the variety 'Widusa' was found to be 10 Krad for X- and γ-rays, while the optimal concentration of ethyl-methane-sulfonate lies between 0.125% and 0.250%. Unfavourable weather conditions have stopped this year's work prematurely. This work was carried out by PRAKKEN and HILDERING of the Department of Genetics, Agricultural University, Wageningen.

PEA:

Seeds of the variety 'Elfjespeul' were irradiated with γ- and X-rays and neutrons or soaked for 24 hours in different concentrations of ethyl-methane-sulfonate (EMS). These treatments did not prevent the plants to flower normally although minor growth effects were noted. A widespread sterility was observed in the EMS treated plants, due to both pollen- and ovary-damage. This sterility increased with temperature at treatment time (WELLENSIEK, Department of Horticulture, Agricultural University, Wageningen).
The M_3-X-ray treated – tested for mutation frequency – gave an unexpected high percentage of wax-less plants. M_2-plants of the 'blue peas', which showed growth- or chlorophyll-aberrations, were grown and compared to normal plants for yields (SPECKMANN and HERINGA, Foundation for Agricultural Plant Breeding, Wageningen).

TOMATO:

Germination capacity was not decreased by irradiations of up to 100 Krads, but almost complete sterility was noted when dosages higher than 30-40 Krads were used. Two mutants had a chlorophyll-content per unit leaf area greater or smaller than the controls. Following tests with and without additional artificial light in winter before planting it appeared that the mutant with the lower chlorophyll-content had reacted in a markedly positive way to extra light. Nevertheless fruiting was delayed as compared to controls. The mutant with the higher chlorophyll-content showed normal growth but also delayed fruiting (VERKERK, Department of Horticulture, Agricultural University, Wageningen).
Although optimal dosages of EMS and ethylene imine (EI) as

chemical mutagens were determined, unfavourable weather conditions have not allowed to pursue this project further (PRAKKEN a.o., Department of Genetics, Agricultural University, Wageningen).

POTATO:

Although bud sprouting was previously inhibited with 2–4 Krads, results in 1962 indicate than 4–6 Krads were necessary. Various changes in shape and colour of the leaves were noted in X-ray treated 'Burmania' and 'Bintje'-varieties (FERWERDA, Institute of Agricultural Plant Breeding, Wageningen).

The uncovering and re-arranging of chimaeras

Induction of chimaeras of asexually propagated plants to increase variability of flower colour without altering the remaining geno- and phaenotype has been continued.
Pink flowered Chrysanthemum varieties 'Asta Lee', 'Breitner' and 'Hortensien Rose' have been selected. Only the last mentioned showed mutants from pink to bronze and red-brown flowers. Irradiation dosages higher than 5-6 Krads suppress rooting and may be lethal to *Begonia-* and *St. Paulia*-varieties tested. Lower dosages showed morphological and chlorophyll-mutations in St. Paulia.
A radiosensitivity study was carried out with the dark red flowering *Rose*, variety Baccara, and a flower colour mutation was found. The first flower was dark red with 3 pink petals. A new shoot at the pink side was forced to grow and gave a pink flower with 2 small red sectors (BROERTJES).

Radiosensitivity studies

Tissue cultures or cell suspensions of which all cells are in a certain phase may provide ideal possibilities to study stage sensitivity. Tissue cultures of *Chrysanthemum indicum* have been started and suitable culture media are being determined (SAUER).

RADIATION PRESERVATION OF FOOD.

Under normal conditions of temperature and humidity, ripe soft fruit deteriorates rapidly due to microbial activities and/or biochemical changes.
Studies on the increase of shelf life of soft fruit have been continued.

Tomatoes irradiated at dosages of less than 200 Krad maintained their firmness; higher dosages caused tissue damage and softening. Respiration studies indicate that the effect of γ-rays is greater than that of electron rays (1 MeV) (VAN KOOY).
A joint project with the Institute for Storage and Processing of Horticultural Produce (IBVT) has been started to screen irradiated fruit. Results so far indicate that, under experimental conditions:

Strawberries	: storage periods can be extended at room temperature by approximately 5 days following treatments with 250-350 Krads.
Raspbarries	: storage life increased 1-2 days following 200 Krads e-irradiation.
Cherries (Var. May-cherry)	: shelf life can be increased by 3 days following 300 Krads treatments.

Blackberries, redblack current, plums and cucumbers showed either small effects or damages (DOESBURG and STADEN, Institute for Storage and Processing of Horticultural Produce, Wageningen).

BEHAVIOUR OF SPECIFIC RADIONUCLIDES IN SOILS, PLANTS AND ANIMALS

The introduction of radio-contaminants in the food chain following nuclear weapons explosions or possible accidents in industrial and experimental power plants makes it imperative to determine the fate of iodine, ceasium and strontium in soils, plants and animals.

Movement of Sr in soils

An attempt to predict with existing theories the behaviour of Sr in soils on the basis of general physico-chemical factors using data available in the literature is being carried out. It combines factors such as rainfall, soil type, etc. and is intended to give an estimate of the flow of Sr^{90} as a function of time (FRISSEL).
Laboratory studies of the movement and distribution of Sr^{85} in soil columns are in progress. A preliminary experiment indicated that it is possible to measure the Sr in layers of 1 cm thick provided suitable shielding and window slit opening is applied (POELSTRA).

Uptake of Ca and Sr by plants under different moisture stress conditions

Experiments to study the absorption of Ca^{45} and Sr^{85} by oat plants under controlled conditions have been initiated. Results available indicate that at harvest time Ca is still in a water-extractable form. Relatively high osmotic pressures (up to 7.5 atm) do not affect Ca uptake by young plants. In older plants however Ca concentration in leaves decreases with increasing osmotic pressure. The Ca concentration in stems is not affected (RINGOET).

Foliar penetration and distribution of radio-active contaminants
Studies to determine the non-held fraction of foliar deposited contaminants have been continued. The retention of P^{32} and Rb^{86} by bean leaves with time was found to follow an S-shaped curve. Variations in root temperatures in as much as they affect the general metabolism influenced also the amounts held or transported but did not appear to influence the areas receiving the fed isotope. Leaf adsorption and absorption appear to be directly connected to the metabolic activity of the growing plant.
The absorption and distribution of Cs^{134} following foliar application to bean plants under controlled conditions has been studied. Preliminary results indicate a higher retention for primary leaves (33-66%) than for the subsequent ones (13-33%) or fruit (20%) of the Cs applied as a water solution (LEVI).

The effect of accumulation of Sr^{90} in geese in their natural environment
Carried out in cooperation with the Institute for Biological Field Studies (ITBON) this project has indicated in preliminary results that the level of Sr^{90}/gr. Ca in the skeleton of geese was 28 pC or approximately half the MPL bone burden in man. Younger birds appear to have a higher Sr/Ca ratio than older ones. Uptake of fresh Sr^{90} by young birds was approximately seven times greater than that by older ones (VAN DEN HOEK).

The influence of extra gifts of calcium to grass on the excretion of Sr^{90} in milk of cows (Carried out in cooperation with the laboratories of animal physiology and of soils and fertilizers of the Agricultural University, Wageningen). Preliminary results indicate a reduction of Sr content of milk of 10-35% when the Ca content of grass was doubled (BROUWER, VAN DEN HOEK, SCHUFFELEN)

USE OF RADIO-ACTIVE SUBSTANCES IN BIOLOGICAL RESEARCH

The increasing use of radio-isotopes in biological research calls for a constant improvement in methods and instrument development. An awareness and search for solutions to possible artefacts in faulty techniques must be considered. Improvements brought to the *macro-autoradiographic technique* following freeze drying of plant material have been successfully adopted during the past year for studies with P^{32}, Rb^{86} and Cs^{134}. Exposure of the films under pressure offers much clearer pictures even with strong β-emitters. No artefacts due to pressure were noted (LEVI).
Macro-autoradiograms of oven dried grasses showed an artificial accumulation of Ca at the ends of stem pieces and in the nodes. Freeze drying eliminates this artefact (RINGOET).
Micro-autoradiographic techniques using stripping films and liquid emulsions are being further developed (RECHENMANN).
In connection with cytological studies an attempt has been made to bring the emulsion into the cells. Difficulties have been met in the past year related to the toxicity of the silver ion to plants. Chelates of glycine and alanine are being tested as possible alternatives (SAUER).

Possible applications of *semi-conductor radiation absorbers* in biological research are being investigated. The small size, efficiency and possibility of measuring β-particles in a γ-contaminated environment at an extremely rapid impulsion time as well as the possibility to discriminate the detection of 2 β-emitters of different energies, make semi-conductors an attractive tool for biological research. Preliminary results are encouraging (RECHENMANN and DE SWART).

Attention was paid during the past year to *counting techniques* and *sample preparation*. The following reproducibilities were obtained:

End window G.M.:	energy of 0.35 MeV: 99%
End window G.M.:	energy of 0.25 MeV: 98% (Ca^{45})
Gas flow :	energy of 0.16 MeV: 97% (C^{14})
Liquid scintillation:	energy of 0.15 MeV: 99.5% (Ca^{45}, C^{14})
	(FRISSEL)

Considerable improvement in the determination of Cs^{137} was obtained using ammonium molybdo phosphate to separate the Cs through ion exchange and counting it at 661 KeV with a channel width of 12 V (POELSTRA).

II. TEACHING AND TRAINING

During the year under review a 4-weeks training course on 'Radio-isotope techniques in soil and plant sciences' was given.
Furthermore a 3-weeks training course for the Association's reactor operators was received at the reactor-centre at Petten, Netherlands. Again a number of the Association's scientific staff were given the opportunity to attend scientific meetings or to work abroad. A short description of these activities is given in Appendix I.

III. TECHNICAL AND SCIENTIFIC SERVICES

ELECTRONICS

A simple, cheap, battery operated monitor for use by scientists while working with radio-isotopes was successfully developed. It records radio-activity with an audible sound only and is equipped with a thin window G.M.-counter. The life of the battery is lengthened considerably by applying the principle of damped oscillation.
For low level counting, a special scintillation counter designed by the Association and built by industrial companies has been used with success. The background of this counter, which is of the well-type and has a shielding of old lead, is 0.88 counts per minute; the efficiency is equal to that of commercially available instruments. Within a reasonable measuring time it can count acitvities of for instance 5 pC Cs^{137} or 7 pC I^{131}.

MASS SPECTROMETER

The mass spectrometer is installed, and calibration of N^{14}-N^{15} has been completed. Phenol samples were successfully examined for impurities.

X-RAY MACHINE

Since the installation of a Philips 250/25 X-ray machine, much service has been given to other laboratories and institutes in the field of mutation breeding.
Technical assistance was also given to other institutes in the field of food-irradiation. For this purpose the 3 MeV electron generator of the Shell Laboratories at Amsterdam was kindly made available to us.

PUBLICATIONS

External Report No. 2:	Preservation of soft fruit by ionizing radiation in the Netherlands; a feasibility study – D. I. Langerak and D. de Zeeuw.
External Report No. 3:	The simultaneous determination of C^{14} and H^3 in biological material, using the Schöniger combustion technique – M. J. Frissel and W. E. Kisieleski.
External Report No. 4:	An artefact in plant-autoradiography – E. Levi, also Science **137** (1962) 3527, pp 343-344.

APPENDIX I

MEETINGS, SYMPOSIA, STUDY VISITS, ETC.

Members of the scientific staff attended the following symposia and conferences:
D. DE ZEEUW attended on 3 and 4 May the 5th session and on 14 and 15 November the 6th session of the OEEC/ENEA Study Group on food irradiation held in Paris. Brief statements were made by the various delegates on programs and progress of work in their own countries; a summary of studies and discussion on potato storage, a discussion of a proposal by the Australian delegate for the internationalization of their research program on fruit-juice irradiation, and a discussion of a report on the preservation of soft fruit presented by the Netherlands delegate were the main topics of the 5th session. The 6th session considered the feasibility of potato, fruit-juice and soft fruit irradiation, and the recommendations of a working party on food irradiation research. These recommendations dealt mainly with wholesomeness research. Official delegates from the United States and Canada were also present following a change in the organization.
H. HEKMAN represented the Association at the IAEA Symposium on Reactor Safety and Hazard Evaluation Techniques held in Vienna from 14-18 May. This symposium was designed to collect available information on the subject and discuss in particular the regulations and techniques developed for the prevention of accidents. Although in some cases there was no agreement, the general impression was that in all countries vigorous efforts are made to decrease possible hazards.
The Eucarpia General Meeting held in Paris from 21-25 May was attended by C. BROERTJES. This meeting of specialists dealt with a number of subjects. The most important papers for the association's research program were those of Dommergues and of Singleton. Dommergues gave a historical survey of chimera research and Singleton reported his experience with maize, in which various phases of development of the egg and the zygote were irradiated. On May 15 the Association organized a meeting for the Mutation

Breeding Working Group at Wageningen. This group (founded in 1959) aims at co-ordinating wherever possible research and exchange ideas and results. Six members of the group reported the results of their research.

C. BROERTJES and G. SAUER followed the International Symposium on Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells held in Leiden from 15-18 August. Most papers referred to experiments on Drosophila.

From 1-6 October D. DE ZEEUW attended the Scientific Days of the Centre National de Coordination des Etudes et Recherches sur la Nutrition et l'Alimentation at Strasbourg. The subject of these meetings was the use of radio-isotopes in nutritional sciences and that of ionizing radiations in food technology. The symposium gave a good survey of research work carried out in France on food irradiation. The Lyons Conservatome group acts as a centre for food irradiation and any scientist can obtain assistance from them on the subject.

An International Conference on Retention and Migration of Radio-active Ions in Soils was held at Saclay from 16-18 October and M. J. FRISSEL and P. POELSTRA were present. Experiments reported on radio-active waste indicate that Cs usually moves about 10 times as slow as Sr in soils.

M. J. FRISSEL and W. F. OOSTERHEERT attended a seminar on the practical applications of short-lived radio-isotopes produced in small research reactors on 5-9 November in Vienna.

Conclusions reached following this seminar were that for reasons of economy and efficiency it is usually only worthwhile to make very short-lived isotopes in one's own reactor.

The Symposium on Operation of Water-moderated Research Reactors, organised by the European Atomic Energy Society at Mol (Belgium) on November 19 and 20 was attended by W. F. OOSTERHEERT. This symposium was divided in 3 sessions: I. General reports and material problems; the reactors described in this section all use MTR-type fuel elements. These did not seem to cause much trouble during and after years of operation. II. Various measuring techniques. III. Operation and maintenance problems; the main operation problem seems to be an exact and reproducible power level measurement.

H. HEKMAN followed the IAEA Symposium on Neutron Detection, Dosimetry and Standardisation held at Harwell on 10-14 December.

Although many papers presented dealt with techniques already known in principle. some new methods developed in practice were demonstrated, for example the increasing use of solid-state detectors for flux measurements.

Members of the scientific staff paid study visits to the following centres:
12-13 February, G. SAUER at 'Physikalisches Institut der Bundesforschungsanstalt für Milchwirtschaft' – Kiel, to familiarize with procedures for making electron-micrographs of leaf surfaces.
25-28 May, C. BROERTJES at 'Institut National Agronomique', Paris, and 'Institut National de Recherches Agronomiques', Versailles, to obtain details of chimera research.
28-30 May, C. BROERTJES at 'Comitato Nazionale per l'Energia Nucleare', Casaccia.
September to November, G. SAUER at 'Laboratoire de physiologie botanique de la Sorbonne', Paris, to acquaint with and develop methods for tissue culture.
4-10 November, R. V. RECHENMANN and J. G. DE SWART at 'Centre de Recherches Nucléaires', Strasbourg, for technical details on semi-conductor detector techniques and information on radiation measuring instruments in general.

APPENDIX II

BOARD OF GOVERNORS AND ADVISORS

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APPENDIX III

STAFF OF THE ASSOCIATION

Director:	D. DE ZEEUW
Scientific staff:	
plant breeding:	C. BROERTJES.
chemistry:	M. J. FRISSEL
health physics:	H. HEKMAN
animal science:	J. VAN DEN HOEK
food technology:	J. G. VAN KOOY
plant physiology:	E. LEVI
reactor physics:	W. F. OOSTERHEERT
soil science:	P. POELSTRA
physics:	R. V. RECHENMANN
plant physiology:	A. RINGOET
cytology:	G. SAUER
biochemistry:	H. L. TEIJEMA (MISS)
Technical staff:	
Technical manager:	H. DIRKSE
Instrument development:	J. G. DE SWART
Documentation:	H. J. SONIES
Administration:	P. H. VAN NIEROP

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N.V. DRUKKERIJ TRIO • 'S-GRAVENHAGE

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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