MASSEY COLLEGE

An Investigation of the effects of Colchicine and Radiation on Citrus from the point of view of:

(i) Dose range effects

(ii) Cytological effects

(iii) Phenotypical effects.

A thesis presented in part fulfilment

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INTRODUCTION

This is a preliminary work on Citrus to lay the foundation for future long term irradiation work which would consist mainly of the improvement of the techniques, methods and types of pre-treatment to be required for future radiation-induced mutation work in Citrus breeding. To effect rare but desirable types of genetic as well as somatic change are the ultimate aims of this present research.

The investigations reported here deal with

(i) Dose range effects.

(ii) Cytological effects.

(iii) Phenotypical or morphological effects.

LITERATURE REVIEW

I. Ionizing radiations as the possible sources of producing useful mutations at very high frequency for crop improvement.

From the cytogenetical aspects of mutations, the analysis indicates that spontaneous and induced mutations are not fundamentally different, although their end results may turn out differently from case to case. In both, three main types of mutations are encountered:

(a) chromosome breakages

(b) intragenic changes (point or true mutation)

(c) direct rearrangement of chromosomes (crossing over)

The only difference between the two cases is their frequency of appearance. In the latter case, the mutants produced are not only higher in their frequency of appearance but also easily achieved as desired.

Goodspeed (58) in 1928 showed in his experiments on Nicotiana, that ionizing-radiation could greatly increase the mutation frequency of genes under certain conditions up to 10,000 times that of control. Gustafsson (28) in a series of experiments maintained that mutation processes can be experimentally controlled; that if cell environment were altered in different ways, or that irradiation effects were varied, quite different mutation and mutant types would arise. Thus "drastic" mutants which require a very strong irradiation effect upon the treated seeds would certainly arise without fail. Delaunay (7) in Russia working on ionizing radiation in wheat independently, supported the general idea that radiation-induced mutations were becoming valuable methods of plant breeding.

II. The types of radiations that are being utilized by radiation biologists for the production of radiation-induced

fast neutrons and slow neutrons and ultra-violet light. The U/V light is the only effective non-ionizing type of radiation while others mentioned are known as ionizing types.

2. Types of effects caused by ionizing-radiations. Lea (40) puts forward two theories for the effects of radiation on a biological system:i.The "Target Theory" and ii. The "Indirect Theory".

(i) The "Target" or the Direct impact theory presupposes that if a swiftly moving charged particle struck a complex of biological material, the biological function of the complex was altered or destroyed. This "direct-hit" is responsible for some specific biological effect. It is obvious to connect this theory with the chromatid or chromosome breakages caused by the ionizing particles traversing and breaking the chromosome or the chromatids.

(ii)The second theory i.e. the "Indirect effect"or radiation. It presupposes that the irradiation by ionizing radiation of water containing material causes ionizing and excitation of a portion of the H_2O molecules, thus highly reactive (H) and (OH) radicals are formed and they contribute considerably to the biological effects of ionizing radiation. Hence there is an indirect effect of radiation on moist tissue caused by these free radicals due to activated water. These radicals are chemically very reactive and can act as reducing and oxidizing agents.

It is believed that the "Indirect" effect of radiation causes the depolymerization of Deoxyribonucleic acid of the chromosomes resulting in "Stickiness" of chromosomes (noticeable at metaphase and anaphase). It shall suffice to mention that the destruction of DNA is caused by the removal of a critical NH_4 or OH group of bases, resulting in the cessation of DNA synthesis also caused by the destruction of an enzyme which brings about DNA synthesis.

3. Comparative difference of the different ionizing radiations. Brock (3) considers that different ionizing radiations differ in the pattern of ionization they produce in the cell.

This difference is associated with their biological effect. Neutrons (also α-particles) of high ion density radiations produce ionization in dense clusters, whereas X-rays, Y-rays and β-rays of low ion density radiations, produce scattered ionizations. When compared on the basis of total dose (ionization per unit volume) the high ion density radiations cause more genetic changes than the low ion density radiations. In addition to this quantitative difference there are also differences in the type of changes to both the genetic and physiological systems produced by the different radiations.

4. Effects caused by non-ionizing radiation. As for the non-ionizing radiation by U/V rays, it causes only excitation and photochemical reaction through selective absorption by cellular constituents, mostly nucleic acids, in producing genetic effects. The U/V rays do not penetrate tissues appreciably thus it is quite limited to its use only for irradiation of pollen grains.

5. The practical aspects of the ionizing and non-ionizing radiation in the production of mutations. Ehrenberg and co-workers (57) hold the opinion that X-rays and neutrons exert their effects in different manners - the latter chiefly by a direct action on the chromosome material and the former by means of combined direct and indirect effects. The end results of the irradiation will differ widely, depending upon the actual metabolic state of the irradiated material (seeds), temperature and O_2 pressure during irradiation, as well as the ionizing density of the agent used. Elliott (11) noted from others work that U/V rays apparently induce a higher frequency of so-called "point-mutation" per chromosomal aberration than do X-rays. Smith (59) in his experiment on maize pollen with U/V rays and X-rays shows that the former produce more frequently, though not exclusively, chromatid breaks and so-called point-mutations without associating with pollen defects. X-rays, on the other hand, induce in this material, in many cases, it not always,

extragenic alteration incidental to chromosome breakage.

6. The accessibility and the practibility of X-rays and Υ-rays from Cobalt^{60} source in the production of induced-mutation. Of all the radiations mentioned, X-rays are readily available in a range of energies from commercial machines. Radioactive Cobalt^{60} source provides the most widely used source of Υ-rays which differ in energy from and are consequently more penetrating than X-rays. Though both have high penetration compared with β- and α-rays, X-rays and Υ-rays are used to irradiate seeds or vegetative parts of the plant and the dosage can be delivered either over a short period (acute irradiation of 100r to 1000r/ minute) or by growing plants close to a continuous source of radiation, e.g. Co^{60} source, spread over the growing period of the plant (chronic irradiation, dose rate of 0.08r to 2000r / day). Such differences in technique can be important for the genetic effect depends on the total dose received whereas the physiological effect, as maintained by Brock (3), is influenced by the rate at which the dose is received.

III. Types of Chromosomal aberrations caused by radiations.

1. Lea (40) and Bacq (1) in their work on ionizing radiations conclude that ionizing radiations cause three general classes of chromosomal aberration depending upon the stage of nuclear development at the time of irradiation viz:

(i) Irradiation at resting stage results in chromosomes responding as though they were single strands - but at Meta- and Ana-phase, the induced aberrations involve both of the two chromatids. In general, the chromosomes aberrations induced are: Dot eletion, rod deletion, Dicentric and Ring chromosomes. Inversion and free translocation are also produced but usually are undetectable.

(ii) Irradiation at Prophase results in chromatid aberrations,

as at this stage sister chromatids are formed. Thus breaks can evolve at both or one of the sister chromatids at any locus - it may be deletion of only one chromatid or both chromatids to form iso-chromatid aberrations. The broken sister chromatids often reunite to form sister-reunions. In such cases, one of the pairs will be without a centromere and is termed an acentric fragment. Such fragments are inert and are left in the cytoplasm having no centromere for spindle attachment. Breaks and reunion of two chromatids of different chromosomes followed by illegitimate reunion will form dicentric fragments. Sister chromatid reunions may form ring or exchange chromosomes. The chromatid exchange also known as translocation is detectable at metaphase, while others such as chromatid deletion, iso-chromatids, dicentrics, rings etc. are recognizable at metaphase or anaphase.

(iii) Irradiation at very late prophase or pro-metaphase produces half chromatid or "sub-chromatid" aberration such as "stickiness" in Tradescantia microspore after irradiation of 50r to 400r of X-rays. This stickiness persists only for a short while depending on the total dose received. This includes half chromatid exchanges between chromatids between sister chromatids, rarely, half chromatid exchange between chromatids of different chromosomes as observed by Sax (45), and is usually observed at anaphase.

2. The types and the cause of stickiness.

The stickiness due to adherence of sister chromatids is referred to as "clamping" while that due to adherence of different chromosomes or chromatids is known as "stickiness". Darlington (60) postulates that chromosome stickiness by irradiation is due to the depolymerization of the Deoxyribonucleic acid (DNA) of the chromosomes. However, Kaufmann disagrees with this hypothesis, he assumes that X-ray induced chromosome stickiness can be due to

partial dissociation of the nucleoproteins and an alteration in their pattern of organization.

3. Bridge formations and their consequences.

Dicentric chromosomes which survive till the end of mitosis usually form bridges which join two chromosome groups together. However, these abnormalities are not important as they degenerate unperpetuated. Should these bridges break, however, and each daughter cell reclaim an almost equal amount of chromosome material, new dicentric chromosomes, formed by sister reunions after the reduplication of the broken chromosomes, result at the resting stage. These are of genetical importance, as these rearrangements of structure make it possible for chromosomal material to divide unequally giving daughter cells which differ in their genetical composition.

4. Stages of chromosome sensitivity to ionizing radiation.

As for the varied degree of sensitivity to ionizing-radiation of the chromosome, it shall suffice here to emphasise Bacq's (1) findings that the chromosomes at resting stage are the most resistant and that their sensitivity increases as the nucleus enters prophase. It increases several fold at early prophase and remains high during prophase of mitotic division. As for meiotic chromosomes, the sensitivity is greatest at meiotic prophase. The diplotene chromosomes of Trillium sp. show about 60 times the sensitivity of those at resting stage. This shows that meiotic chromosomes are more sensitive than mitotic chromosomes. This differential sensitivity of the chromosomes to radiation is assumed to be associated with chemical changes in the chromosomes and particularly with the concentration of DNA though it has not been proved.

5. Differences between Intra-genic changes and ordinary chromosomes aberrations.

Finally, Gustafsson and co-workers (32) point out that that there is no distinct boundary line between Intra-genic

change i.e. point mutation and chromosome break. They assume that a minor duplication, or a minor deficiency - possibly also a large one - may certainly originate as the consequence of intragenic changes. In other words, gene mutation may involve chemically an addition of material or loss of material.

IV. Factors which influence the radiosensitivity of an organism to irradiation.

1. Smith (60) in his studies with over eighty species of plants with ionizing-radiation concludes that the four factors in an organ that influence radio-sensitivity are its genotype, age of tissue, stage of chromosome and chromosome number.

2. Species differences. Differences in response to irradiation have been found among species, varieties and genetic strains within a variety. For example, a daily dose of 30r/day produced severe effects in the trumpet lily, whereas it required a dose of 6,000r/day to cause comparable radiation damage to gladiolus. Gustafsson (27) in his observations of radiation of seeds of various cultivated plants, reports that the "critical dosage" ranges from 5000r for seeds of sunflower to 90,000r for seeds of rutabaga. (Here critical dose is referred to as the dose that while still producing a sufficiently large surviving population for further observation, induces a maximum of mutation and chromosome rearrangements).

3. Seed size differences. Gustafsson (28) also discovered that seed-size plays an important role in the sensitivity to radiation. Large-seeded varieties suffer less from irradiation than the small seeded ones especially shown in barley grain treatment.

4. Chromosome size differences. Sparrow and co-workers (52) discovered that plants with very large chromosomes have a high radiosensitivity, those with smaller chromosomes tend to be less sensitive while those with fewer and bigger chromo-

somes are the most sensitive of all.

5. Differences of polyploidy. Polyploids are in general more resistant to radiation than related diploids, however, there are exceptional cases like wheat and oats which show that the frequency of induced chlorophyll seedling mutations is lower in the sp. with higher chromosome number.

V. The effects of pre-treatment to the irradiation of plant material.

1. Pre-soaking of seeds in water. It has been shown that pre-soaked seeds are more sensitive to X-rays than dry dormant seeds. Caldecott (5) in his research on "the effects of Hydration on X-ray sensitivity in Hordeum seeds", shows that this sensitivity decreases as the water content of the embryo increases from 4% to 8%. At this upper level a plateau is reached and further addition of water to the embryo (up to 60%) results in no additional modification of sensitivity. He points out that X-ray tolerance can actually be increased by "stepping" at both temperatures of 22°C and 0°C.

2. Effects of abnormally high O_2 pressure as pre-treatment. Ehrenberg and co-workers (57) show that when low doses of radiation are applied, chromosome aberrations and mutation increase with the dose and with a yield per dose unit that is determined by several factors such as metabolic state (moisture content), oxygen pressure and temperature. When higher doses were given however, there is a reduction in the frequency of genetical changes due to a cumulative physiological killing of the most radio-sensitive cells. It is also shown that in some cases, certain types of mutation can be induced by abnormally high oxygen pressure while others are not. In such cases an external concentration of about 20% O_2 is needed. Marked increase in the irradiation effects is observed with the change from air to O_2 (pure), which is perhaps due to the high oxygen consumption of the germinating seeds. This is particularly

so in the case of X-radiation which effects a mutation rate of 2.5 times that of neutron-radiation.

3. Using Colchicine as a pretreatment. Gustafsson and Nybom (30) in their experiments on combined effects on the mutation process of various chemical substances and X-rays, claim that X-radiation treatment with colchicine as pre-treatment can cause the origin of rare mutations which do not readily appear in X-raying of ordinary dormant seeds, while the more common types of mutants appear to be reduced by such an effect.

In the case of barley the rare mutants are the xantha mutant types which are caused by very low concentrations of colchicine as pre-treatment.

4. Low temperature-pretreatment and its effects. Nilan (61) in his findings shows that radiation-induced "injury" as measured by seedling height and survival to maturity, was reduced by low temperature but not affected either by O_2 or CO_2. He also shows that radiation-induced chromosomal aberration frequencies were increased by each of the gases applied at room temperature and were reduced by low temperature (-80°C). The combination of either gas with low temperature resulted in a much greater reduction. Finally he shows that radiation-induced mutation frequencies as measured by chlorophyll deficient seedlings amongst plants were not affected by either of the gases or by low temperature.

5. The effectiveness of the combination of low temperature and gases as pretreatment. That low temperature combined with either CO_2 or O_2 significantly reduces the irradiation-induced aberration frequency below that of low temperature alone suggests (as Nilan assumes) an interaction of gas and low temperature on the radiation-induced chromosomal aberration frequency. Nilan then puts forward two hypotheses to explain the fact that the induced chromosomal aberration frequencies are altered while the mutation rate remains

virtually constant, with the treatments used:-

(a) Chromosomal aberrations and "point mutation" are independent events, either of kind or of degree, and only the former are affected by the treatments accorded in his work.

(b) That the amount of ionizing effect, resulting in both gross aberration and "point mutation" is unchanged, but the amount of reunion of primary breaks is altered. In view of the present knowledge, some of which is reviewed, hypothesis (b) seems more likely - that the treatments do not alter the primary breaks but affect reunion. That is, low temperature in the presence of air, acts to facilitate restitution. This action is enhanced in the presence of CO_2 and O_2 but at room temperature the presence of the gases, or substances produced by these gases under X-radiation, react with the broken ends to reduce restitution.

VI. How and why radiations can be beneficially utilized to induce useful mutation for crop improvement.

Brock (3) in his conclusion states that although we have little control over the process of mutation, the biological results can be influenced by manipulation of the environment, choice of the material to be treated and the selection of techniques used. Variations in the water content of seeds or the gaseous environment and metabolic activity of cells are known to alter both the physiological and chromosomal changes occurring after X-radiation.

2. The importance of selection of the plant materials prior to radiation treatment for improvement. This success of any attempt at plant improvement by irradiation-induced mutation will be greatly influenced by the selection of plant material. The type of plant tissue to be treated depends upon the type of mutation desired, the reproductive system of the plant and the selection of technique used. More important still is the selection of the species to be improved. Induced mutation provides in the plant a fresh

burst of variability which is the restrictive factor in plant improvement. In some species, particularly the cross-fertilized, this factor is not the variability but lack of adequate selection techniques - so the provision of more variability in such plants does little to improve it. However, even in such species there may be a lack of a particular character (e.g. disease resistance) in which case mutation is justified.

Though most work has b_een done with self-fertilized plants, nevertheless there are quite a number of cross-fertilizing species being improved by induced mutation. Induction of specific mutations in vegetatively propagated plants of extreme heterozygosity and long reproductive cycles appears more promising than the conventional methods of breeding. Similarly the technique is promising where varieties lack a particular desirable characteristic, and in flowers and other ornamentals where the unusual mutant is of commercial value.

3. The optimistic view for utility of radiation in plant improvement. The early scepticism of Stadler of the utility of radiation induced mutation for improvement in plants has been completely disproved. Nilan (61) in his conclusion points out that the effects of radiation can be modified differentially, which is of immediate practical importance to the plant breeders who advocate and use ionizing radiations to provide viable and useful mutations. His data indicates that it is possible to maintain a radiation-induced mutation frequency and at the same time reduce the induced chromosomal aberration frequency and injury. Gustafsson (28) emphasizes that the mutation can be experimentally controlled. If cell environment is altered in different ways, or the irradiation effect is varied, this causes quite different mutations and mutant types to arise.

4. Production of rare mutations by irradiation. Gustafsson (29) claims that some very rare mutations can be induced after pre-treatment with weak colchicine solutions. He also claims that irradiation with low and medium dosages produces immediate X_1 heterosis.

5. Specific aims which can be obtained by radiation-mutation induction. Konzak (37) postulates that induced mutation by irradiation can offer the following advantages:

(a) Improvement in simple characteristics of a variety may be induced, such as resistance to diseases and viruses.

(b) Time required to effect simple improvements may be less than for any other method.

(c) Improvements should seldom be accompanied by undesirable alterations in other characteristics; should this happen however, a few back-crosses would restore the desired residual factors. Some mutants do have pleiotropic effects, but if valuable enough, they sometimes can be utilized such as X-ray induced good quality tobacco, but low leaf number.

(d) The possibility of duplication of desired improvement in characteristics known to be controlled normally by a number of genetic factors, such as induced mutations in barley, shorten plant height as well as rachis internodes length.

(e) To render the plants incompatible to disease attack through a biochemical action.

(f) Induction of mutation for early maturity or for hardiness, is quite feasible through a physiological action.

(g) High frequencies of mutation can be induced in polyploids as well as diploids. Polyploidy is useful for genetic buffering and permits the organism to withstand a greater amount of genetic damage.

VII. Some practical achievements obtained in certain crop improvement by radiation-induced mutations.

1. Rare recombination. The best known work on radiation-induced mutation is contributed by Sears (49,48). He has successfully transferred the leaf-rust (Puccinia trilicina) resistance of Aegilops umbellulata to wheat Triticum aestivum through an amphiploid Triticum dicoccoides x Ae. umbellulata which serves as a bridge. Sears finds that the resistance of Ae. umbellulata to leaf-rust is epistatic (dominant) to the susceptibility of wheat material. The amphiploid i.e. Ae. umbellulata x T. dicoccoides is produced having a full 21 pairs of chromosomes and also a single Aegilops chromosome which possesses the resistance and also a deleterious effect on the plant, paticularly on pollen formation. This new amphiploid with an added iso-chromosome involving the resistance-carrier is X-rayed shortly before meiosis and then used in crosses with normal T. aestivum. Of the 6000 offspring 150 were resistant and were normal; which must have involved most of one arm of Aegilops chromosome.

2. Affecting improvement by induced physiological action in the biological systems. Konzak (37) in his studies of various diseases of plants concludes that obligate parasites such as rusts, mildews or even viruses must live on, or in, and derive food only from specific hosts. Thus, if a mutation altered the availability of a food substance required by the pathogen, an incompatible or resistant type of host-parasite interaction would result. Such a mechanism does operate in nature in the apple scab fungus Venturia inaequalis. Konzak further points out that when a facultative saprophyte incites a disease, as in the case of the Victoria blight or root-rot disease of oats (Helminthosporium victoria) it appears that the toxic substance produced by the fungus kills the host tissue prior

to the fungus invasion. Or in the case of the wildfire disease of Tobacco, the pathogenic toxin acts as an anti-metabolite to the plant system. Resistance to this type of disease is induced by low doses of radiation, thus rendering the plants no longer subject to the toxic effect or to the metabolic interference. Konzak recommends Wheeler's method of inducing such mutants. The technique consists of harvesting the seeds from individual irradiated plants. These seeds are rolled in moistened paper towels allowed to germinate in a moist chamber for about 2 days, and then sprayed with diluted toxin solution, after which the seeds are left to germinate for another 3 to 4 days at 75°F to 80°F; the rolls are then opened. During the process the toxin kills the roots of all sensitive plants but does not affect those resistant individuals in the same lot. The resistant mutants are then transferred to the soil for propagation.

3. Induction of heterosis. One of the most striking heterotic effects of radiation is seen in the case of the production of Penicillium during World War II. From natural selection the high yielding strain gives 200 to 250 units per millilitre of cultured solution, but when the strains are X-rayed or treated by U/V radiations, the yield is up to 3000 units. Similarly is the case in the irradiation induced mutants which produce Streptomycine from Streptomyces griseus.

4. Inducing very early maturity in vegetable crop. (35) In Holland in 1957, radiation plant breeders have been successful in producing early-maturing peas from a normally very late variety by X-radiation of a low dosage. The late variety normally flowers from the 16th stem node, but in the mutant evolved from the R_2 line some flowered earlier while some segregated back to mother line. Some which are true to the R_3 and R_4 generation flower at the 8th and some at the 6th node. This means 13 and 28 days

earlier respectively.

5. Induced acceleration in initial seedling growth in vegetable crop. Kuzin (39) in Russia who worked on the "critical" dose of X-radiation on vegetable crops found that a very narrow optimum dose is essential to stimulate the growth of each crop which in turn has its own critical dose and when that particular dose is surpassed will cause an inhibitory effect. This acceleration of the initial stages of plant development is of primary importance since it may essentially influence the yield in the arid districts as well as those where the sowing period is limited. This acceleration of growth at the initial stage of plant development results in earlier ripening and a higher yield.

6. The use of radioactive isotopes in vegetable crop improvement. Kuzin (39) also studied the influence of a number of radioactive isotopes upon the growth and development of plants, particularly those on vegetable crops. His findings show that prolonged action of diluted solutions of radio-active β- and Y- emitters is more active than that of concentrated solutions with a lesser exposure time. The best result is with solutions of a mixture of β- and Y- emitters with an activity ranging from 0.2 to 0.5 mc/l.; The seeds being soaked for 24 hours. As a result of such treatment, the flowering of the crops takes place 2-5 days earlier and their yield increase ranges between 25% to 55% as compared to the mother strain.

7. Induction of short culm rice by radioactive P^{32} isotopes. Kainde (35) reports that the Japanese radiobiologists have been able to convert a normally long culm variety of rice to short culm form without impairing its productivity by treatment with radio-active P^{32}. Here the seeds germinated are grown for 14 days in $NaHP^{32}O_4$ solution with dose rate of 12.5 mc (microcuries) per seed which then absorbs the radioactive P^{32}. In the progeny test the short culm rice breeds true even in the F_4(i.e. R_4) generation without impairing the yielding quality.

8. Improvement of rice by X-ray treatment. Kainde (35) also reports that in Formosa, radiobiologists experimenting with X-rays to induce mutants in Rice,after three years of intensive research have evolved 37 promising lines derived from 6 of the 10 X-rayed varieties. The said promising lines consist of 20 high-yielding (normally morphologically as well as physiologically), 5 short culm, 3 erectoid of short culm with strong stiffness of culm, 2 early types. All these excelling their mother varieties in yield. When subject to varying levels of fertilizer trials especially nitrogen, 15 of them showed higher productivity than their mother varieties, one suppassing the mother variety by as much as 27%.

9. Overcoming the incompatibility factor in Brassica species by irradiation. In the same report (35) is also quoted that in the United Kingdom ionizing radiation workers exploited the destructive properties of radiation to overcome the natural barriers to hybridization that exist between many closely related species of plants. In the case of the genus Brassica, successful interspecific hybrids are produced after irradiation of either male or female gametes of one species which are otherwise incompatible by the conventional methods of breeding. Likewise, incompatibility is overcome in other crops. In a hypothetical explanation of this effect of irradiation it is assumed that the effects of certain modifiers, suppressors or inhibitors may be changed due to a certain "critical" dose of radiation which presumably acts by way of the indirect action of ionization (as explained), thereby allowing the expression of new characters with or without the heterotic effect.

10. Retaining a desired genetic complex by irradiation. Gregory (23) in his work with radiation on peanut (Arachis hypogea) points out that if an organism possesses a complex of desired characteristics which might be lost by out-

crossing, but can be retained by radiation. This is very true of certain inbred lines of corn (Zea mays).

11. Improvement on apples by ionizing radiation. Bishop (2) in Canada has been working on mutation in apples induced by X-radiation. This chiefly consists of dormant scions of diploid and polyploid apple varieties. The scions are X-rayed prior to grafting on grown trees by the "framework method". In his observations of the mutants he finds more morphological changes than genetical types. The morphological changes are of the type due to chromosome effects from radiation rather than gene effects and in general did not persist beyond the first season's growth. The type of gene mutations which he gets, as expected, are colour changes in fruits or possibly chlorophyll deficiencies in the leaves. Of economic importance are the colour changes in fruits - the presence of red overcolour. This, has been shown to be due to the action of one or more dominant genes. These genes, as emphasized by apple breeders and geneticists, appear to be additive in their effect, so that in varieties which are already partly red it is still possible to produce darker red sports. (In such varieties it is also possible to produce apples of a lighter colour which is a recessive mutation). These gene mutations resulting in bud sports probably arise in single meristematic cells of the shoot meristem and in most cases are probably confined to a single layer of the plant structure. This is the case of periclinal chimeras. Thus to affect the skin of an apple one of the outer layers of the tissue must be involved, possible the second. A genetic change in one of the internal layers being unseen is undetectable. Another striking change seen in fruit from the X-rayed scions is that most of them are of giant size and very irregular in shape. Some of these X-rayed scion branches in the 2nd year produce fruits of a more normal size. The explanation for this is that the 1st

year large sized fruits are produced largely from flower buds induced by the X-ray treatment, an abnormal growth; and those scions producing normal fruits in the 2nd. year are largely unaffected scions. The spurs producing flower buds the first year become vegetative the second year and outgrow the stunting effect of the 1st. year's abnormal growth.

12. Colour improvement in apples. Bishop (2) finds that X-radiation is most useful in the production of deep colour sports and that 50% survival dosage being about 5000r of X-rays as being the optimal dose for the purpose. Granhall (19) in his radiation work on apples and pears has produced a triploid apple by X-radiation, but in his major work he has been successful in producing "green-yellow" striped and brown russeted fruits in pears which are strikingly different from those evolved through spontaneous mutations in that these coloured stripes in the induced fruits are more pronounced and broader in their bands. The manner of inducing such mutations is that of irradiating the scion wood bud prior to grafting.

13. The need for further investigation into the utility of radiation in fruit crop improvement. Granhall et al (21) in their evaluation of X-radiation in the improvement of fruit trees draw the following conclusions: that X-ray treatment consequently seems to increase already existing abnormal tendencies and on further observation may reveal whether these types of effects are real vegetative mutations or more likely derived from local damage at the points of vegetation. The indication of real vegetative mutation is insufficiently investigated in fruit trees but the observations made on pears and apples seem to be rather promising. For the immediate future, interest will be directed especially towards the investigation of mutation type, frequency and utility and also the use of other types of radiations. If vegetative mutations can be induced on a

a larger scale in this way it means that single particularly important characters or complexes of characters can be changed: e.g. colour, size and shape of fruits, time of ripening and also essential physiological characters like winter hardiness, disease resistance, vitamin content and palatibility. In comparison with the common crossing method of effecting variation the mutation method means that the main mass of genes remains unchanges.

14. The possible use of radioactive isotopes in horticultural plants. Ehrenberg et al (10) in their investigation aim to see if radioactive isotopes, by way of injection into the stem of fruit trees, can be used in fruit tree breeding i.e. to see if they cause genetical changes and further to study the most suited condition of application. Their conclusions for their evaluation has been drawn on the comparison with other effects of ionizing radiation. They do find it hard to give a direct comparison. Take for instance, the tolerance dose for survival for cherry trees, which seems to be in the range of 6000r - 8000r of P^{32} treatment. A direct comparison of this figure with 5000r of X-rays causing a decreased survival of scions cannot be made due to the very different conditions of radiation application and energy uptake. Scions irradiated by X-rays in the dormant state probably do not die because the buds are damaged but because of a decreased ability to make new callus formation after grafting. The X-ray dose necessary to kill individual dormant buds will probably be higher. In the case of radio-active isotope treatment most of the radiation occurs in the more sensitive state of swelling and growing.

It shall suffice to cite that their investigation demonstrates that it is possible by aid of radioactive P^{32} and S^{35} to apply radiation doses in buds of a magnitude which from experiments with X-rays is known to give mutations in frequencies perceptible in a limited material. From

the experiences of Ehrenberg in barley it is noted that the isotopes exert their effects on the tissue in a state more sensitive also from a mutational point of view and its mutagenic effects are qualitatively different from those of X-rays (unpublished).

Thus, radioisotopes seem to constitute a very valuable complement to ionizing radiations in systematic mutation work in fruit tree improvement. Their value in this respect is still more increased through the fact that the emitte emitted radiation is concentrated on to the embryonal parts of the trees, leaving the rest relatively undamaged.

15. Irradiation work on Citrus. As for Citrus, little work has been done with it by way of ionizing-radiation treatment for its genetical improvement. In 1935 Haskins et al. (34) in their work with X-radiation on Citrus primarily to investigate into the physiological aspects of radiation on Citrus, i.e. to see the effects of X-rays on growth modification in Citrus seedlings grown from X-rayed seeds. In this case the seeds are soaked in distilled water for 15 minutes and then left in moisture saturated atmosphere for 12 hours prior to irradiation. The seeds are then germinated under control temperature and then left in the glasshouse for one year for observations.

16. Effects of radiation on Citrus. The most striking effect they observe is "precocious" or premature flowering which is effected only three weeks after sprouting of the X-rayed seeds. This premature flowering however can be had spontaneously but not so effectively early. The other abnormalities which they find in the one year observation are chlorotic seedlings which are best known as 'virscent' seedlings. Fasciation is also commonly observed and associated with stuntedness. Almost all the badly affected seedlings do have twisting of the first pair of leaves which usually remains or persists for quite a long time. There are other minor abnormalities observed.

VIII. The Study of the Genetical Behaviour and Breeding Habit of Citrus.

1. The main purpose of the present research is to investigate the possibility of using ionizing radiation to induce useful mutation to improve Citrus breeding. Thus it would certainly be advantageous to have some idea of the unusual genetical behaviour and breeding habit of Citrus.

2. The genetics of Citrus. Frost (14) in his study of the genetics and breeding habit of most of the varieties of Citrus finds the occurrence of the following behaviour in Citrus:

(1) The occurrence of nucellar embryony in most Citrus species. That is the purely vegetative development of cells in the nucellus or integument into the embryo sporophyte forming a supermumary embryo.

(2) The occurrence of polyembryonic seedlings in most species except Citrus graudis (pumelo) and C. reticulata (mandarin orange). That is, the presence of more than one embryo in a seed: often one embryo is sexually produced and the remainder vegetatively by diploid parthenogenesis.

(3) There is great genetic diversity resulting in the abundance of heterozygosis of citrus varieties and the consequent high and extreme variability among the genetic progmies from both selfing and crossing.

(4) The feasibility of interspecific crosses in the Citrus genus, and of intergeneric crosses between related genera such as Poncirus, Fortunella and Citrus.

(5) The tendency towards increased vigour of progenies of crosses of unlike parents and less extreme intergeneric crosses.

Hamilton (33) in his review of literature on inheritance of disease resistance in Citrus and related genera, shows that various Citrus types and varieties show great variation in inheritance of resistance or susceptibility to various diseases. In the case of Citrus scab - Sphaceloma

fawcetti, only a few species of Citrus and kumquat of Fortunella are resistant and the susceptibility under certain climatic conditions may not be apparent. In the case of citrus withertip or anthracnose - Gloeosporium limetticolum the susceptibility seems not to be a dominant character in F_1 hybrid progenies. Citrus canker - Pseudomonas citri quite commonly has as hosts varieties and related genera of Citrus that are susceptible, but hybrids involving reciprocal crosses between species and genera such as Fortunella and Poncirus and Citrus seem to show great resistance. This is more evident in the backcross to the Fortunella and Citrus parentals.

3. Breeding habit of Citrus. Frost (14) emphasises that hybridization in Citrus species and also between related genera, contributes a high variability among the gametic progenies and consists mainly of the visible differences of size, shape and colour of leaves and fruit. Genetically this variability is due to changes in chromosome number and structure of the F_1 hybrids, such as triploid hybrids, from crosses of diploid Citrus and tetraploid Fortunella. Most of these polyploidizations are effected mainly in the nucellus before any fecundation in the gametic cell. Beside polyploidizations, chromosome aberrations arise frequently enough to account for the great F_1 variability.

4. Polyploidy and its utility in Citrus breeding. Polyploidy is quite a common occurrence in Citrus and its related genera. It occurs more often in the tetraploid form. The seedlings of the tetraploids are usually inferior phenotypically, the evidence being smaller sized fruit with thick rind and quite an irregular shape. The acid and sugar content may be lower as compared to the diploid. Fruit maturity and colouration are usually delayed and the seedlings show a general retarded growth. However, from a breeding point of view, tetraploid plants exhibit a greater fertility than their diploid counterparts and

have abundant seed production which is due to the fact that autotetraploids produce a lesser proportion of pure recessive gametes, therefore a much smaller proportion of homozygous recessive progenies than do the corresponding diploids. The segregation of recessive genes unfavourable to vigour and fertility, which occurs abundantly in polyembryonic Citrus seedlings, produces less sterility in the tetraploids than in diploids. This tendency is specially marked in tetraploid Lisbon lemon which has a tendency to autosynapsis (i,e. the pairing in a polyploid of chromosomes phylogenetically derived from the same diploid species.) Horticulturally the tetraploids are used for the production of triploid seedless hybrids by a cross with a diploid.

5. Spontaneous mutation and its role in Citrus breeding. Spontaneous mutation in Citrus among gametic seedlings does occur but at low frequency, besides it is hard to distinguish it from the extremely numerous variation due to normal recombination and segregation of genes heterozygous in the parents. The more easily noticeable gene mutation is under the class of "bud-mutation" which is capable of perpetuation through bud propagation by grafting. This is commonly known as somatic mutation or a "bud-sport". The physiological somatic mutations have some horticultural value such as the Washington Navel which is derived from bud sport mutation.

6. Frost's optimistic view of the use of radiation-induced mutation in Citrus improvement. Having made the following study of the genetics and breeding habits of Citrus, Frost comes to the conclusion that one of the two methods of more systematic breeding of new forms of citrus would be by ionizing radiations which have been proved capable of inducing gene mutation or other genetic changes in other plants. He is of the opinion that varieties like pumelo (C. grandis) which have no nucellar embryony, selfing and crossing of selected parents are indicated as desirable,

possibly with ionizing radiation treatment to increase variability of desired types as bud variations seem to be rare in these varieties, probably because of a comparatively low prevalence of heterozygosis.

7. Review of Citrus growing in Cook Islands. From the report on Citrus growing in the Cook Islands by Fletcher (63) one sees clearly that Citrus grown there exhibit certain inferior qualities compared to the Citrus grown in Australia, such as a lacking in attractive colour, keeping quality and flavour. The authority on Citrus in Rarotonga (Cook Island) seems to believe that these poor qualities are the consequence of the tropical and humid climate of these islands and seems to be quite pessimistic about the improvement by breeding methods. Thus the concentrated work there is not one of genetical breeding which of course involves long term work but rather consists of bud selection and therefore the emphasis is on the selection of good root stock for bud grafting. Here again their choice of root stock is quite limited to a few varieties of Citrus and what is more "discouraging" is that all the "supposed" suited types of varieties for this area seem to be inferior in one way or another such as:

(1) The Rough lemon root stock seems to be the most dependable one as it is vigorous in growth, early bearing and gives fruits earlier. It is best adapted for nursery work and can "bud" with ease and has high resistance to common citrus virus and disease. The fruits it bears are very inferior - low sugar content and acid is poor resulting in an insipid flavour. The fruits are often coarse in texture and poor in rind colour. These conditions can be aggravated by mineral deficiencies.

(2) Sweet orange seems to be a better root stock, the fruits tested are good quality but the drawback is that the tree is slow in growing and a light bearer besides the scion bud "take" on this stock is very poor.

(3) Mandarin root stock appears to be vigorous in growth, has good resistance to disease and produces standard sized fruits but again is slow in developing.

(4) Poncirus trifoliata seems to be quite promising as it bears excellent quality fruits, bearing is increased, but this species is very susceptible to trace element deficiencies especially in the Cook Islands.

Such drawbacks undoubtedly can be overcome either by breeding by the conventional methods which involve long term work or by means of ionizing radiation to induce suitable and desired types of mutations which can eliminate such inferiority in the production of Citrus in the Cook Islands.

8. Dr Brooks' recommendation. Dr. Brooks (4) (through personal communications) suggested that radiation can be of great use in the improvement of Citrus to overcome some of the drawbacks faced by the Citrus breeders. Atempts can be made to induce disease resistance particularly that of root rot of Citrus by radiating rooted cuttings and then screening them for resistance after innoculation with pathogen toxin. Then attempts can be concentrated on the study of the heterotic effects of ionizing radiation of a specific optimum dosage on the qualitative aspects of Citrus. Of course, the emphasis should be placed first on the study of the techniques of radiation and also the behaviour of Citrus to ionizing radiation, only then irradiation-induced mutation can be applied to Citrus breeding to great advantage.

EXPERIMENTAL

A. A study of the effect of different dose rates of one common total dose of Y-ray irradiation on 13-day old germinating Lisbon lemon seedlings.

I. METHOD

1. Germinating of seeds. 185 healthy Lisbon lemon seeds of uniform size (large) were selected and put into germination in a mixture of moist peat and pumice, (45% and 55%). The mixture with the seeds was put into a polythene bag and buried in sawdust in an electrically heated frame with the temperature maintained at 30°C (±3°). It was kept in the frame for ten days (in College). On the 10th day the whole bag was removed from the frame and taken to Wellington for irradiation. The bag was then put into a special container in a hot water bath prior to irradiation. The temperature of the water bath was 30°C (±2) and maintained for three days.

2. Sorting of germinating seeds. On the 13th day the seeds were taken out for examination and sorting.

Observation of germinated seeds.

Rotted	17	percentage	9%
Non-germinated	56		
Germinated	111	"	60%
Missing	1		
Total	185		

3. Observation of length of difference of germinated (radicles)

1mm to 3mm	22
3mm to 5mm	20
5mm to 10mm	22
10mm to 15mm	25
15mm to 20mm	12
20mm to 25mm	3
above 25mm	7
Total	111

(The 56 non-germinated and those germinated with radicles of 1mm to 3mm length were put back into water bath at 36°C (±6°) for further germination for 24 hours, thus giving an extra 35% germination as 19 germinated)

4. Pre-radiation of seedlings with radicles from 5mm to 15mm

long. Those with straight radicles are selected for irradiation for root elongation study while those with crooked radicles were for cytogenetical study after irradiation. The seedlings were grouped into four groups each having both types of seedlings (straight and crooked radicles), one group for control and the other three for the different dose rates of Y-radiation as shown below.

Dose rate	9r/minute	45r/min.	184r/min.	Control
Radiation samples	Y-1	Y-2	Y-3	Y-con.

5. Radiation Laboratory detail.

Source	Co^{60} source, ½life of 5.3 years 180 Curie source.
Room temperature	22°C (±2°)
Dose rate	9r/min 45r/min 184r/min
Distance of Irradiation	58cm 35cm 9cm
Total dose	11,000r each
Duration of Irradiation	20hr 4hr 1hr
Finishing	All the three samples were timed so as to finish at the same time. (While Y-1 was being radiated, Y-2 and Y-3 were kept in the water bath)
Seedling conditions	13-day old germinating seedlings of radicle length between 5mm and 15mm. About 15 seedlings for each group.
Method of radiation	All seedlings in each group were placed in small bottles with moist cotton wool and placed at their respective distances from the Co^{60} source.

6. Samples for cytogenetical study. At 3½ and 36 hours after irradiation, six root tips at each interval of time for each treatment including the control were taken and put into Carnoy Fixative solution for root-squash for cytogenetical observation. (The technique for staining and root-squash is described in Appendix I).

7. Post-radiation treatment. After irradiation the seedlings of each sample were put in peat and pumice mixture in the respective grouping. The 1st post-irradiation measurement of root length was taken at the 2nd day while in Wellington. Subsequent measurements were taken after a 2 or 3 day interval. Two days after irradiation (when back at College) the seedlings were put into the heated frame with temperature maintained at 20°C (± 3°).
One week after irradiation, the seedlings were put into water culture in de-ionized water with sufficient quantity of "Zest" fertilizer. The seedlings were arranged in rows in a nylon-mesh frame which just floated on the water. The de-ionized water was warmed to 20°C (± 3°) before the seedlings were put into it. The changing of the de-ionized water took place at four day intervals and with each change of water a fresh supply of "Zest" was added. As there was no significant recovery of the root, the measurement was stopped at 22 days after irradiation.

II. RESULTS.

1. Rate of root elongation. From the various measurements of root elongation, the following analysis has been made to find if there is significant difference between the 3 treatments.

(a) Total root length of seedling 22 days after irradiation.

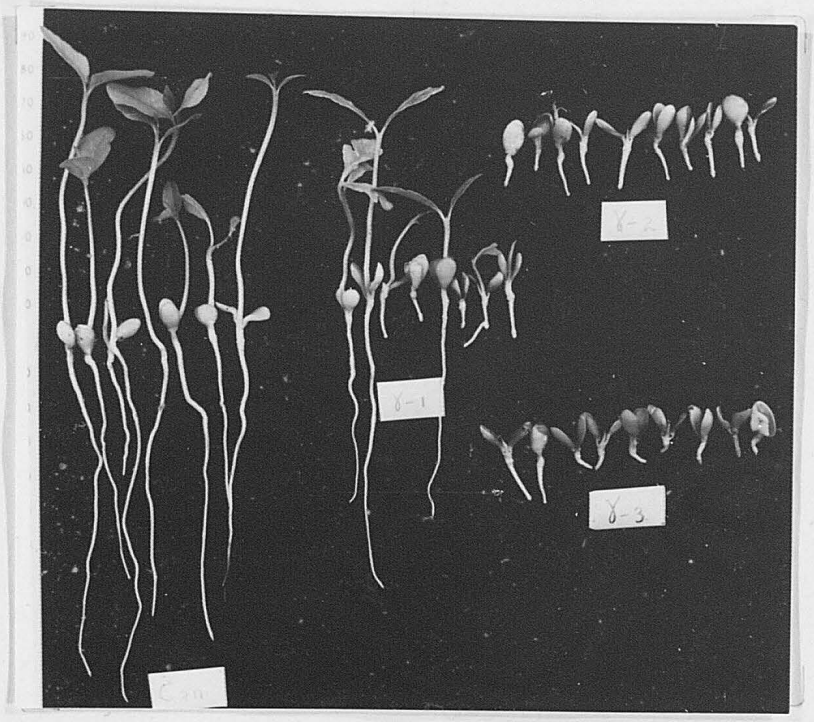
	Control	Y-1	Y-2	Y-3
Mean in mm	39.40	20.6	9.73	8.56
Standard error	±3.80	±3.90	±0.44	±1.12
Variance %	49	70	20	52

(b) Rate of increment in ten days.

	Control	Y-1	Y-2	Y-3
mean in mm	37.50	14.79	2.42	2.67
standard error	±3.80	±3.90	±0.60	±1.08
variance	51%	100%	109%	122%

Note Please refer to Table I for more details of root elongation on other dates.

Fig.1. Irradiated and non-irradiated seedlings of Lisbon Lemon.
This is to show the effects of Y-radiation of three different dose rates of one common total dose on 13-day-old germinating seedlings, as shown by the difference of root elongation. The photo was taken six weeks after radiation. (Magnification 0.5x)

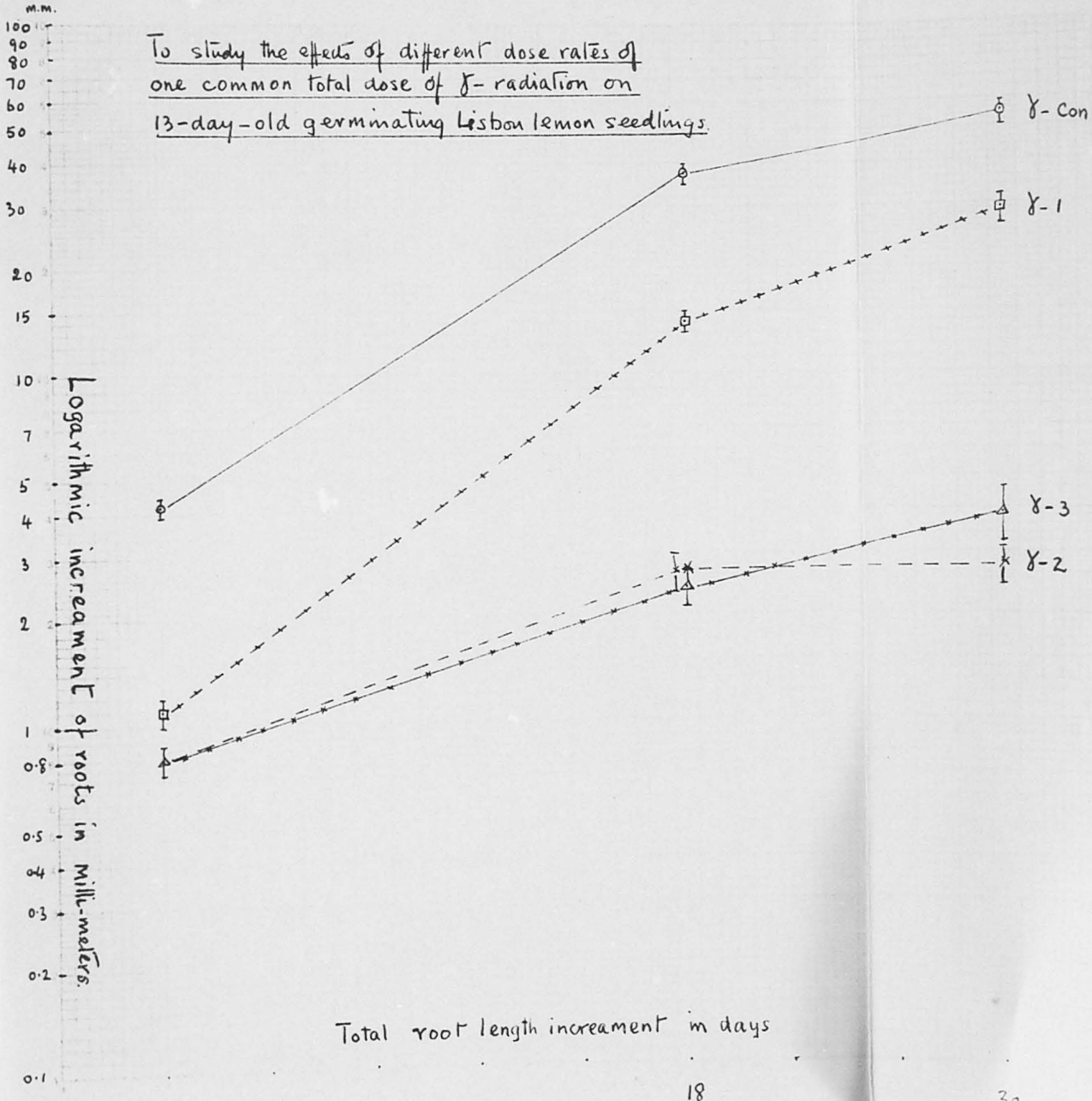


Reference:

Con: control
Y-1: 9r per minute
Y-2:45r per minute
Y-3: 184r per minute

GRAPH "F"

To study the effects of different dose rates of one common total dose of γ- radiation on 13-day-old germinating Lisbon lemon seedlings.



2. Cytological observation. From both samples of the tips taken 3½ and 36 hours after irradiation the following observations were made.

(i) stickiness of chromosome (anaphase) (ii) micronuclei (resting stage)	9r/min.
(i) irregular shaped chromosomes in group (could be bridges at anaphase)	45r/min.
(ii) micronuclei (iii) irregular shaped resting cells	184r/min.

As for control, all chromosomes at various stages were normal.

III. CONCLUSION

(a) 1. For all measurements of root elongation, there is a significant difference between (i) control and Y-1
(ii) Y-1 and Y-2

There is no significant difference between Y-2 and Y-3.

2. Y-1 has a mild effect on the root elongation while Y-2 and Y-3 which show no significant difference between the two show "some great" retardation to root elongation and have some lethal effect.

3. Judging from the curves of Graph "F" which is drawn in terms of the Logarithmic scale, Y-2 seems to be a more suitable dose rate for the purpose of radiation-mutation study on seedlings as it has a smoother curve, while Y-3 shows a more inhibitory effect on elongation toward the later stage of experiments.

4. If the experiment had continued longer it is possible that there could be some significant difference between Y-2 and Y-3.

5. At two days and sixteen days after irradiation the coefficients of variance were similar for Y-2 and Y-3, but after 30 days there was an indication that variability was less in Y-3 than in Y-2, so that Y-2 may not be so useful a dose rate for examination of root growth in older plants.

(b)1. Due to the fact that the Citrus chromosomes are very small it was hard to draw much conclusion from the cytological observations to make any difference cytogenetically between the various treatments.

2. The only inference that could be made would be that for Y-1 there was some stickiness of chromosomes which it may be inferred was the temporary injury caused by ionizing radiation.

IV. REMARKS.

From the above observations it could be assumed that for the cytological study of chromosome aberrations and for the 50% survival of the treated material, a dose rate intensity of between 45r/min. and 184r/min., but nearer to 45r/min. is most suitable.

B. To study the effects of X-rays on 15day-old seedlings of Lisbon lemon and by plotting the curves to find the "near" critical dosage which shows the best growth consistency with the maximum expectation of mutation.

(This experiment is based on the findings of the previous experiment in which the most suitable dosage for mutation study was between 45r/min. and 184r/min.)

I. METHOD.

1. Germinating of seeds. 220 healthy and freshly extracted Lisbon lemon seeds were dried and put into germination in a peat and pumice medium (45% and 55%) in polythene bags in a heated frame maintained at 20°C (±3°). After ten days the whole bag of seeds was taken to Wellington and placed in a container in a hot water bath at 30°C (±1°) for 4½ days more.

2. Sorting of germinated seedlings after 15 days.

Rotted	40	18%
Lost	15	8%
Non-germinated	27	12%
Germinated	138	62%
Total	220	

3. Observed difference in radicle length.

5mm to 10mm	38
10mm to 20mm	32
20mm to 25mm	35
25mm to 35mm	25
above 35mm	8

4. Pre-radiation. Seedlings of radicle length between 10mm and 25mm were selected for irradiation and 25mm to 35mm were used as control and some for post-irradiation cytological study. The seedlings were to be irradiated with one common dose rate intensity but of different total dosages.

5. Radiation laboratory detail.

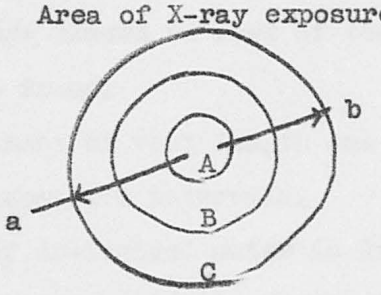
Room temp.	18°C (±2°)
Source	G.E.C. Maxima X-ray Hospital therapy unit
Max. energy	210 k.r.
Current	10 m.a.
Filter	0.5mm Cu plus 1mm Al
Distance from filter	16.5 cm.
Dose rate	86r/min (95%± 3%)

Samples	Time of exposure (min.)	Total Dose
x-con	0	-
x-011	10	860r.
x-022	35	3010r
x-033	70	6020r
x-044	105	9030r

Seedling conditions. 15 day old actively germinating seedlings of radicle length between 25mm and 35 mm, about 35 seedlings for each group.

Method of radiation. The seedlings were irradiated in two groups at a time, one group occupying the area of a semi-circle. They were placed in a tray in the central circumference "B" of the direction of the beam as shown in the diagram so as to avoid the maximum error due to difference of soft and hard rays from radiation. The error is about ± 5%. Thus the seedlings were placed in the "B" area shown in the diagram. This is to cut off the extreme effects due to soft or hard rays.

Diagram 1. Area of X-ray exposure on the tray



The outer periphery "C" receives mostly soft rays.
The inner periphery "A" receives all hard rays.
The central periphery "B" receives averagely even mixture of rays.
The calculated dose rate is 86r/min. exposed at a distance of 16.5cm. This includes an error of 3% only. The whole beam of exposure on the tray at a specified distance is marked by the arrow "ab".
To avoid rapid drying up of the seedlings, a thin sheet of paper was placed over the tray of seedlings which were placed on moist filter paper.

x-033 and x-044 had split exposures as the machine had to have a compulsory rest of 5 minutes after every 55 minute exposure.

6. Post irradiation treatment. Immediately after irradiation the different groups of seedlings were placed separately in a moist filter-paper-lined container. Later the seedlings in the respective groups were transferred to moist peat and pumice mixture when the root length including those of the control were taken.

Those for cytological studies were taken out at 5 hours and 36 hours after irradiation respectively. Their root tips and shoot tips plumules were taken and put into Carnoy fixative solution.

Two days after irradiation, the seedlings were put into water culture of de-ionized water with sufficient "Zest" as fertilizer. The water temperature was maintained at 20°C (±2°) in the heated frame controlled by a thermostat. The seedlings were placed in rows of the respective groups in a nylon mesh frame.

Measurement of root length was done at five day intervals for five consecutive intervals.

The change of de-ionized water is done every seven days and in each change the water is warmed up to 20°C (±2°) before the seedlings were put back. (This is done by de-ionizing warm water).

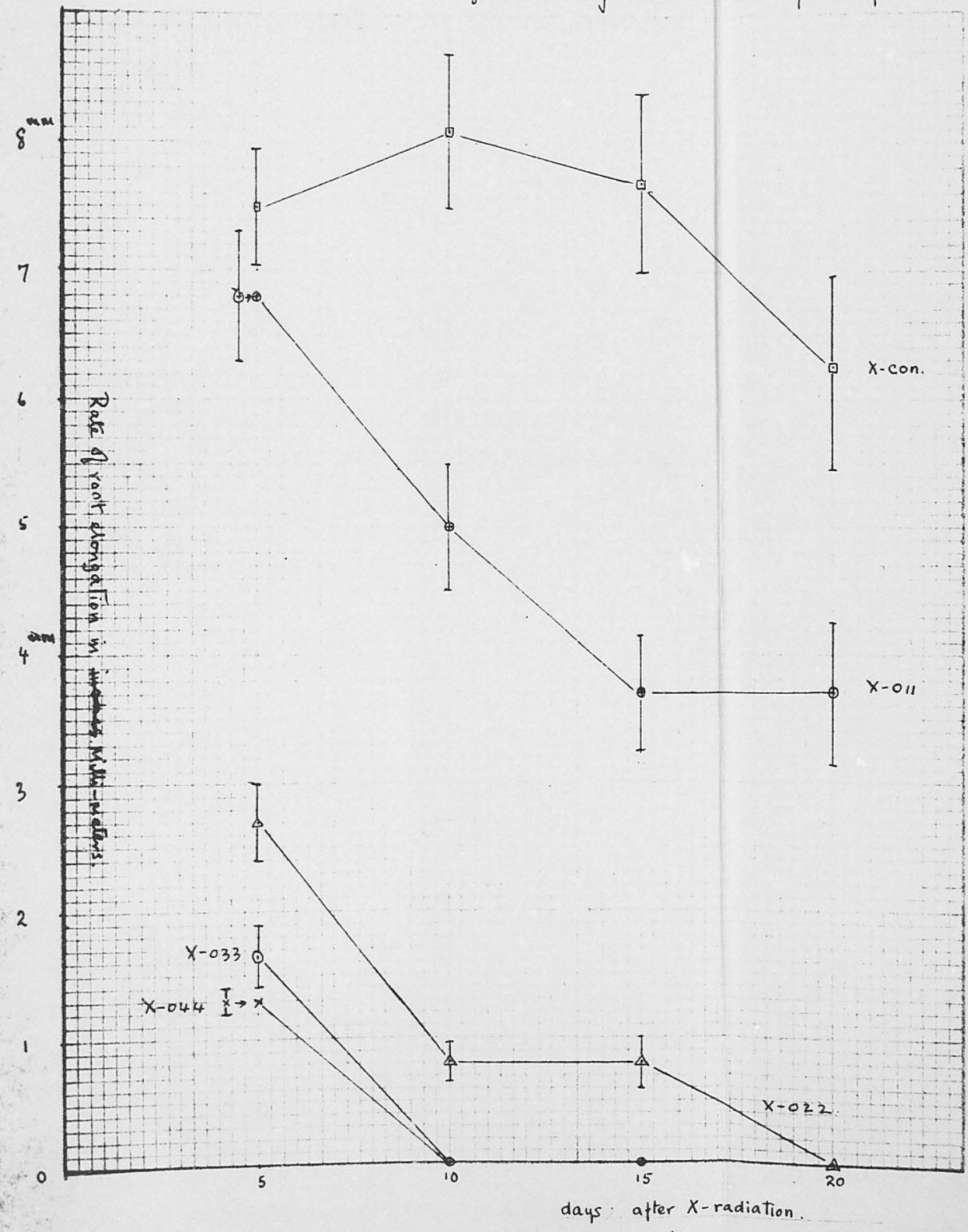
7. Cytological study of the chromosome aberrations caused by the radiation treatment was carried out by the modified Gomori's Hematoxylin staining and smear of root tips and plumules. This technique has been recommended by Mr de Latour of Grassland Research at Palmerston North. (The method of squash or smear is described later).

II. RESULTS.

1. Measurement of rate of root elongation. As general inhibition is seen in all samples (including controls) this could be due to the effect of "over-dose" of Zest

GRAPH I

To study the effects of X-rays on young Citrus seedlings and from the Graph study to find which dosage shows the best growth constancy with the maximum expectation of mutation.



fertilizer. Thus the last measurement was taken at 21 days after irradiation. From the four measurements and recordings, the analysis was made. The respective mean length of root increment with the respective standard error of each are calculated and tabulated below. (The full detailed analysis is recorded in Table II as shown at the end)

Rate of root elongation.

Dates	5 days	10 days	15 days	21 days
Treatment				
x-con	7.5±0.9	8.1±1.2	7.7±1.4	6.24±1.6
x-011	6.8±1.0	5.0±1.0	3.7±0.9	3.80±1.1
x-022	2.7±0.6	0.8±0.3	0.8±0.4	0
x-033	1.7±0.5	0	0	0
x-044	1.3±0.2	0	0	0

Footnote. As the controls are taken from slightly faster growing seedlings strict comparison of growth rate is not quite practicable between the treated and control as far as rate of elongation is concerned, but in other respects such as lethality and chromosome aberration comparisons are valid.

2. Cytological observation.

(a) 5 hours after irradiation
(i) normal cell divisions - control
(ii) disfigured micronuclei, chromosome stickiness - x-011
(iii) no observable division (due to inhibition of cell division caused by radiation) - x-022, x-033, x-044

(b) 36 hours after irradiation
(i) good number of normal cell divisions - control
(ii) quite a few normal cell divisions of metaphasic and anaphasic stages - x-011
(iii) no division encountered - x-022, x-033, x-044

III. CONCLUSION.

(a) Root elongation study.

1. From the root increment analysis and from the study of the shapes of the curves in Graph I it indicates that treatment x-011 had a very minor effect and behaved quite steadily, while treatment x-022 initially showed a severe effect in inhibiting the elongation of the roots at later stages and finally proves lethal. Treatments x-033 and x-044 had similar but more severe inhibitory effects and

lethality was evident much earlier.

2. Thus the near critical dosage (needed for mutation study) should be between dosages of treatments x-011 and x-022 (i.e. between 860r and 3000r) but more towards the dosage of treatment x-011, as its rate of survival approaches 50%.

(b) Cytological observations.

1. From the cytological observation of all samples prepared (including the control) at 5 hours and 36 hours after irradiation the following conclusions were made.

(i) Treatment x-011 seemed to have a temporary effect of inhibition which lasted 36 hours. Thus this dose might not be suitable to effect the desired type of mutation changes.

(ii) This fact would suggest that the optimum dosage needed for mutation study would be one above that of treatment x-011 (860r) but not much greater.

Though the next two experiments are not directly connected to this experiment B, they are nevertheless important in this series of experiments. They are conducted to elucidate the effects of Y-radiation and also the different mode of action of Y-radiation and X-radiation on Citrus seeds. The essence of these two experiments is to find the "near" critical dose for Y-radiation for Citrus seeds and also the lethal dose for Y-radiation on Citrus seeds.

C. To study the effects of a series of dosages of one common dose rate on high intensity of γ-radiation on seeds of Lisbon lemon in order to find:

(i) the near critical dosage which will produce minimum lethal effect but simultaneously produce a high rate of mutation (and chromosome aberration).

(ii) the lethal dosage for Lisbon lemon seeds.

I. METHOD.

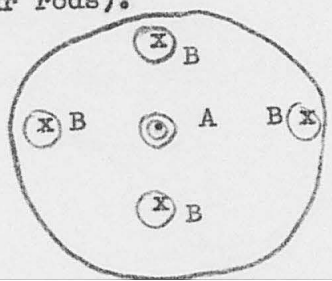
1. Pre-radiation. Some 360 Lisbon lemon seeds of uniform size were selected and grouped into six lots. They were dried at room temperature prior to irradiation.

2. Radiation Laboratory detail.

Laboratory room temperature	22°C (±2°)
Source	Co^{60} 180Curies ½life 5.3yrs.
Dose rate	1540r/min.
Distance from source	2.5cm.
Seed condition	dry and dormant

Method of radiation. As shown in Diagram II, the source is encased in a special cylinder housed in a special lead container. The source as shown in the diagram consists of four rods "B", while at the centre "A" is another cylinder in which is placed the material for very high intensity radiation. Both "A" and "B" are operated by a hydraulic mechanism. The distance from the centre to the source is 2.5cm. The seeds of each lot are placed in a suitable glass container which just fits into the central compartment "A" and by means of the hydraulic system, the seeds in "A" compartment are lowered to where the source is. The glass cylinder has a central partition in the centre so that two lots of seeds can be irradiated at the same time.

Diagram II. To show the method of radiation by Co^{60} at very high density. (A-centre for placing material and B-site of the four rods).



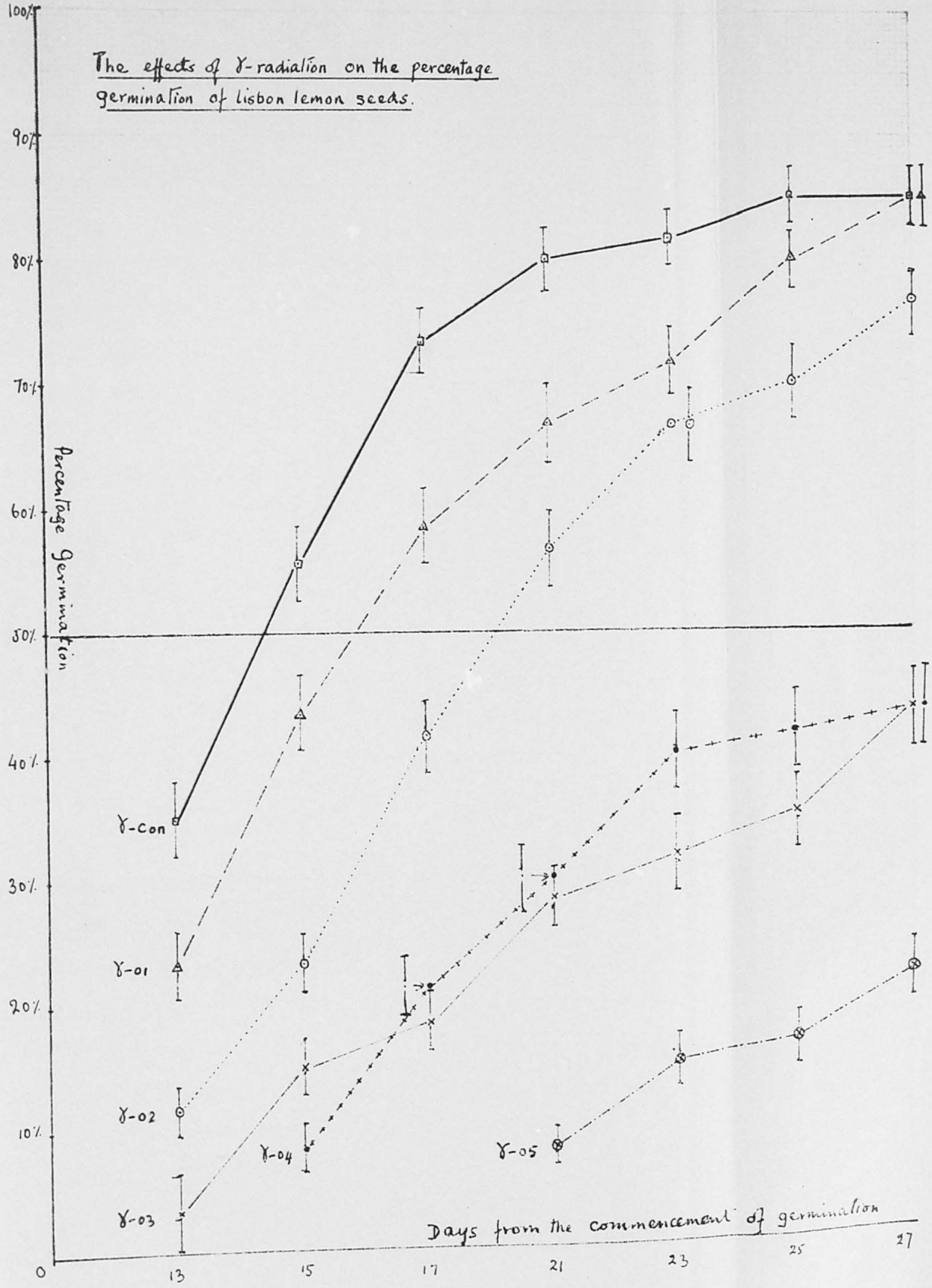
Sample	Total dose	Time in minutes
Y-con	0	-
Y-01	25,000r	16
Y-02	50,000r	32
Y-03	100,000r	64
Y-04	150,000r	96
Y-05	200,000r	128

Post-radiation. The next day the seeds were put to germinate in petri dishes which had moist filter paper at the bottom to keep the seeds sufficiently moist. The petri dishes were so covered that air could easily flow in and out for necessary respiration. They were then buried in sawdust in a heated frame at 20°C (±2°).

Twelve days later the petri dishes were taken out at night and the seeds were examined quickly under ordinary red light for the number of seeds germinated, which was then recorded. The germinated seeds were then removed and put into separate containers in peat and pumice mixture and buried under the same frame. Subsequent examination of the germination of seeds and subsequent transference of the germinated ones to another container was done at two day intervals.

When the germination of seeds was completed, the seedlings in the respective lots were put into water culture using de-ionized water in the heated frame. This was to measure and study the rate of root elongation. The seeds were placed in rows in nylon mesh frame and the measurement of root length taken at two day intervals. Due to the effect of the black paint applied to the nylon mesh, the seedlings rotted, including the controls. The experiment terminated two weeks after the completion of germination of the seeds. From the data obtained the analysis of rate of germination and rate of root elongation of each treatment was made and tabulated.

GRAPH "E"


The effects of γ-radiation on the percentage germination of lisbon lemon seeds.
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0
Percentage Germination
γ-Con
γ-01
γ-02
γ-03
γ-04
γ-05
13
15
17
21
23
25
27
Days from the commencement of germination

II. RESULTS.

Rate of germination

Treatment / Dates	Y-con	Y-01	Y-02	Y-03	Y-04	Y-05
13 days	35% ±6.2	23.3%±5.6	11.6%±4.1	3.3%±6.1	-	-
15 days	56.6%±6.4	43.3%±6.4	23.3%±5.5	15%±4.6	8.3%±3.6	-
17days	73.3%±5.7	58.3%±6.4	41.6%±6.4	18.3%±4.9	21.6%±5.3	-
21days	80.0%±5.2	66.6%±6.1	56.6%±6.4	28.3%±5.8	30%±5.9	8.3%±3.6
23days	81.6%±5.0	71.6%±5.8	66.6%±6.1	31.6%±6.0	40%±6.3	15%±4.6
25days	85.0%±4.6	80.0%±5.2	70.0%±5.9	35.0%±6.2	41.6%±64	16.6%±4.8
27days	85.0%±4.6	85.0%±4.6	76.6%±5.5	43.3%±6.4	43.3%±6.4	21.6%±5.3

Total root elongation in millimetres.

Treatment / Dates	Y-con	Y-01	Y-02	Y-03	Y-04	Y-05
17days	7.8±1.2	3.7±0.5	2.0±0.1	1.5±0.6	1.3±0.4	0
23days	15.8±2.5	6.1±0.9	2.0±0.3	1.6±0.1	1.3±0.1	1.1±0
27days	16.1±1.7	6.4±0.9	2.3±0.1	2.0±0.1	2.0±0.1	1.1±0

For full detail analysis refer to Table III and VI

III. CONCLUSION.

(i) From the study of the rate of germination and from the study of the shape of the curves of Graph E it shows that even the lowest dosage of 25,000r would affect the speed of germination initially. Later at the 27th day of the commencement of germination ther was virtually no difference in the percentage germination between the control and the treatment Y-01.

Thus the "near" critical dosage lies between the dosage of treatment Y-01 and that of the control.

(ii) Dosages below the treatment Y-02 (50,000) delay the germination without apparently reducing the final germination percentage. Those above the treatment Y-02 have a severe effect on final percentage of germination. Therefore dosages up to 50,000r and not above are more suited for radiation study.

(iii) The lethal dosage for Lisbon lemon seeds was evidently higher than dosage of Y-05 (i.e. 200,000r).

D. To study comparatively the effects of the "near" critical range of dosages of Y-radiation and X-radiation on germination of seeds and root elongation of the seedlings of Lisbon lemon. Also to find the lethal dosage of Lisbon lemon by Y-radiation. (This experiment is based on the findings of the previous experiments which indicate that the "near" critical dosage for Lisbon lemon seeds by Y-radiation is between the control and a dosage of 25,000r. That also the lethal dosage for the same material is much higher than 200,000r).

I. METHOD.

1. Pre-radiation. About 500 uniform sized and healthy Lisbon lemon seeds were selected and divided into two lots; one for Y-radiation and the muxh smaller lot for X-radiation. For Y-radiation there were five lots of 65 seeds each and for X-radiation two lots of 45 each and one lot of 65 seeds for the control.

The seeds were dried at room temperature prior to irradiation.

2. Radiation laboratory detail.

Room temperature maintained at 18°C (±2°).

Y-radiation

Source: Co^{60} 180 Curie source

½life 5.3 years

Dose rate: 1540r/min.

Distance from source: 2.5 cm.

Sample	Time (min.)	Dosage
Y-con	-	0
Y-06	3.25	5,000r
Y-07	6.5	10,000r
Y-08	9.75	15,000r
Y-09	13.00	20,000r
Y-10	195.00	300,000r
Y-11	325.00	500,000r

X-radiation

Source: G.E.C.Maxima X-ray hospital therapy unit.

Filter: ½mm Cu + 1mm Al

Max. energy: 210 kv.

Current: 10ma.

Dose rate: 205r/min.

Distance from source: 21.4 cm.

Sample	Time	Dosage
x-01	24	5,000r
x-02	98	20,000r

3. Method of irradiation. In the case of Y-radiation it was the same method as in the previous experiments in which the materials were placed in the centre surrounded by the source

(refer to diagram in previous experiment 'C').

In the case of X-radiation the seeds were put on a tray, well spread in the area covered by the X-rays. The X-02 treatment had to have two-split exposure as the machine had to have an intermission of five minutes after every 55 minutes.

4. Post radiation. The next day after irradiation, the seeds were put into petri dishes with moist filter paper at the bottom. The petri dishes having been covered in such manner as to allow free air passage for the oxidation of seeds were then buried under sawdust in the heated frame whose temperature was maintained at 20°C (± 2°). Ten days later the seeds were taken out for examination at night under red-light. Those germinated were then put into separate containers for the respective groups in a peat and pumice medium and kept in the same frame. Subsequent observations were made at two day intervals and at the same time measurement of the root elongation of those already germinated was taken. The data were then recorded.

The last measurement was taken at 30 days after the date of putting the seeds into germination. This time the seedlings instead of being put into water culture were put in a moist peat and pumice medium and exposed to light in the heated frame.

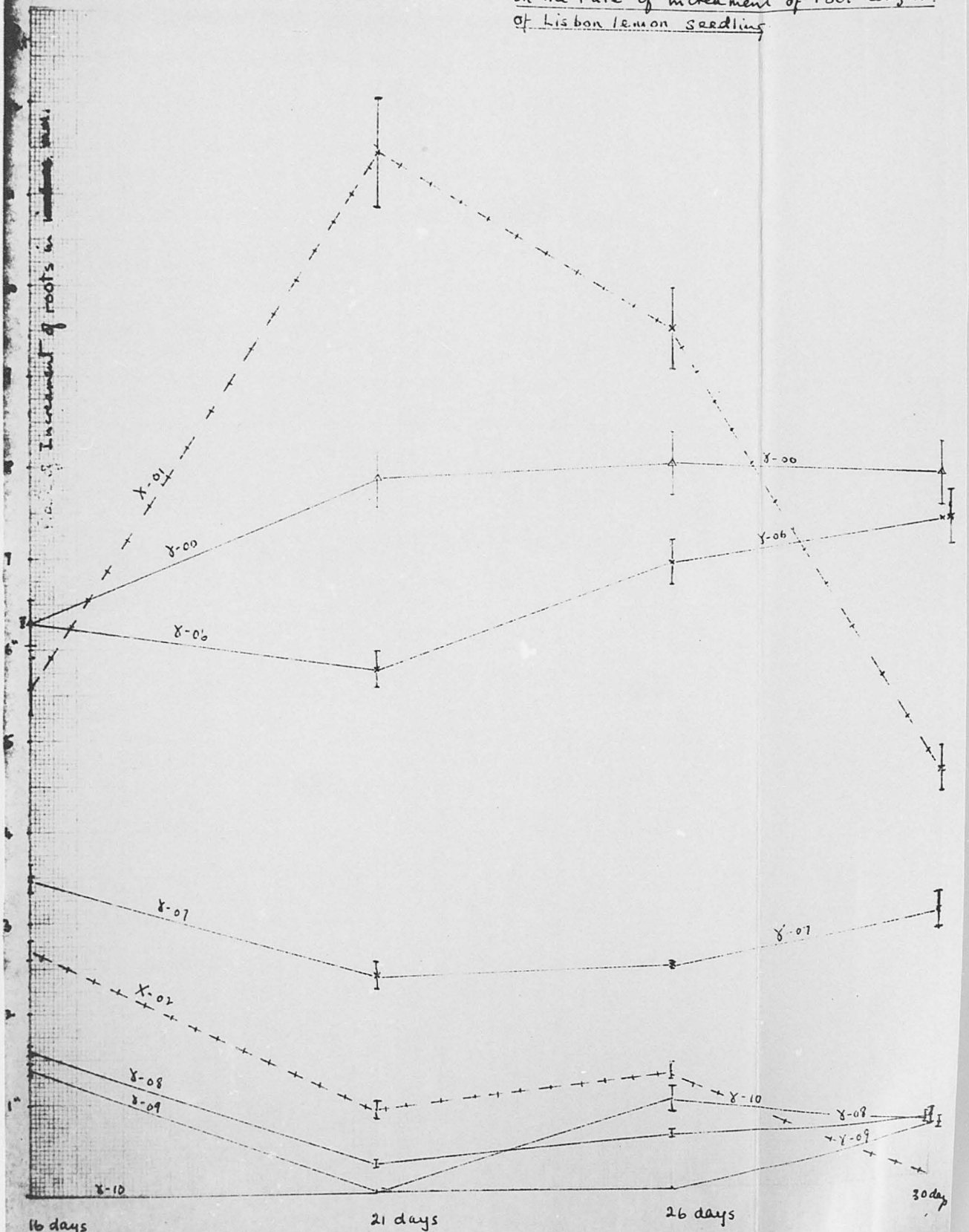
Two months later, the whole box of seedlings in the respective order of treatments was photographed to show the apparent difference between various groups of seedlings of different dosage effect. Refer to photoplate, No. .

II. RESULT.

From the data recorded analysis of the percentage of germination of each lot of treatment with the respective standard error are calculated and tabulated as are the results of the rate of root elongation. By means of these

GRAPH "A"

The effects of γ-radiation and X-radiation on the rate of increament of root length of Lisbon lemon seedling



..... root elongation in days.

Since the commencement of germination

16

statistical analyses graphs were drawn to scale to show the various dosage effects as compared to the control. From the study of these curves in Graphs A and B conclusions were made of the various treatments. From the study of these three graphs comparisons were made of the effects of the two types of radiation.

(a) Rate of germination in days from commencement of germination test. (expressed as a percentage)

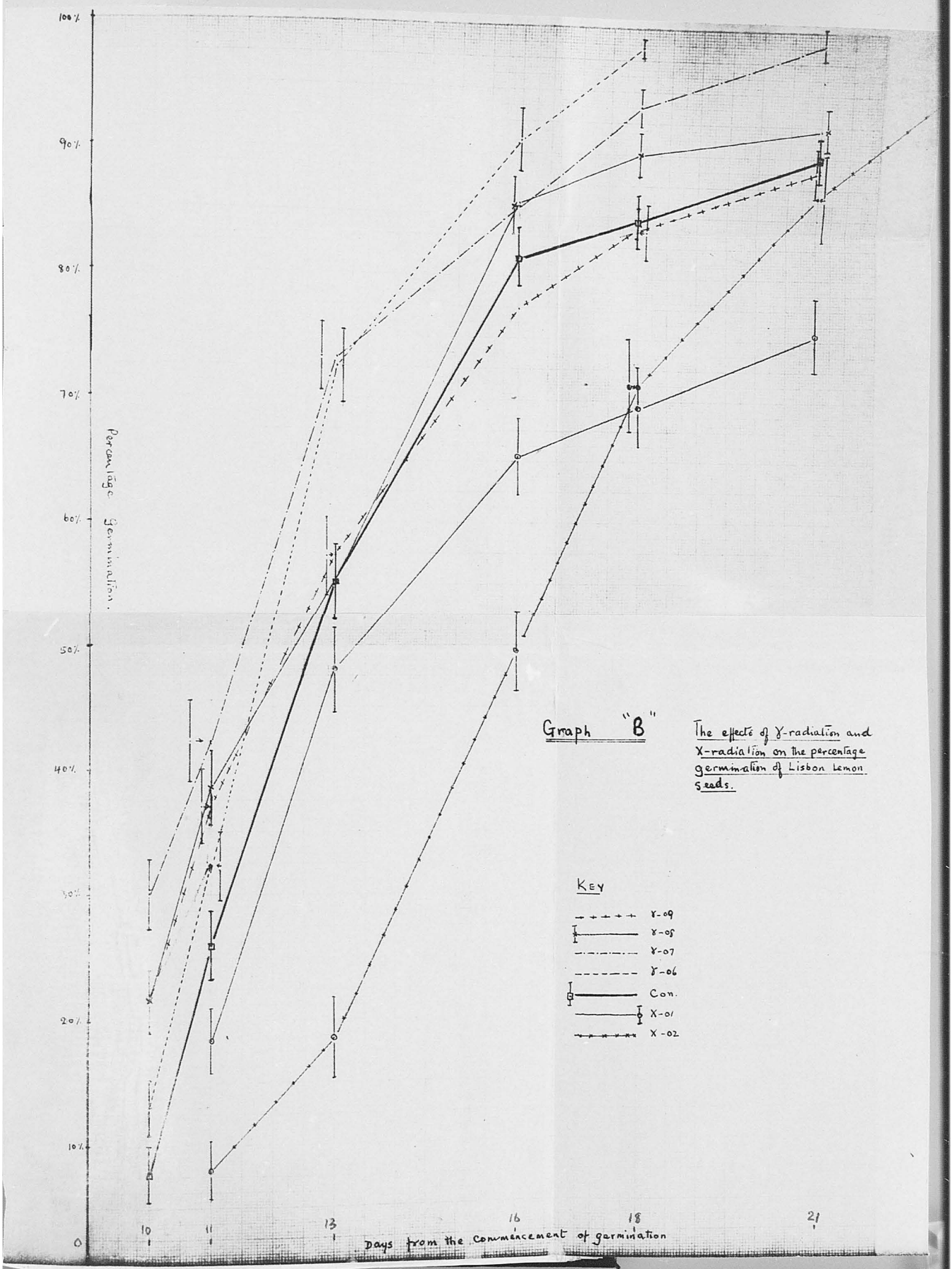
Treatments / Days	Y-con	Y-06	Y-07	Y-08	Y-09	Y-10
10	7.6±4.4	13.2±4.9	30.1±5.6	21.5±5.3	21.0±5.3	-
11	26.2±5.5	32.4±5.7	44.4±6.3	38.5±6.0	37.1±6.1	-
13	55.4±6.2	72.5±5.4	73.1±5.6	55.4±6.2	57.3±6.3	-
16	81.5±4.8	91.0±5.0	85.7±4.4	86.2±4.3	77.4±5.3	-
18	84.6±4.5	98.5±1.5	93.7±3.1	90.7±3.6	83.9±4.7	-
21	89.2±3.8	98.5±1.5	98.4±1.6	92.3±3.3	88.7±4.0	6.7±3.7
26	-	-	-	-	-	20.0±5.4
39	-	-	-	-	-	33.3±5.7

(continued)

Treatments / Days	x-01	x-02
10	-	-
11	18.4± 5.5	7.9±4.4
13	48.2±7.1	18.5±6.3
16	65.3±6.8	49.5±6.3
18	69.4±6.6	71.1±8.1
21	75.5±6.1	86.8±7.3
26	75.5±6.1	100%

Note: (i) On the 21st day from the commencement of germination the germination study terminated for Y-con and Y-06 to Y-09 as the remaining seeds rotted.

(ii)There was no sign of germination for Y-11 treatment.



Graph "B"

The effects of γ-radiation and X-radiation on the percentage germination of Lisbon Lemon seeds.

(b) Rate of root elongation of the treated germinated seedlings, measured in millimetres.

Samples / Days	Y-con	Y-06	Y-07	Y-08	Y-09	Y-10
16	6.3±0.5	6.3±0.1	3.5±0.3	1.6±0.1	1.4±0.1	0
21	7.9±0.6	5.8±0.4	2.4± 0.3	0.3±0.1	0	0
26	8.1±0.7	7.0±0.5	2.5±0.1	0.6±0.1	0	1.5±0.3
30	8.0±0.7	7.5±0.6	3.1±0.2	0.7±0.2	0.6±0.1	0.7±0.1

(continued)

Samples / Days	x-01	x-02
16	5.6±0.6	2.7±0.2
21	11.5±1.2	0.8±0.2
26	9.6±0.9	1.3±0.2
30	4.7±0.5	0

For detailed analysis refer to Tables IV and VII

III. CONCLUSION.

(a)Germination. (i) From the above analysed data of rate of germination of the treated materials including the control and also from the study of the curves of Graph B, it indicates that the treatments Y-06, Y-07, Y-08 and Y-09 have definitely accelerated the rate of germination at the initial stages of germination. Treatment Y-07 had a high percentage of germination initially and then maintained a constant rate of germination above that of the control. The percentage germination of treatment Y-09 is high initially but slackens later. Two weeks later its percentage germination does not differ significantly from the control.

(ii) Treatment X-01 showed a constant lower germination rate while X-02 showed a more retarded percentage germination initially but accelerated the germination rate higher than that of X-01 eight days later. At the later stage of this experiment (39 days) the percentage germination of X-02

Fig.2. 9-week-old germinated seedlings of the irradiated seeds by X-radiation and Y-radiation.

This is to show the rate of germination of the irradiated seeds of the various dosages and also to show the rate of seedling growth. (Magnification 0.5x)

Reference:

Con:	Control	
Y-06:	5,000r	(Y-radiation)
Y-07:	10,000r	"
Y-08:	15,000r	"
Y-09:	20,000r	"
Y-10:	300,000r	"
X-01:	5,000r	(X-radiation)
X-02:	20,000r	"

is higher than that for any other treatment.

(b) Root elongation study. (i) From the analysed data of the rate of root elongation of the seedlings concerned and also from the study of the curves of Graph A, treatment x-01 does accelerate the rate of root elongation very greatly, and those of the other treatments are retarded.

(ii) From the study of the curves of Graph A treatment x-01 shows an abnormal mode of action on the elongation of roots e.g. as indicated below:

On the 16th day the elongation is below that of control.
" " 21st " " " is accelerated to twice that of control.
" " 26th " " " acceleration dropped.
" " 31st " " " was below and half that of control.

The other treatments show fairly severe effect but relatively uniform effects on retarding root elongation.

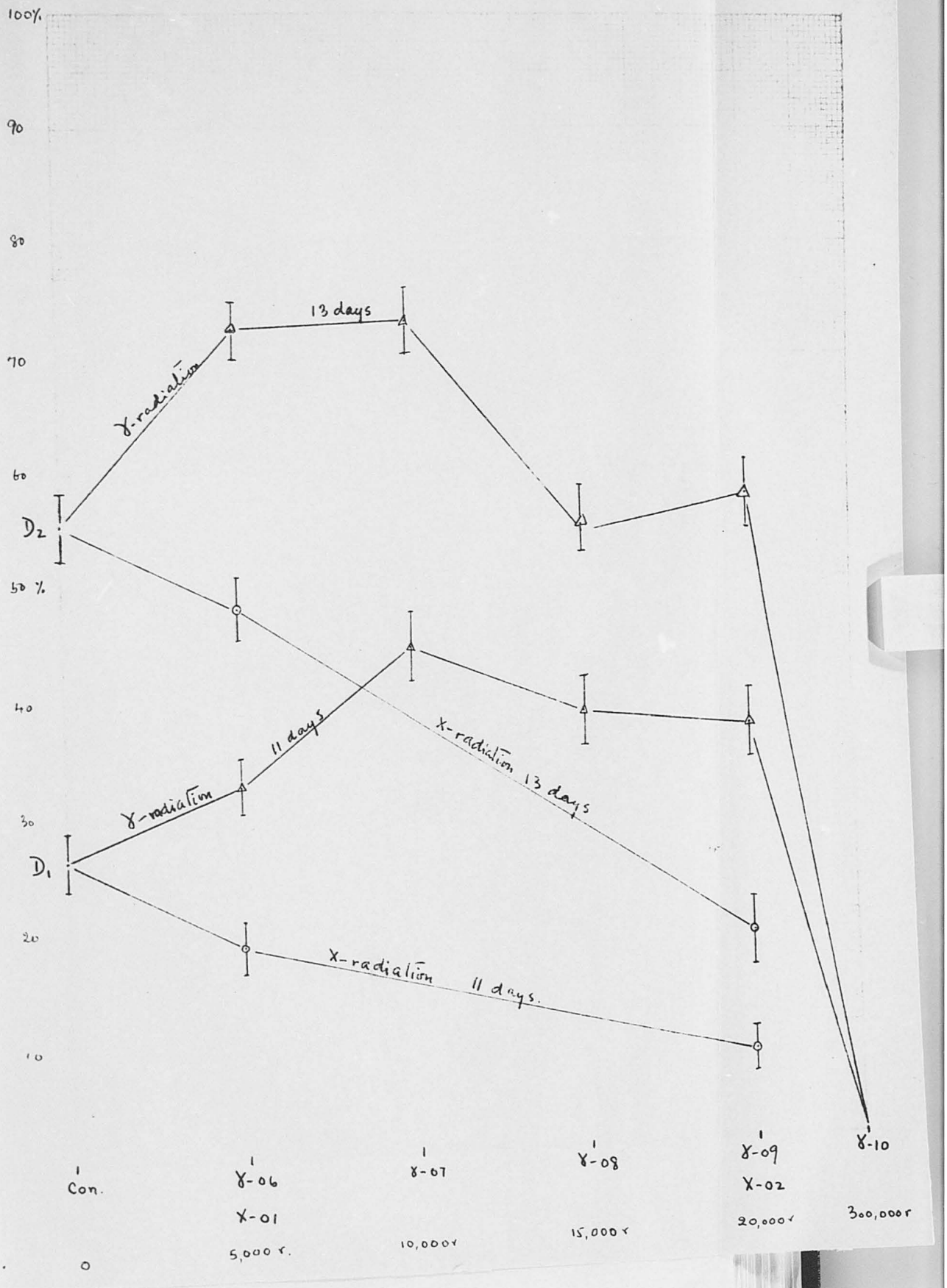
(iii) Treatment Y-07 shows a 50% inhibitory effect compared to the control and treatment Y-09. This can be the possible "near" critical dosage of Y-radiation suitable for mutation study of useful mutation.

(c) The comparative study of rate of germination caused by Y- and X-radiation. From the study of the curves in Graphs D1, D2, D3 and D4, the following brief conclusions can be made of the comparisons. D1 shows that all dosages of X-radiation reduce or retard germination, while Y-radiation of treatments Y-06 to Y-09 does accelerate the initial germination rate. Treatment Y-10 has a greater inhibitory effect on germination.

D4 shows that treatments Y-06, Y-07 and Y-08 of Y-radiation increase the final germination percentage. Only on this date does treatment Y-10 show some delayed germination. On the other hand treatment x-01 checks the rate of germination but treatment x-02 shows its effect of accelerating the rate of germination towards the later stages of germination.

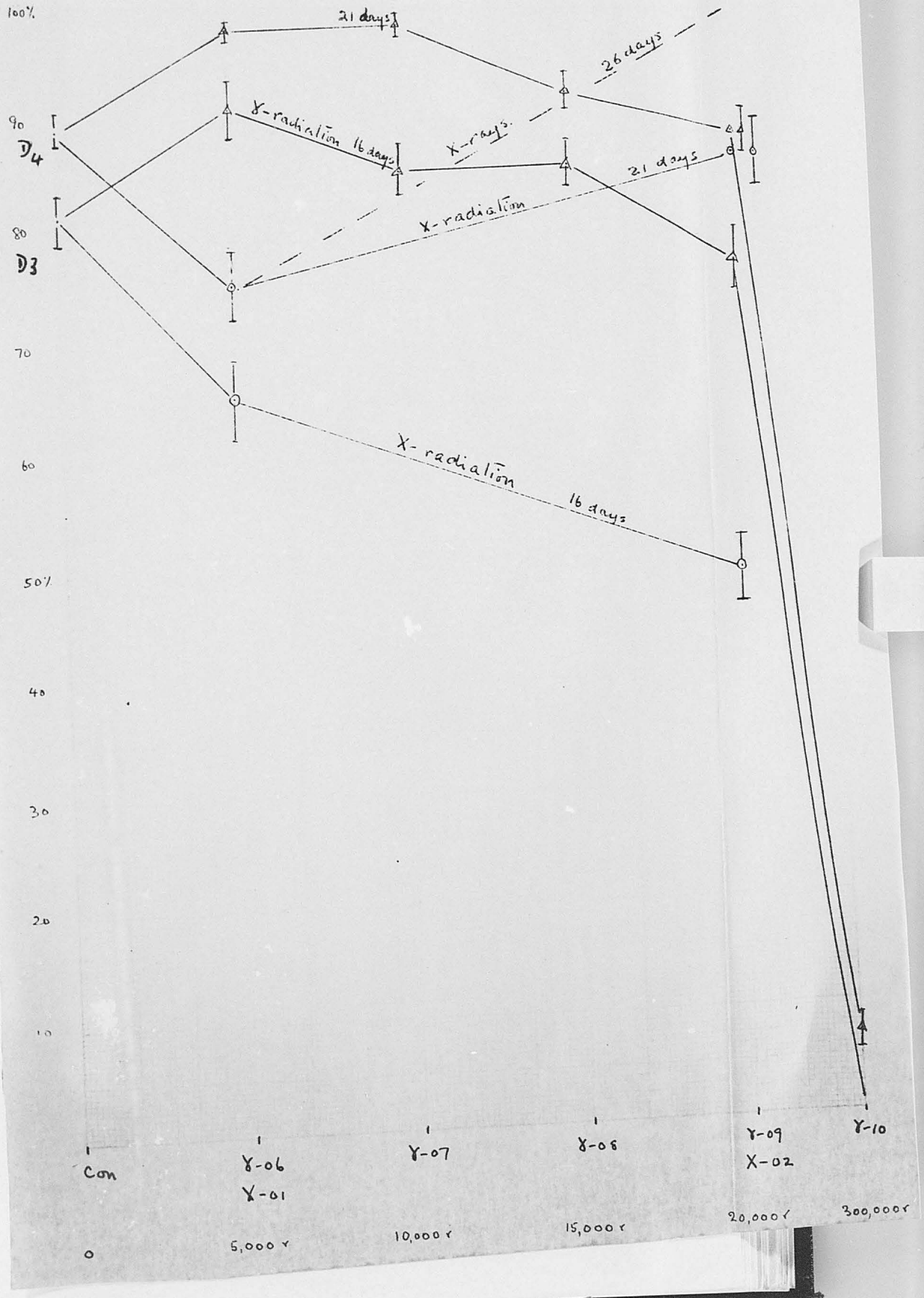
GRAPH "D" (1 and 2)

The effects of γ-radiation and X-radiation on the percentage germination of citrus seeds.

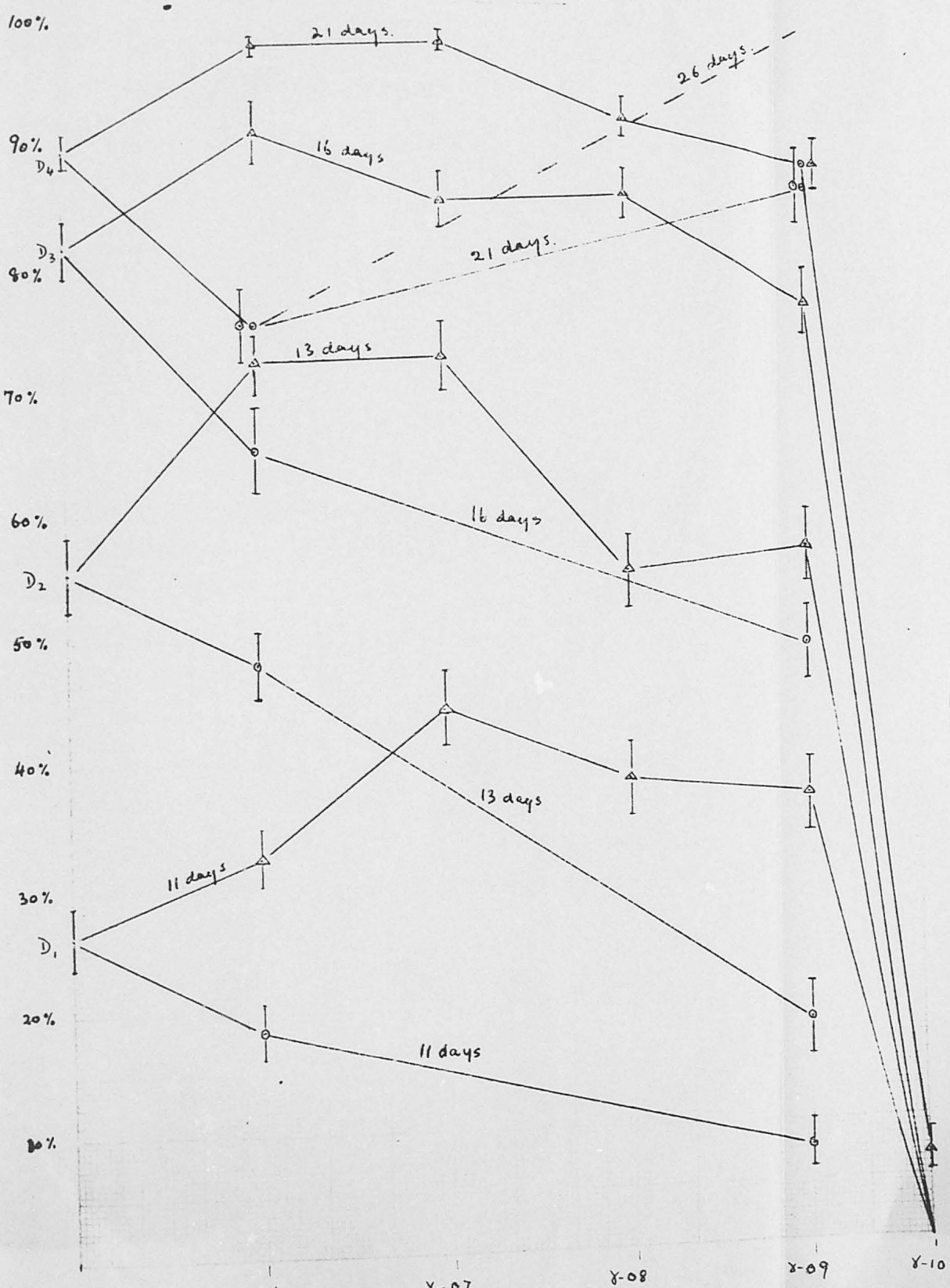


GRAPH "D" (3 and 4)

The effects of γ-radiation + X-radiation on the percentage germination of Citrus seeds.



Graph "D" The effects of γ-radiation and X-radiation on the percentage germination of Lisbon lemon seeds.



IV. REMARKS.

From the above experiment D in which the seeds were being irradiated by Y-rays of a series of dosages ranging between 5000r and 20,000r, it is indicated that the "near" critical dose for suitable mutation study is between 5000r and 10,000r but more towards 10,000r or Y-radiation. However, in the comparative study of the effects of Y-radiation and X-radiation of the same dosage (5000r) of the same experiment, it was shown that X-radiation had a retarding effect on the rate of germination and later in the course of the experiment andaccelerating effect on the rate of root elongation of the seedlings, while the Y-radiation of the same dose accelerated the rate of seed germination. This "difference" in the action of Y-radiation and X-radiation on the germination of Citrus seeds could be due to the two types of "effects" caused by ionizing radiation as previously mentioned in the Literature review; one type being the "direct" effect which causes a biological complex to be altered or destroyed (depending on the dosage); while the second type being the "indirect" effect of a chemical nature causing the depolymerization of the DNA of the chromatid materials. It has been strongly maintained that X-radiation does cause more of the "indirect" effect while Y-radiation causes mainly the "direct" effect.

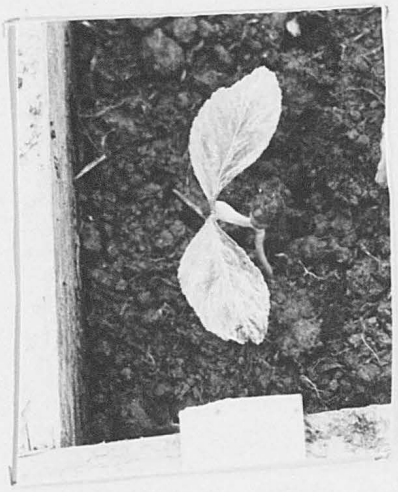
Another more probable explanation for the above "differences" in action of Y-radiation and X-radiation is that X-rays consist of soft and hard rays which have a longer wavelength compared to the Y-rays which have a very short wave length, but are many times more penetrating than the X-rays, thus accounting for the "delayed" or "retarded" action of X-radiation on rate of germination of seeds of Citrus.

Whatever it may be it does need a more thorough and more detailed investigation with a closer and narrower

Fig.3.
9-week-old germinated seedlings of the irradiated seeds by X-radiation and Y-radiation.
To show the morphological deformities due to irradiation. (The most common of which were mosaic pattern of chlorosis and leaf serration and irregularly shaped leaves.)(Magn. 0.5x)

Reference: Con: control Y-06: 5000r; Y-07: 10,000r (Y-rad.)
X-01: 5000r (X-radiation)

Fig. 4.
9-week-old germinated seedling of X-ray treated seeds.
This is to show the type of chlorotic mutant of the morphological nature due to X-radiation.
(Magnification 0.75x)



range of dosages for the similar comparative study of the two types of radiation in order to draw a strict conclusion of the findings.

(The next experiments though having no direct connection are conducted to investigate whether the different types of pretreatments could be of any specific utility when radiating any biological materials so as to enhance the production of rare mutations of some importance).

E. Pretreatment study of ionizing radiations on seeds, to find if there can be any significant difference caused by the effect of high moisture content of dormant seeds when irradiated by X-rays and Y-rays.

I. METHOD.

1. Preradiation. About 250 apparently healthy and uniform sized seeds were selected and divided into five lots of 50 seeds each lot. Two lots of seeds were soaked in distilled water and another two lots of seeds were put into a dessicator of silica gel (which remains blue when dry). The soaking and dessicating of the seeds continued for 25 hours. The seeds for control were left at room temperature and conditions. Just prior to irradiation the soaked seeds were removed lot by lot and were surface dried on filter paper for a minute or so.

2. Radiation Laboratory detail.

Room tmperature: 18°C (±2°)

Y-radiation	X-radiation
Source: Co^{60} 180 curie	Source: G.E.C. Maxima X-ray Hospital therapy unit
½life 5.3 yrs.	Filter: ½mm Cu + 1mm Al
	Max. energy: 210 kv.
	Current: 10 ma.
Dose rate: 184r/min.	Dose rate: 203r/min.
Distance from source: 9cm.	Distance from source: 21.4cm.
Total dose: 30,044r	Total dose: 30,012r
Time: 163 min.	Time: 142 min.

3. Methods of radiation. In the case of Y-radiation, the seeds were placed in small glass bottles which were placed on a disc rotating round the source and at respective half time each bottle was turned right round so as to give an even Y-radiation to all seeds.

In the case of X-radiation each lot was placed over the circular area on the tray which would be placed directly under the X-rays during irradiation. In both cases the

seeds were irradiated by fractionated dosages.

Samples	X-rays	Y-rays	
soaked	SO_1	SO_2	
dessicated	De_1	De_2	
control			Con.
dosages	30,012r	30,044r	0

4. Post-radiation. The treated seeds and the control were put into germination soon after treatment by radiation. The seeds in the respective lots were put into petri dishes which had soaked cotton wool and filter paper at the bottom and were then covered with some allowance for air to enter for respiration of the seeds. Then they were buried under sawdust in the heated frame at temperature 22°C (±2°). Ten days later the seeds were examined under red light at night for the rate of seed germination. Subsequent examinations of seeds were made at two day intervals. It was noted that towards the end of germination there were quite a number of seeds rotted in the "treated" as well as the "control". This is probably due to faulty seeds and not to the treatment. From the germinated seedlings, the measurement of the root length in millimetres was taken for the study of rate of elongation.

II. RESULTS.

1. As there was a great number of seeds rotted the data for seed germination could not be dependable for a "strict" comparison of rate of germination due to the various treatments and the control. However, for the comparative study of the different treatments, the analyses were statistically made from data collected from the study of elongation of root length of the remaining seedlings.

Root elongation at three and a half weeks.

Samples: S-01	S-02	De-1	De-2	Control
Type of X-rays	Y-rays	X-rays	Y-rays	-
Radiation.				
Mean length and Standard error in mm.	7.4±1.7	4.3±2.2	7.0±1.8	6.07±1.3 14.5±2.1

For full detailed analysis refer to Table V

III. CONCLUSION.

From the above statistical analysis of the seedlings taken at 3½ weeks from the date of commencement of the germination test, the following are the conclusions:

(i) All treatments retarded the root growth considerably.

(ii) In the case of X-rays, there was no significant difference shown between soaking and dessication. In the Y-rays the soaked seeds showed a reduction which was more obvious than that of the dessicated at 2% level. (i.e. significant at 2% level).

(iii) There seemed to be no difference between X-rays and Y-rays effect by dessication. Y-rays were more severe than X-rays for soaked seeds.

(iv) For soaked seeds Y-rays were likely to be more useful for mutation on the basis of higher lethal effect.

(The aims of the next two experiments are to investigate into the possibility of using colchicine as a pre-treatment agent for the ionizing-radiation so as to induce rare but desirable types of mutation. The nature of the work suggests that these experiments are only the very fundamental steps for the "proposed" investigation that should ultimately lead to the future detailed work on the production of the "wanted" mutant).

F. To study the effect of Colchicine as a pre-treatment for the ionizing radiation of seeds by Y-rays and X-rays.

I. METHOD.

1. Pre-radiation. About 260 apparently healthy and good Lisbon lemon seeds of uniform size were selected of which 20 were kept for control. The others were divided into three lots to be soaked in three different concentrations of colchicine of 0.1%, 0.2% and 0.5%. They were soaked in the solution for 25 hours. After which, the seeds from each concentration were taken out and divided into four groups. Of the four, two groups were for Y-radiations of dosages of 1,500r and 3000r. The remaining two groups were for X-radiation of dosages of 1,500r and 3000r. Each of the groups of the respective colchicine treatments has its own group label as shown below.

	1500r		3000r	
25hrs	Y-rays	X-rays	Y-rays	X-rays
0.1%	Col-Y_1	Col-X_1	Col-Y_4	Col-X_4
0.2%	Col-Y_2	Col-X_2	Col-Y_5	Col-X_5
0.5%	Col-Y_3	Col-X_3	Col-Y_6	Col-X_6

Prior to irradiation the seeds were surface dried on filter paper for a few minutes.

2. Radiation Laboratory detail.

Laboratory room temperature 18°C (±3°)

Y-rays

Source: Co^{60} 180 curie source.
½life 5.3 years

Dose rate: 184R/min.
Dist. from source: 9cm.

Total dose:	Time.
1,500r	8min.
3000r	16min.

X-rays

Source: G.E.C. Maxima X-ray Hospital therapy unit.
Filter: ½mm Cu + 1mm Al
Maxim. energy: 210 kv.
Current: 10 ma.
Dose rate 203r/min.
Dist. from source: 21.4 cm.

Total dose	Time
1,500r	7.25min.
3000r	14.5min.

3. Post-radiation. After irradiation the seeds were sown in rows in a peat and pumice medium in a box and kept in the heated frame under sawdust at temperature 20°C (±2°). Along with the treated seeds were the control seeds. (It was expected that Colchicine would delay greatly the rate of germination of the treated seeds thus this experiment was set up primarily for the study morphologically of the combined effects of colchicine and radiation. In this respect, the data for the rate of germination of the treated and that of the control was not needed).

II. RESULTS.

1. Twelve days after sowing, the whole box of seeds with the treatee and the control was taken out for observation. The controls had more than half the number of seeds germinated while for the treated, only two seedlings germinated they were:

(i) Col-Y_1 - the seedling had unusual enlarged radicle twice the size of a normal radicle.

(ii) Col-X_1 - Similar effect as the above, enlarged radicle.

On closer examination of the non-germinated seeds, there were quited a considerable number of seeds that had rotted in each treated lot including one or two in the control.

2. Fifteen days later subsequent observations were made. Only a few more seeds from treatment Col-Y_1 and Col-X_1 germinated showing enlarged or swollen radicles. As for the other treatments there were signs that the seeds were about to germinate.

As for the seedlings of the treatments Col-Y_1 and Col-X_1 which had germinated earlier, they showed signs of vigorous growth compared to the control. The newly elongated portion of the radicles showed no sign of enlarging as they had earlier. The average length of the control radicles was 16mm while that of Col-Y_1 was 12mm and 9mm.

3. Twenty days later another observation was made. It was observed that quite a considerable number of seeds in all

treated lots germinated. These seedlings did show the usual effects due to colchicine treatment i.e. had enlarged and swollen radicles more particularly those seedlings from the colchicine treatment of 0.2% and 0.5% which also seemed to have retarded the root growth quite significantly, while those seedlings of treatment Col-Y_1 and Col-X_1 (i.e. 0.1% of colchicine) did show sign of normal growth with normal elongation.

From the count of the germinated, rotted and non-germinated seeds it was shown that there was a greater number of seeds germinated in the X-ray treatment of dosage 1500r. The treatments Col-Y_6 and Col-X_6 showed the greatest effect of delayed germination.

Table to show the number of seeds germinated, non-germinated and rotted of the treated and the control.

Treatment		Germinated	Non-germinated	Rotted
Dosage	Control	14	1	5
1500r	Col-Y_1	10	4	6
	Col-Y_2	9	4	7
	Col-Y_3	9	5	6
3000r	Col-Y_4	8	6	6
	Col-Y_5	9	6	5
	Col-Y_6	7	5	8
	Total	52	30	38
1500r	Col-X_1	12	3	5
	Col-X_2	13	3	4
	Col-X_3	13	2	5
3000r	Col-X_4	9	5	6
	Col-X_5	9	6	5
	Col-X_6	8	4	8
	Total	64	23	33

III. CONCLUSION.

1. Due to the great number of non-germinated and rotted seeds in each of the treated including the control no statistical analysis was made to determine the rate of germination - thus no specific or solid conclusion was made of the rate of germination due to the various treatments.

2. However, judging from the morphological study of the germinated seedlings of the various treatments and from the comparison of this study with that of the control the following tentative conclusions were made:

(i) Colchicine did inhibit the elongation of the seedlings but did not affect so much the germination of the seeds especially for colchicine 0.1 and 0.2%.

(ii) Colchicine treated seeds did show unusual swelling at the radicle at early germination especially for 0.1%.

(iii) The more tolerable colchicine concentration for pre-treatment for Citrus was 0.1% and not higher so as to have an option pre-treatment for radiation effect.

(iv) X-radiation could probably be more suitable than Y-radiation for irradiation with colchicine as the pre-treating agent.

G. To study the effect of colchicine as a pretreatment for ionizing radiation on 12 day old germinating seedlings by X-radiation. (Strictly for morphological and cytological studies and not statistical study).

I. METHOD.

1. Pre-radiation. About 250 good Lisbon lemon seeds were put into germination in a peat and pumice medium in a heated frame at 18°C (±2°). In seventeen days 100 seeds germinated with radicles ranging from 5mm to 20 mm in length. They were then evenly divided into two groups to be pre-treated with Colchicine of concentrations of 0.025% and 0.05%. The seedlings were put in the colchicine solutions for pre-treatment for 26 hours.

Samples treated.

A -0.025%
B -0.05%
} 26 hours.

These colchicine pre-treated seedlings along with the control were taken down to Wellington and put into a special container in a hot water bath at 25°C (±2°). They were in the bath for two days prior to irradiation so as to keep the seedlings actively growing.

2. Radiation Laboratory detail.

Room temperature: 18°C (±2°)

Source: G.E.C. Maxima X-ray hospital therapy unit.

Filter: ½mm Cu + 1mm Al.

Max. energy: 210 kv.

Current: 10 ma.

Dose rate: 86r/min.

Distance from source: 32 cm.

Total dose	Control	500r	1000r
Time (min.)	-	6	12
Sample	A_0	A_1	A_2
	B_0	B_1	B_2

3. Method of radiation. As the seedlings had to be placed at a considerable distance from the source, the extreme effect due to the mixture of soft and hard rays may not have been so effective (as the soft rays could make only part penetration in such thick seeds). In this case the seedlings were placed in the centre of the arc of exposure. Heating facilities were provided and set up in the room to maintain a constant temperature of 18°C (±2°) for the period of irradiation.

4. Post-radiation. One hour after irradiation three seedlings from each sample were taken and their radicles and plumules put into Carnoy fixative for cytogenetical observation. The other seedlings of the various treatments were put into separate containers and placed in a hot water bath at 20°C (±2°) for two days before taking them back to College and placing them in the heated frame with temperature maintained at 18°C (±2°). In the heated frame the seedlings were put in separate compartments in a wooden box in a peat and pumice medium for further morphological observation.

5. Cytological study. For the cytological study of the effects of pre-treatment and irradiation the materials which had been already fixed in Carnoy fixative were taken for squash by the modified Gomori's Hematoxylin staining and squashing method as described later.

II. RESULTS.

(a) Cytological.

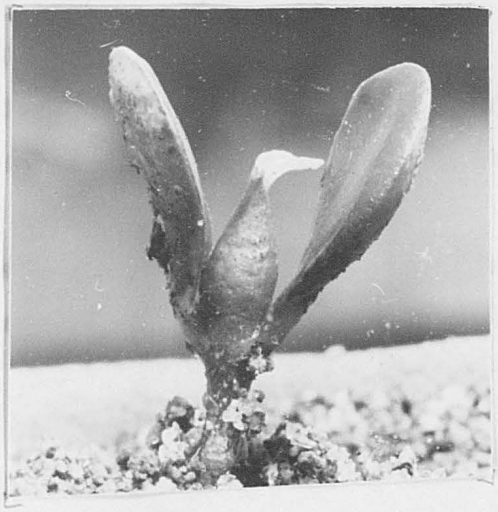
(1) From the squash preparation of sample A_o and B_o (i.e. the colchicine treated but non-irradiated) materials, it was found that only A_o materials had a few polyploid cells and none in B_o. These polyploid cells were those of the metaphasic stages. The chromosome number of such cells exceeded the normal diploid number of 18 chromosomes.

2. The squash preparation of samples A_1 materials showed some affected cells which had irregular resting cells which indicated that there could be chromosome "stickiness"

Fig. 5. Colchicine pre-treated seedlings prior to X-radiation
Photographed at 3½ weeks after irradiation. (magnif. 0.25x)

Reference: Con: control
A_0: 0.025% colchicine non-irrad.
A_1: 0.025% colchicine, 500r; A_2: 0.025% colchicine, 1000r (X-ray)
B_0: 0.05% colchicine, non irradiated.
B_1: 0.05% colchicine, 500r; B_2: 0.05% colchicine, 1000r (X-ray)

Fig. 6. Colchicine pre-treated seedling prior to X-radiation.
Showing the effect of 0.05% colchicine as pre-treating agent prior to X-radiation of 1000r. Note the unusual swelling of the plumule. Photographed at 3½ weeks after irradiation. (Magnification 3.5x)



Reference: B_2: 0.05: colchicine, 1000r (X-ray).

and bridges formed at anaphase stages.

3. The squash preparation of A_2, B_1 and B_2 materials showed a few irregular cells which could be stickiness.

4. Polyploid cells were also observed, they were more in the squash preparation of A_1 and A_2 materials, than in B_1 and B_2 preparation.

(b) Morphological observation of seedlings.

1. Four days after irradiation all seedlings showed active growth but at the same time still showed the effects due to colchicine treatment which were different from the normal untreated control. Treatment A_1 showed two to four seedlings with active plumule growth while the other seedlings in the group remained less active with plumules just appearing.

2. Ten days after irradiation. Treatment A_0 had most of its seedlings actively showing plumule growth. These plumules showed signs of swelling, while treatment B_0 had seedlings growing less vigorously than those of A_0.

Treatment A_1 seedlings showed some gradual progress in their growth, most of which had their plumules swelling the same way as their radicles had behaved earlier. Treatment A_2 had most seedlings showing active growth and most of their plumules were swollen.

Treatment B_1 seedlings showed evidence of retarded growth with swollen radicles. Treatment B_2 seedlings showed the similar effect to that of B_1 seedlings.

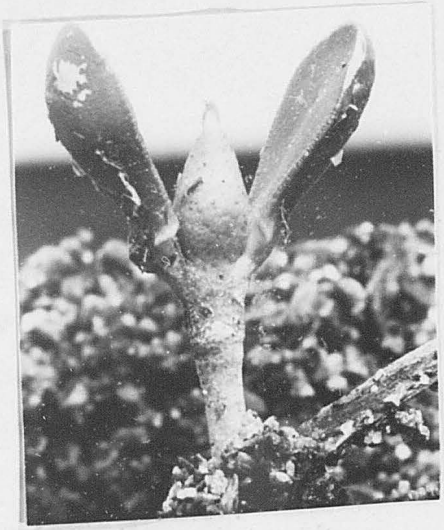
Control. The seedlings had been growing actively and their plumules were thin and their first pair of leaves well developed. They were three times the size of the treated.

3. Two months after irradiation. In fact none of the treated seedlings gained much in height while those of the control had gained extra height. Treatment A_0, A_1 and A_2 showed signs of active growth, their stems were thick and long.

Fig. 7. Colchicine pre-treated seedling prior to X-radiation. Showing the effects of 0.025% colchicine as pre-treating agent prior to X-radiation of 1000r. Note the slight swelling of the plumule, the extra leafy growth at the axil of the cotyledons and the rough texture of the larger leaves. (In some cases there were lateral shoots at the axil of the cotyledon). Photographed 3½weeks after irradiation. (Magn.2.5x)

Reference: A_2: 0.025% colchicine, 1000r (X-ray).

Fig.8. Colchicine pre-treated seedling prior to X-radiation. Showing the effects of 0.05% colchicine as pre-treating agent prior to X-radiation of 500r. Note the great swelling of plumule at its basal end and initial swelling of radicle before tapering off. Photographed 3½ weeks after irradiation. (Magnification 3.5x)



Reference: B_1 : 0.05% colchicine, 500r (X-ray)

Treatment B_o, B_1 and B_2 seedlings had lateral shoots coming out from the axil of the cotyledons but a few seedlings from treatment A_o also had such lateral shoots which were more obvious in B_1 and B_2 seedlings. There was no sign of the above observed in the control and A_2 and A_1.

4. Four months later. Seedlings of treatments of A_o, A_1 and A_2 seemed to have overcome the effect of the pre-treatment and showed signs of active growth. Their first pair of leaves were growing quite actively. The seedlings of treatments B_1 and B_2 in particular were still badly affected and checked from further growth; some still with their plumules swollen and some showed lateral buds forcing their way out from the axil of the cotyledons. In all cases the cotyledons turned very gree.

III. CONCLUSION.

1. From the cytological findings it can be concluded that colchicine concentrations of 0.25% and 0.05% can effect polyploidy in Citrus seedlings at the actively growing stage, but 0.025% would be a better concentration which could effect polyploidy without adversely affecting the growing condition (physiology) of the seedlings. This was evidenced by the fact that after four months the seedlings of treatments B_1 and B_2 seemed to be adversely checked in their growth, while the seedlings of treatments A_1 and A_2 which had 0.025% colchicine pre-treatment were actively growing.

2. Since the seedlings of treatments A_o, A_1 and A_2 showed the same active growth after four months, it could be inferred that the radiation dosages of 500r and 1000r of X-radiation at 86r/min. intensity were below the optimum dosage which would show a 50% survival necessary for a suitable mutation study. However, further study of trials of various dosages with pre-treatment would be necessary in order to have a better understanding of the effects of pre-treatment for ionizing radiation.

H. To study morphologically the phenotypical characters of X-radiation effects of citrus seedlings from Lisbon lemon seeds irradiation by a series of dosages of one common dose intensity.

I. METHOD.

1. Pre-radiation. About 200 healthy and uniform sized seeds were selected and dried at room temperature and divided into lots of 25 seeds each.

These seeds were to be irradiated in the Palmerston North Hospital by X-rays.

2. Radiation Laboratory detail.

Room temperature: 18°C (±2°C)

Scource: G.E.C. Maxima hospital therapy unit.

Max. energy: 210 kv.

Filter: ½mm Cu + 1mm Al.

Current: 10 ma.

Dose rate: 196r/min.

Distance from source: 22cm.

Samples and the respective dosages of irradiation.

Samples	Total dose (r)	Time (minutes)
M	196	1
N	392	2
O	588	3
P	784	4
Q	980	5
R	1176	6
S	1470	7.5
T	1960	10
U	2450	12.5
V	2940	15
W	3430	17.5
X	3920	20
Y	4410	22.5

3. Method of radiation. The seeds were spread on the cardboard tray in the circular area which would be directly under the beam of the X-ray. With the exception of a few, most of the treatments were two split doses, in fact the treatments were done two groups at a time for each group and the seeds were spread in a semicircle.

4. Post-radiation. All the treated and the control seeds were put into germination in a peat and pumice medium in

Fig. 9. Rare characteristics induced by X-radiation of the seeds. To show the unusual branching just below the 1st pair of leaves. X-A_6 (3000r) had a rough stem not round like the control. Branching below the first pair of leaves. X-A_7 (800r) made a loop before branching below the 1st pair of leaves. Note the stunted size of the treated compared to the control: (con). Photographed six months after germination of treated seeds. (Magnif. 0.5x)

Reference: X-A_6:3000r (X-ray); X-A_7: 800r (X-ray)
Con: control

Fig. 10.
The close-up of X-A_6 and X-A_7 mutants. (Magnif. 0.75x)

a long box. The seeds of each treatment were sown in a single row. The rows were spaced far enough apart for proper observation of the seedlings later. As it was summer time, the whole box was kept in an enclosed frame without heating facilities. The day temperature was 18°C (±5°) and the night temperature was about 15°C (± 5°). The humidity in the frame was very high which kept the medium moist for a long time.

II. RESULTS.

1. Two and a half weeks after radiation all treatments had a few seeds each germinated including some of the control. One seedling from treatment R showed an unusual plumule which was split.

2. Two and a half weeks later most of the seeds in all treatments germinated (those remaining could have rotted)- a very high rate of germination. There were many abnormal seedlings (phenotypic mutants) observed in the following treatments. Treatment X had one seedling having four complete chlorotic leaves which had a greenish mid-rib, the stem was also chlorotic. This seedling had a poor root system. The seedling was transplanted to a pot in soil medium and labelled X-A_3.

Treatments O, W, and Y had seedlings showing partial chlorosis. Most of these had irregularly shaped leaves. One seedling of treatment W had one of its first pair of leaves completely chlorotic with green mid rib while the other leaf was normal. This seedling was transplanted and labelled X-A_2. One seedling of treatment O, X-A_1 had its first pair of leaves badly shaped and wrinkled.

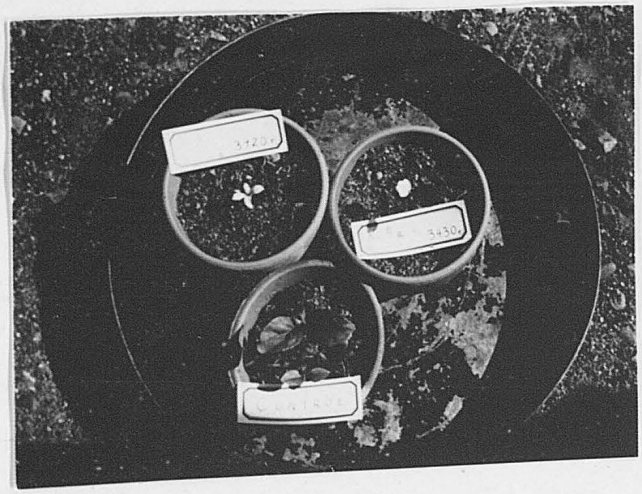
3. Two months later most of the seedlings of all treatments had partial chlorosis, in the 1st and 2nd pair of leaves. The chlorotic effect was on or near the leaf margin. Some of the effect was in mosaic pattern and some in strips. None of these chlorotic effects were seen in Control.

4. Ten and a half weeks later seedling X-A_3 which did

Fig. 11. Rare characteristics induced by X-radiation of seeds. Note the unusual deformed and curled first pair of leaves which were exceptionally greener than other leaves of the same plant. This condition persisted for a long period. Photographed six months after the germination of the treated seeds. (Magnification 0.75x).

Reference: X-A con: Control; X-A_1: 600r (X-radiation)

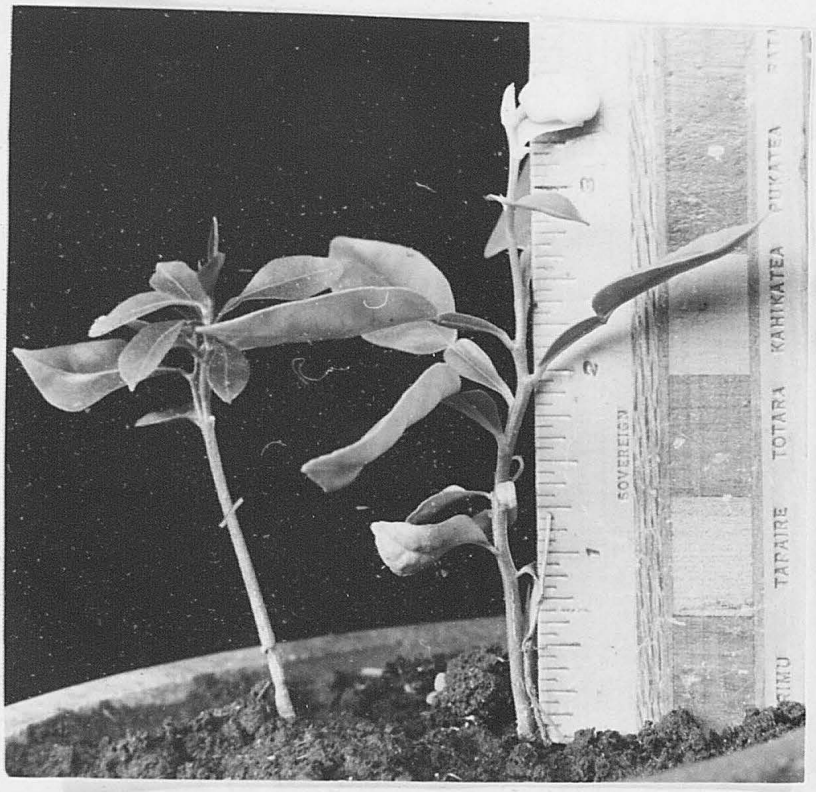
Fig. 12. Chlorosis induced by X-radiation. Sample X-A_3 was a complete chlorotic mutant with four completely chlorotic leaves with greenish midribs and the stem chlorotic. (This mutant had a poor root system and survived ten and a half weeks only). Sample X-A_2 was a partial chlorotic mutant which had only one of its first pair of leaves completely chlorotic while the other leaf was completely normal. The chlorotic leaf later defoliated. (The second pair of leaves had a mosaic pattern of chlorosis with a whitish midrib as shown in Fig. 4) Photographed eight weeks after germination. (Magn. 0.5x)



Reference: Con: control
X-A_2: 3430r (X-radiation)
X-A_3: 3920r (X-radiation)

Fig. 13. Pre-mature flowering in Citrus plants.

This was a six-month-old seedling of the Jamaican Grapefruit . This precocious mutant, however, was not from the group of mutants induced by radiation, it had mutated spontaneously. Note the height of the plant. The flower which was quite normal however was not fertilized.



not show sign of any growth died.

5. Three months later from treatment V one seedling had its plumule looped in a circle and then branched before the first pair of leaves appeared, it was repotted and labelled X-A_6. In treatment P one seedling X-A_7 had a very unusually shaped stem which had branched about 1.5 cm. above the cotyledons. The X-A_2 seedling had its single chlorotic leaf defoliated, while its 2nd pair of leaves had a mosaic pattern of chlorosis with whitish midrib which was more clear at the leaf base. Its normal leaf still remained green and normal. (This could have been a sectorial chimeral mutant). The seedling X-A_1 still had its 1st pair of wrinkled and deformed leaves growing more green than before while its second pair of leaves grew and developed quite normally. (Refer to photoplates of the various seedlings).

III. CONCLUSION.

1. Most of the phenotypic effects of X-radiation of dosages of 2000r to 25,000r were in the form of partial or complete chlorosis and quite often accompnaied by serration and irregularly shaped leaves. These were normally found in the first pair of leaves but quite often these could be found in the 2nd pair.

2. Some other deformatives such as split stem branching before the formation of the first pair of leaves were also seen.

3. Sectorial chimeral mutants were also seen.

DISCUSSION

From the dose rate trials of Y-radiation on young actively growing Citrus seedlings, there is evidence to show that dose rates between 45r/min. (treatment Y-2) and 184r/min. (treatment Y-3) are more suitable for effecting any mutation and that dose rates below 45r/min. seem to show a temporary effect followed by a gradual recovery as is evidenced by the cytological studies of the root squash of the treated materials. (Please refer to Graph F). As for the optimum total dose which would cause the best growth consistency with maximum expectation of mutation, it lies between the dosages of 860r (treatment X-011) and 3000r (tratment X-022), but closer to the dose of 860r as the materials (from cytological studies) of this dosage treatment did show a gradual recovery from the radiation effect. (Please refer to Graph I).

In the treatment of seeds by radiation it is found that Citrus seeds (Lisbon lemon) can withstand very high dosage of very high dose rate of Y-radiation. The lethal dosage seems to be between dosages of 300,000r (treatment Y-10) and 500,000r (treatment Y-11) but the "near critical" dosage to effect useful mutation lies between 5000r (treatment Y-06) and 10,000r (treatment Y-01), more towards the 10,000r dose. (Please refer to Graph B and Table VII).
It is of interest to note that there is a contrast of effects being caused by Y-radiation and X-radiation of the same total dose on Citrus material, for example: when the seeds are radiated by a same total dose of 5000r, it is found that in the case of rate of germination of the treated seeds, Y-radiation seems to have an accelerating effect initially, while X-radiation exhibits retardation on the germination rate initially when compared to the control. In the case of the rate of root elongation of the same seedlings treated, Y-radiation of the same total dose does show a steady inhibitory effect throughout the duration of the

experiments, while X-radiation exhibits a very abnormal mode of action on the rate of root elongation. e.g. at the 16th day from the commencement of germination, the rate of root elongation of the seedlings of this particular dosage treatment is below that of the control. Then at 21st day the rate is accelerated to twice that of the control, but on the 26th day its acceleration dropped and by the 31st day the rate of root elongation of this 5000r X-ray treatment was half that of control. (Please refer to Graph A of Experiment D and Table IV). This abnormal effect of X-radiation can be attributed to the effects of the mixture of the soft and hard rays emmitted by X-radiation.

From the study of the pre-treatment by hydration and dessication of theCitrus seeds prior to irradiation, it is evident that hydrated seeds when irradiated will be affected with a higher frequency of mutation than dessicated seeds. This is due to the fact that hydration does cause or initiate the activation and germination of seeds, thereby enhancing a higher rate of mutation, while dessication does keep the seed dormant, thus accounting for the lower rate of mutation. In this connection it is necessary to recall what has been stated earlier in the review of literature, that the higher rate of mutation encountered by radiation biologists in the study of irradiation effects on plant material is found in most actively growing materials where there is a maximum number of cells undergoing mitosis or meiosis, while resting cells show a greater resistance to radiation, thus having a lower frequency of mutation in the form of chromosome aberrations. (Please refer to Table V). On comparing the effects of Y-rays and X-rays on the pre-treated seeds, it is found that Y-rays seem to be more effective than X-rays in terms of inhibiting the root elongation. This can be explained from the point of pact that Y-rays, due to their shorter wavelength are many times more penetrating than X-rays which consist

of a mixture of soft and hard rays. This mixture however, is not found in Y-rays. (However, further experimental studies on the duration of hydration and percentage of moisture content in the seeds prior to irradiation will be of beneficial importance for the production of useful mutation in Citrus).

In the study of the different doncentrations of colchicine as a pre-treatment for ionizing radiation, it is found that a concentration of 0.1% of Colchicine is most suitable as a pre-treatment for the irradiation of Citrus seeds to effect rare types of mutation. Concentrations higher that 0.1% seem to have a severe inhibitory effect on germination, while that of 0.1% is a more tolerable one in which the pre-treated seedlings did show the usual abnormal effects of colchicine treatment at the same time they also exhibited a fairly active growth. Of the two radiations tried (Y-rays and X-rays) X-rays seem to be the more probable radiation to be used with colchicine as pretreatment to effect the production of rare mutation.

As for the use of colchicine as a pre-treatment for the irradiation of two-week old Citrus seedlings it is found that a concentration of 0.025% is the more tolerable concentration than that of 0.05%. Though both seem to have effected polyploidization of the seedlings, however, the pre-treatment of 0.025% concentration seems to show no adverse effect on the growing condition which is seen in the case of the seedlings of the 0.05% concentration pre-treatment.

From the morphological study of all the seedlings raisdd from irradiated seeds, a variety of morphological deformaties are observed; the most common of which are the chlorotic types most of which were showing mosaic pattern of chlorosis, but there were a couple of seedlings showing complete chlorotic leaves. One of the seedlings had all leaves completely chlorotic and lived for only a few weeks.

Other forms of deformaties encountered are twisting of the 1st pair of leaves and most of the leaf margins were serrated. Of special interest is the splitting of the plumule before the formation of the 1st pair of true leaves. In one case the plumule looped first before splitting.

These observed morphological deformaties and changes do not differ much from those observed by Huskin and Moore who assumed these changes to be the general effectsoof radiation, most of which are not permanent changes. However, it is unfortunate not to have encountered any premature flowering.

From the experiments conducted as well as some earlier experiments carried out on some six-month-old seedlings, the data of which, however, were not recorded, there is good indication that the treatment of seedlings or well developed vegetative growth by ionizing radiation for the improvement of Citrus could achieve promising results. In this connection it may be worth the trouble of trying to irradiate the scion bud wood which has just broken out from dormancy. This method of irradiation can enhance the induction of sports mutation for colour changes in the rind (as had been achieved by Bishop (2) in his attempt to improve the colour of apples). Similarly root-rot resistance in Citrus might also be effected by radiation of the rooted cuttings followed by innoculation of the pathogen. This method is also useful in improving the root-stock varieties which are susceptible to root-rot diseases.

Whatever may be the aim in the radiation work it is very essential to find out the "narrow critical" dosage necessary for each radiation treatment.

Another method of irradiation that would effect true gene or point mutation and be of value from the breeding and genetical point of view is the treatment of pollen grains just before the opening of the flower bud. There

may be a great possibility that this method of irradiation could overcome certain difficulties encountered in the breeding of Citrus such as nucellar embryony, polyembryony and high and extreme variabilities among genetic progenies from both selfing and crossing as discovered by Frost (16,14). Such methods can also effect rare recombination by translocation. This of course is meant to be a long term work involving entirely different techniques and approach in the treatment. A more useful purpose of this method is the transference of rare but horticulturally important characteristics of one species to another species of Citrus without introducing any undesirable characteristics of the former. This however is certainly not achieved by ordinary conventional methods of breeding Citrus. Thus, irradiation of anthers prior to fertilization will certainly be of great use in the breeding of new and rare varieties that can be acclimatized in any unfavourable climate.

It is known that the "Rarotonga seedless" variety of oranges from the Cook Islands are as sweet as the Australian "Murray River" but unfortunately the former lack the rind colour as caused by a climatic factor - lacking the optimum daylight for ripening. For this reason "Rarotonga seedless" has an inferior commercial value compared to the "Murray River" oranges. Also, there is the "Morrisons Seedless" grapefruit in New Zealand. It lacks only the sweetness, otherwise it would have been an excellent fruit, even so it is still popular commercially.

The Citrus breeding in New Zealand faces not only a disadvantage in the fruit quantity but also the difficulties of getting a suitable root stock which could produce a reasonably good harvest at the same time possessing the desired good characteristics of root stock from the propagation point of view. It is thus very likely that by the use of ionizing radiation these two main disadvantages can be overcome in order to produce excellent quality

Citrus fruits in N.Z. and at the same time can excell in the competition with foreign Citrus fruits commercially.

SUMMARY

The aim of these experiments is primarily to investigate into the behaviour of Citrus to ionizing radiation and also lay the foundation for future intensive work on radiation induced mutation in Citrus so as to create new and rare varieties of Citrus of commercial value.

To sum up, the results of the experiments conducted are as follows:

1. The irradiation of 13-day-old germinating Lisbon lemon seedlings showed that the dose rate for mutation study of severe aberration with about 50% survival was between 45r and 184r/min. but nearer to 45r/min.

2. In another treatment of two-week-old seedlings it was found that the optimum dosage needed to induce a 50% survival suitable for inducing maximum mutation was about 860r.

3. In the treatment of dormant seeds with high dose rate of Y-radiation, the lethal dosage was higher than 200,000r and the dosage of 500,000r seemed to have delayed the percentage of germination initially of the treated seeds without apparently reducing the final germination percentage. The effect was slight at 25,000r.

4. The lethal dosage of dormant seeds by Y-radiation at very high intensity between 300,000r and 500,000r was found. In the case of Y-radiation, the critical dosage was between 5000r and 15,000r. These dosages showed an acceleration of the rate of germination at the initial stages when compared to the control. The same dosages of Y-radiation did retard the elongation of the root length of the seedlings of the treated seeds, while 5000r of X-radiation showed a retarded rate of germination and an accelerating effect on the elongation of the roots of the seedlings of the same lot of treatment. However, this "contrasting" effect of X-radiation was shown to be quite abnormal as shown by the graphs.

5. In the study of hydration and dessication of the Citrus seeds as pre-treatment for radiation it was shown that there was significant effect in both types of pre-treatments for bith types of radiation i.e. X-radiation and Y-radiation. However, there was no significant difference shown between soaking and dessication.

In the case of Y-rays, hydration showed more effect than dessication, the significance was at 2% level. For dessicated seeds, Y-radiation seemed to be more useful for the induction of mutation on the basis of inducing a higher lethality to the seeds.

6. In the use of colchicine as a pre-treatment for the radiation of Citrus seeds by X-rays and Y-rays, it is shown that concentrations of 0.1%, 0.2% and 0.5% did show the inhibitory effect on germination and root elongation. The seedlings of the colchicine pre-treated seeds showed unusual bulging of the radicles at early germination. The more tolerable colchicine concentration for pre-treatment purposes was 0.1% and not higher. This could effect the optimum change needed for irradiation.

Of the two types of radiation, X-rays seemed to be the more suitable for radiation with colchicine as pre-treatment agent.

7. In the colchicine pre-treatment of two-week-old Citrus seedlings for irradiation, concentrations of 0.025% and 0.05% did effect polyploidization but 0.025% seemed to be a better concentration as it did not adversely affect the growing conditions of the seedlings while the 0.05% did inhibit the seedling growth for quite a long period of time. It showed that the radiation of the pre-treated seeds with dosages of 500r and 1000r at 86r/min. intensity were below the optimum which should have a 50% survival necessary for a suitable mutation study.

8. In the study morphologically of the effects of ionizing radiation on Citrus it was shown that most of the phenotypical results of X-radiation of dosages of 200r to 25,000r were in the forms of partial and complete chlorosis, more often of deformed shapes of the first pair of leaves, but these chlorotic effects were also observed in the 2nd and 3rd pair of leaves.

Other effects of radiation were split stem and branching before the formation of 1st pair of leaves; these were particularly so in the case of seedlings of the treatment at higher dosages.

APPENDIX

Comment on the cytological technique.

1. Earlier techniques used in the study of Citrus chromosomes. Kung (64) in his study of the chromosome number of Citrus and related genera has mentioned the use of a modified root tip smear technique described by Brown (65). All who work in the cytogenetical study of Citrus chromosomes find that these chromosomes, 18 in number in the whole complement in the diploid, are exceptionally small, besides which they fail to take stain or else stain very porrly in smear preparations prepared by any of the usual smear methods. Thus Brown has recommended a new revised method of smearing for Citrus first used by Warmke (66) in 1935. This method proves quite satisfactory. The essence of this technique consists mainly in the killing and fixing of root tips for 12 hours in glacial acetic acid and absolute alcohol in the ratio of 1:3. The materials are then depectenized by a solution of 95% alcohol and conc. HCl (equal parts) for 5 to 10 minutes. After which, the materials are transferred to Carnoy's fluid for 5 minutes before they are ready for the smear on the slide under a cover slip.

2. The latest technique recommended for the study of Citrus chromosomes. However, for the present cytological study of the chromosomes of Citrus treated by radiation, a more recent modified method of the root tip smear (squash) "the Fuchsin-Orcein smear method" has been recommended by Mr. de Latour a cytologist of the Grasslands Research Department at Palmerston North.

The schedule is as follows:

(1) Fixation of the materials (root tips or leaf buds) in Carnoy's fixative for a minimum of 3 hours (preferable carried out over night). The Carnoy's fixative consists of:

Abs. alcohol	60%
Glacial acetic	30%
Chloroform	10%

(2) Replacing fixative with 70% alcohol, 50% alcohol and finally distilled water, allowing a couple of minutes in each. The materials should be well immersed in each solution.
(3) the distilled water is replaced by 1N HCl in a vial and it is hydrolysed at 60°C for 15 minutes. (This is to soften the tissue).
(4) The acid is poured off and replaced with leuco-basic Fuchsin stain and is then placed in the dark for a minimum of one hour.
(5) The material is then removed from the L.B.F. stain and placed in a small amount of tap water for only about a minute and not more. This is to increase the intensity of the L.B.F. staining.
(6) The material is then placed on a slide. Under an ordinary dissecting microscope the smallest and most stained portion of the material is dissected out. (This is the most actively growing part of the material). For critical work this most deeply stained part of the root tip is cut again into two longitudinal portions for two separate smears (as the smaller the material the better will be the smear or squash to produce a very flat slide.)
(7) This small dissected portion of the root tip is then transferred to a drop of 0.5% or 1.0% orcein in 45% acetic acid on a clean slide. Then slowly with the aid of a dissecting needle a clean cover slip is placed over the material and it is ready for squashing by means of a gentle tapping with the needle handle and in between pressing under thick folds of filter paper. The slide is warmed gently over a spirit lamp and occasionally is observed under a microscope to see it it is properly flattened.
(8) When properly flattened the slide is sealed by nail polish.
It must be born in mind that the above improved method of smear is primarily used for Lotus. Thus it is tried for Citrus and trials on the duration of fixation in Carnoy solution and also the percentage of Orcein used were

carried out with Citrus root tips as well as leaf buds. From these trials the following conclusions were made:

(i) Fixation in Carnoy solution is best between 3 hours and six hours duration and certainly not below three hours.

(ii) That 0.5% orcein is most suited for study of chromosomes in metaphase, anaphase and telophase while 1.0% is good only for metaphase especially when it is necessary to count the chromosomes as they stained deeply in 1.0% orcein, but those at anaphase and telophase looked quite blurred and the shapes of the chromosomes were not clear.

(iii) If a good and well flattened slide has to be made the orcein solution has to be filtered as it tends to deposit sediment which prevents flattening of the cells.

Thus this Fuchsin-Orcein (double staining) method does not really seem the best method for the study of Citrus chromosomes.

3. A better and modified technique adapted for the use of the study of aberrent chromosomes in Citrus.

Mr de Latour further recommends a trial of the Gomoris Hematoxylin squash method which consists of two things, the preparation of the Gomoris Hematoxylin stain and then the squash schedule.

The making of the G.H. stain consists of mixing equal parts of 1% aqueous solution of hematoxylin and 5% aqueous solution of chrome alum. Added to each 100 ml of the mixture is 2 ml 5% aqueous solution of potassium dichromate and 2 ml 0.5N H_2SO_4. This mixture can be used after only two days standing but is best kept for a fortnight before use and is usually kept away from heat. A good solution is covered by a film of a metallic lustre on its surface after one day's standing. The stain has to be filtered immediately before use.

The squash schedule is as follows. (This is also slightly modified to suit small chromosmes of Citrus).

(1) Fixation in either Carnoy solution (6:3:1) or in acetic alcohol (1:3) for a minimum of 3 hours.

(2) It is then transferred to 70% and 50% alsohol and then through distilled water. The material is in each solution for a minute or two only.

(3) Hydrolysis in 1N HCl at 60°C (±1°) for 15 minutes. The vial is immediately dipped into cold water after 15 minutes of hydrolysis.

(4) The material is rinsed in distilled water before transferring to the G.H. stain.

(5) The material is stained in G.H. stain at 60°C (±1°) for 30 minutes.

(6) After 30 minutes of staining the material is destained in 45% acetic acid for another 30 minutes at 60°C (±1°).

(7) The material is dissected under a dissection microscope and then squashed in 45% acetic acid. (Method of squashing is same as that used in Fuchsin-Orcein method).

4. <u>The need for further investigation to improve this Gomoris Hematoxylin method of staining and squashing</u>.

The above schedule does seem more suited for the Citrus chromosome study, nevertheless there is the need to modify and improve it, so trials on the fixation of the material before staining and also trials on the destaining were conducted in the present investigation.

From the fixation trials the aim was to test which is the better fixative - acetic alcohol or Carnoy and also to see if the materials could be stored in the fixative for a longer period than overnight.

The trials are (A) Acetic alcohol (absolute) fixative - (12 hours overnight)

(B) Carnoy fixative (12 hours overnight)

(C) Acetic alcohol fixative storage (36 hours)

(D) Carnoy's fixative storage (36 hours)

The slides of materials (root tips and leaf buds) made from the above trials were kept over a month after which

they were examined. From the examination of these slides all the four groups showed good and clear chromosomes of the metaphasic stage and of the anaphasic stage. There seemed to be no difference between the two fixations and it also showed that the materials can be stored in these fixatives for a couple of days without showing signs of deterioration.

From the destaining trials from a range of timings of 15, 30, 50, 60 and 90 minutes at 60°C (±1°), it was found that destaining at 60°C (±1°) for 50 minutes was the best as the chromosomes of the anaphasic stage seemed to be the clearest. Timings below 50 minutes still left the chromosomes deeply stained - so much so that it was difficult to distinguish the proper shape of the chromosomes. Treatment for longer than 50 minutes makes the chromosomes over destained.

5. Conclusions on the trials made. Comparing the two types of staining and smearing, that is the Fuchsin-Orcein staining and the Gomori's Hematoxylin staining, it is found that the latter is the better of the two for the following reasons:

(i) it makes a cleaner slide without any stain sediments (as those found in the Fuchsin-Orcein staining)

(ii) it is easier to make the smear as the materials are softened three times at 60°C (±1°).

(iii) this method of staining and squashing can be followed by a procedure to prepare the slide for permanent mounting. This method of permanent mounting has recently been modified by Bradley (67) of Dept. of Botany - Univ. of California. This method does not necessitate the removal of the cover slip, the technique consists of the exchange of the reagents in an absolute alcohol vapour chamber.

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TABLE I

Days.	γ-1 2	γ-1 18	γ-1 21	γ-2 2	γ-2 18	γ-2 21
n	16	14	13	22	19	18
Σ	19	207	367	18	46	70
Σ^2	53	5891	20231	56	236	852
Cor.	22.56	3061.64	10360.69	14.73	111.37	272.22
Cor. S.S.	30.44	2830.36	9870.31	41.27	124.63	579.78
$S^2 = \frac{\text{Cor.SS.}}{n-1}$	2.29	217.72	822.63	1.9	6.92	34.10
$V.\eta M = \frac{S^2}{n}$	0.14	15.56	63.27	0.09	0.36	1.89
$S.E.\eta M = \sqrt{\frac{S^2}{n}}$	0.37	3.94	7.95	0.3	0.6	1.38
$M = \frac{\Sigma}{n}$	1.19	14.79	28.23	0.82	2.42	3.89
M ± S.E.	1.2 ± 0.4	14.8 ± 3.9	28.2 ± 8.0	0.8 ± 0.3	2.4 ± 0.6	3.9 ± 1.4

Days.	γ-3 2	γ-3 18	γ-3 21	γ-con. 2	γ-con. 18	γ-con. 21
n	19	15	14	29	26	22
Σ	16	40	39	123	974	1204
Σ^2	28	196	195	659	45610	77830
Cor.	13.47	106.67	108.64	521.68	3648753	65891
Cor. S.S.	14.53	89.33	86.36	137.32	9122.47	11939.00
$S^2 = \frac{\text{Cor.SS}}{n-1}$	0.81	6.38	6.36	4.90	364.96	568.52
$V.\eta M = \frac{S^2}{n}$	0.04	0.43	0.47	0.17	14.03	25.84
$S.E.\eta M = \sqrt{\frac{S^2}{n}}$	0.2	0.62	0.65	0.40	3.70	5.07
$M = \frac{\Sigma}{n}$	0.84	2.67	2.79	4.24	37.46	54.73
M ± S.E.	0.8 ± 0.2	2.7 ± 0.6	2.8 ± 0.7	4.2 ± 0.4	37.5 ± 3.7	54.7 ± 5.1

TABLE I.

Study of the effects of differen
dose rate of one common
total dose (11,000 r) of
γ-radiation on two-week o
germinating Lisbon Lemon See

TABLE II

Effects of X-radiation on the rate of elongation of roots of two-week-old seedlings of Lisbon Lemon.

	X-011				X-022			
Days	5	10	15	21	5	10	15	21
n	23	23	20	19	16	16	16	15
Σ	156	116	74	73	43	13	12	0
Σ^2	1572	1138	616	665	197	29	42	
Cor.	1058·09	585·04	273·80	280·47	115·56	10·56	9	
Cor. S.S.	513·91	552·96	345·20	384·53	81·44	18·44	23·00	
$S^2 = \frac{Cor.SS}{n-1}$	23·36	25·13	18·17	21·36	5·43	1·23	2·20	
V. of $M = \frac{S^2}{n}$	1·02	1·09	0·91	1·12	0·34	0·08	0·14	
S.E. of $M = \sqrt{\frac{S^2}{n}}$	1·0	1·04	0·95	1·06	0·58	0·28	0·37	
$M = \frac{\Sigma}{n}$	6·78	5·04	3·7	3·84	2·69	0·81	0·75	0
M ± S.E.	6·8 ± 1·0	5·0 ± 1·0	3·7 ± 1·0	3·8 ± 1·1	2·7 ± 0·6	0·8 ± 0·3	0·8 ± 0·4	

Days	X-033 5	10	15	21	X-044 5	10	15	21	X-con. 5	10	15	21
n	21	21	21	21	18	18	18	18	19	17	17	17
Σ	35	0	0	0	24	0	0	0	142	138	130	106
Σ^2	157				50				1312	1496	1536	1352
Cor.	58				32				1061·26	1120·24	994·11	660·92
Cor. S.S.	99				18				250·74	375·76	541·89	691·06
$S^2 = \frac{Cor.SS}{n-1}$	4·95				1·06				13·93	23·49	33·87	43·19
V. of $M = \frac{S^2}{n}$	0·24				0·06				0·73	1·48	1·99	2·54
S.E. of $M = \sqrt{\frac{S^2}{n}}$	0·49				0·24				0·85	1·21	1·41	1·59
$M = \frac{\Sigma}{n}$	1·66	0	0	0	1·33	0	0	0	7·47	8·12	7·65	6·24
M ± S.E.	1·7 ± 0·5				1·3 ± 0·7				7·5 ± 0·9	8·1 ± 1·2	7·7 ± 1·4	6·24 ± 1·6

TABLE III

	γ-01			γ-02			γ-03		
Days	17	23	27	17	23	27	17	23	27
n	37	42	43	28	43	48	12	18	24
Σ	135	255	273	55	87	108	18	29	47
Σ^2	863	2961	3281	125	211	294	32	53.00	105
Cor.	492.56	1548.21	1733.23	108.04	176.02	243.00	27	46.72	92.04
Cor. S.S.	370.44	1412.79	1547.77	16.96	34.98	51.00	5.00	6.28	12.96
$S^2 = \frac{\text{Cor. S.S.}}{n-1}$	10.29	34.46	36.85	0.63	0.83	1.09	0.45	0.37	0.52
$V.of\ M = \frac{S^2}{n}$	0.28	0.82	0.88	0.02	0.09	0.02	0.04	0.02	0.02
$S.E.of\ M = \sqrt{\frac{S^2}{n}}$	0.53	0.91	0.94	0.14	0.30	0.14	0.63	0.14	0.14
$M = \frac{\Sigma}{n}$	3.65	6.07	6.35	1.96	2.02	2.25	1.50	1.61	1.96
M ± S.E.	3.7 ± 0.5	6.1 ± 0.9	6.4 ± 0.9	2.0 ± 0.1	2.0 ± 0.3	2.3 ± 0.1	1.5 ± 0.6	1.6 ± 0.1	2.0 ± 0.1

Days	γ-04			γ-05			γ-Con		
Days.	17	23	27	17	23	27	17	23	27
n	13	24	26	0	9	14	48	48	49
Σ	17	32	52		10	16	374	757	789
Σ^2	25	48	118		12	20	6098	17 467	19237
Cor.	22.23	42.67	104		11.22	18.71	2914.08	11 938.52	12 704.51
Cor. S.S.	2.77	5.33	14		0.78	1.73	3183.92	5528.48	6532.49
$S^2 = \frac{\text{Cor. SS.}}{n-1}$	0.23	0.23	0.56		0.11	0.13	67.74	117.63	136.09
$V.of\ M = \frac{S^2}{n+1}$	0.18	0.01	0.02		0.01	0.009	1.41	2.45	2.78
$S.E.of\ M = \sqrt{\frac{S^2}{n}}$	0.42	0.1	0.14		0.1	0.03	1.2	1.6	1.67
$M = \frac{\Sigma}{n}$	1.30	1.33	2.00		1.12	1.14	7.8	15.77	16.10
M ± S.E.	1.3 ± 0.4	1.3 ± 0.1	2.0 ± 0.1	0	1.1 ± 0.1	1.1 ± 0.0	7.8 ± 1.2	15.8 ± 2.5	16.1 ± 1.7

TABLE III.

The effects of high intensity γ-radiation on the rate of root elongation of Lisbon Lemon seedlings from irradiated seeds.

TABLE IV

The effects of γ-radiation and X-rays on the elongation of root length of the Lisbon Lemon from Treated seeds.

Days	X-01				X-02			
	16	21	26	30	16	21	26	30
n	33	36	35	35	15	33	38	38
Σ	184	416	335	164	48	25	48	0
Σ^2	1442	6518	4199	1032	138	47	132	
Cor	1025·93	4761	3206·42	788·45	128	18·94	60·63	
Cor. SS	416·07	1757	992·58	243·55	10	28·06	71·37	
$S^2 = \frac{Cor.SS}{n-1}$	13·00	48·81	29·19	7·15	0·59	0·88	1·93	
$V.q.M = \frac{S^2}{n}$	0·39	1·36	0·83	0·22	0·03	0·03	0·05	
$S.E.q.M = \sqrt{\frac{S^2}{n}}$	0·62	1·16	0·91	0·47	0·17	0·17	0·22	
$M = \frac{\Sigma}{n}$	5·58	11·50	9·57	4·69	2·67	0·76	1·26	
M ± S.E.	5·6 ± 0·6	11·5 ± 1·2	9·6 ± 0·9	4·7 ± 0·5	2·7 ± 0·2	0·8 ± 0·2	1·3 ± 0·2	—
n		36		35		33		38
Σ		689		1105		94		138
Σ^2		14189		43310		334		624
Cor.		13186·69		35076		267·76		501·15
Cor. SS		1002·31		8234		66·24		122·85
$S^2 = \frac{Cor.SS}{n-1}$		28·64		242·18		2·07		3·32
$V.q.M = \frac{S^2}{n}$		0·80		6·92		0·06		0·09
$S.E.q.M = \sqrt{\frac{S^2}{n}}$		0·89		2·63		0·24		0·3
$M = \frac{\Sigma}{n}$		19·14		31·66		2·85		3·63
M ± S.E.		19·1 ± 0·9		31·7 ± 2·6		2·9 ± 0·2		3·6 ± 0·3

Rate of elongation

Total increment of root length

Days	γ-Con.				γ-06				γ-07			
	16	21	26	30	16	21	26	30	16	21	26	30
n	55	60	60	59	62	62	63	63	57	66	67	66
Σ	344	473	484	470	389	360	443	474	198	159	169	206
Σ^2	2954	5239	5708	5352	2927	2780	4099	4934	896	777	903	1256
Cor.	2151·56	3728·81	3904·26	3744·06	2440·66	2090·32	3115·06	3566·28	687·78	383·07	426·28	642·74
Cor. S.S	802·44	1510·19	1803·74	1607·94	486·34	689·68	983·94	1367·72	208·22	393·96	476·72	613·04
$S^2 = \frac{Cor.SS}{n-1}$	14·56	25·60	30·57	27·72	7·97	11·31	15·87	22·06	3·72	6·09	7·22	9·43
$V.q.M = \frac{S^2}{n}$	0·27	0·42	0·51	0·47	0·13	0·18	0·25	0·35	0·07	0·09	0·11	0·14
$S.E.q.M = \sqrt{\frac{S^2}{n}}$	0·52	0·64	0·71	0·68	0·36	0·42	0·50	0·59	0·26	0·3	0·11	0·37
$M = \frac{\Sigma}{n}$	6·25	7·88	8·07	7·97	6·27	5·81	7·03	7·52	3·47	2·41	2·52	3·12
M ± S.E.	6·3 ± 0·5	7·9 ± 0·6	8·1 ± ·7	8·0 ± 0·7	6·3 ± 0·1	5·8 ± 0·4	7·0 ± 0·5	7·5 ± 0·0	3·5 ± 0·3	2·4 ± 0·3	2·5 ± 0·1	3·1 ± 0·2
n		60		59		62		63		66		66
Σ		863		1768		812		1713		430		790
Σ^2		13509		67166		12630		55727		4088		14780
Cor.		12412·81		52980		10634·58		45577		2801·5		9456·04
Cor. S.S.		3096·19		14186		1995·42		9160		1286·69		5323·94
$S^2 = \frac{Cor.SS}{n-1}$		52·48		244·59		32·71		14·76		19·79		81·91
$V.q.m = \frac{S^2}{n}$		0·88		4·15		0·53		0·23		0·30		1·24
$S.E.q.M = \sqrt{\frac{S^2}{n}}$		0·94		2·03		0·73		0·47		0·54		1·11
$M = \frac{\Sigma}{n}$		14·38		30·00		13·10		27·19		6·52		12·00
M ± S.E.		14·4 ± 0·9		30·0 ± 2·0		13·1 ± 0·7		27·2 ± 0·5		6·5 ± 0·5		12·0 ± 1·1

Rate of elongation

Total increment of root length

Days	γ-05				γ-09				γ-10			
	16	21	26	30	16	21	26	30	16	21	26	30
n	57	61	61	61	48	55	55	55	0	0	6	15
Σ	89	18	36	41	08	0	0	39			9	9
Σ^2	197	32	92	143	125			48			17·0	9
Cor.	138·96	5·31	21·25	27·56	96·33			21·02			13·50	6·23
Cor. SS	58·04	26·69	70·75	115·44	31·67			26·98			3·5	2·77
$S^2 = \frac{Cor.SS}{n-1}$	1·04	0·44	1·18	1·92	0·67			0·5			07	0·23
$V.q.M = \frac{S^2}{n}$	0·02	0·01	0·02	0·03	0·01			0·01			012	0·02
$S.E.q.M = \sqrt{\frac{S^2}{n}}$	0·14	0·1	0·14	0·17	0·1			0·1			0·34	0·14
$M = \frac{\Sigma}{n}$	1·56	0·3	0·6	0·7	1·42			0·72			1·5	0·67
M ± S.E.	1·6 ± 0·1	0·3 ± 0·1	0·6 ± 0·1	0·7 ± 0·2	1·4 ± 0·1	—	—	0·6 ± 0·1	—	—	1·5 ± 0·3	0·7 ± 0·1
n		61		61		55		55		6		13
Σ		167		229		106		142				18
Σ^2		601		1383		240		390				30
Cor.		451·19		857·68		204·29		276·55				24·92
Cor. SS.		143·81		523·32		35·71		113·45				5·08
$S^2 = \frac{Cor.SS}{n-1}$		2·40		8·72		0·66		2·1				0·42
$V.q.M = \frac{S^2}{n}$		0·04		0·37		0·01		0·04				0·03
$S.E.q.M = \sqrt{\frac{S^2}{n}}$		02		0·37		0·1		0·2				0·17
$M = \frac{\Sigma}{n}$		2·74		3·75		1·93		2·58				1·38
M ± S.E.		2·7 ± 0·2		3·8 ± 0·4		1·9 ± 0·1		2·6 ± 0·2		—		1·4 ± 0·2

Total increment of root length.

TABLE V

The effects of Hydration and Dessiccation as pre-treatment prior to ionizing radiation by X-rays and γ-rays on Lisbon Lemon Seeds.

	So-1 X-rays	So-2 γ-rays	De-1 X-rays	De-2 γ-rays.	Con.
n	15	11	12	14	15
Σ	111	47	84	85	288
Σ^2	1455	735	1037	829	4058
Cor.	821.40	200.82	588.00	516.07	3168.26
Cor. S.S.	633.60	534.18	449.00	312.93	889.74
$S^2 = \frac{\text{Cor. SS}}{n-1}$	45.26	53.42	40.82	24.07	63.55
V.q M $= \frac{S^2}{n}$	3.02	4.86	3.4	1.72	4.24
S.E. of M $= \sqrt{\frac{S^2}{n}}$	1.7	2.2	1.8	1.3	2.1
$M = \frac{\Sigma}{n}$	7.4	4.27	7.0	6.07	14.53
M ± S.E.	7.4 ± 1.7.	4.3 ± 2.2	7.0 ± 1.8	6.1 ± 1.3.	14.5 ± 2.1

TABLE VI

The effects of high intensity γ-radiation on the percentage germination of Lisbon lemon seeds.

G.T. = Grand Total seeds used.
T = Total Seeds germinated.
S.E = Standard error.
% = Percentage germination

DAYS	Con. G.T. 60	γ-01 G.T. 60	γ-02 G.T. 60	γ-03 G.T. 60	γ-04 G.T. 60	γ-05 G.T. 60
13	T. 21 35.0% S.E. 6.2	T. 14 23.3% S.E. 5.5	T. 7 11.6% S.E. 4.1	T. 2 3.3% S.E. 6.1	–	–
15	T. 34 56.6% S.E. 6.4	T. 26 43.3% S.E. 6.4	T. 14 23.3% S.E. 5.5	T. 9 15.0% S.E. 4.6	T. 5 8.3% S.E. 3.6	–
17	T. 44 73.3% S.E. 5.7	T. 35 58.3% S.E. 6.4	T. 25 41.6% S.E. 6.4	T. 11 18.3% S.E. 4.9	T. 13 21.6% S.E. 5.3	–
21	T. 48 80.0% S.E. 5.2	T. 40 66.6% S.E. 6.1	T. 34 56.6% S.E. 6.4	T. 17 28.3% S.E. 5.7	T. 18 30.0% S.E. 5.9	T. 5 8.3% S.E. 3.6
23	T. 49 81.6% S.E. 5.0	T. 43 71.6% S.E. 5.8	T. 40 66.6% S.E. 6.1	T. 19 31.6% S.E. 6.0	T. 24 40.0% S.E. 6.3	T. 9 15.0% S.E. 4.6
25	T. 51 85.0% S.E 4.6	T. 48 80.0% S.E. 5.2	T. 42 70.0% S.E. 5.9	T. 21 35.0% S.E. 6.2	T. 25 41.6% S.E. 6.4	T. 10 16.6% S.E. 4.8
27	T. 51 85.0% S.E. 4.6	T. 51 85.0% S.E 4.6	T. 46 76.6% S.E. 5.5	T. 26 43.8% S.E. 6.4	T. 26 43.3% S.E 6.4	T. 13 21.6% S.E. 5.3

TABLE VII

The Effects of γ-radiadiation and X-radiation on the percentage germination of Lisbon lemon seeds.

G.T = Grand Total seeds used
T. = Total seeds germinated
S.E. = Standard Error.
% = Percentage germination.

DAYS.	γ-00 G.T. 65	γ-06 G.T. 66	γ-07 G.T. 63	γ-08 G.T. 65	γ-09 G.T. 62	γ-10 G.T. 45	X-01 G.T. 49	X-02 G.T. 38
10	T. 5 7.6 % S.E. 4.4	T. 9 13.2 % S.E. 4.9	T. 19 30.1 % S.E. 5.6	T. 14 21.5 % S.E. 6.3	T. 13 21.0 % S.E. 5.3	—	—	—
11	T. 17 26.2 % S.E. 5.5	T. 22 32.4 % S.E. 5.7	T. 28 44.6 % S.E. 6.3	T. 25 38.5 % S.E. 6	T. 23 37.1 % S.E. 6.1	—	T. 9 18.4 % S.E. 5.5	T. 3. 7.9 % S.E. 4.4
13	T. 36 55.4 % S.E. 6.2	T. 49 72.5 % S.E. 5.4	T. 46 73.1 % S.E. 5.6	T 36 55.4 % S.E. 6.2	T. 36 57.3 % S.E. 6.3	—	T. 20 48.2 % S.E. 7.1	T 7. 18.5 % S.E. 6.3
16	T. 53 81.5 % S.E. 4.8	T. 62 91.0 % S.E. 5.0	T. 54 85.7 % S.E. 4.4	T. 56 86.2 % S.E. 4.3	T. 48 77.4 % S.E. 5.3	—	T. 32 65.3 % S.E. 6.9	T. 18 49.5 % S.E. 6.3
18	T. 55 84.6 % S.E. 4.5	T. 67 98.5 % S.E. 1.5	T. 59 93.5 % S.E 3.1	T. 59 90.7 % S.E. 3.6	T. 52 83.9 % S.E. 4.7	—	T. 34 69.4 % S.E. 6.6	T. 27 71.1 % S.E. 8.1
21	T. 58 89.2 % S.E. 3.8	—	T. 62 98.4 % S.E. 1.6	T. 60 92.3 % S.E. 3.3	T. 55 88.7 % S.E. 4.0	T. 3 6.7 % S.E. 3.7	T. 37 75.5 % S.E. 6.1	T. 33 86.8 % S.E. 7.3
26	—	—	—	—	—	T. 9. 20.0 % S.E. 5.9	—	T. 38 100 % S.E. 5.5.
39	—	—	—	—	—	T. 15 33.3 % S.E. 5.7.	—	—