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THE GERMINATION OF SEEDS

Submitted for the Degree of

Doctor of Philosophy at the

University of Waikato,

Hamilton, New Zealand.

October 1971

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ACKNOWLEDGEMENTS

The author wishes to thank Professor A. T. Wilson for suggesting the topic and the supervision he provided, Dr Sutton and Dr Malcom of the Institute of Nuclear Sciences for irradiating the seeds, the gardeners at both Waikato University and Victoria University for tending the plants, Dr Payne and and Simon Crase of Victoria University for help with the computer programme especially when the computer rejected it, Mr Roberts of the Applied Maths Division for advice on the Analysis of Variance, and Professor J. F. Duncan for the help he provided. Thanks are also due to Mrs Singleton for the superb effort she has made with the typing of this thesis. Last but not least, I wish to thank my husband for his patience in bearing the trials and tribulations he would not have had without me.

CONTENTS

ABSTRACT

Literature Reviews of seed dormancy, the germination of seeds (restricted as closely as possible to the period actually preceding radicle protrusion) and the effects of gamma radiation are included in this thesis.

Tritiated water studies of heavily irradiated Sinapis alba seeds showed that these seeds can carry out active metabolism, leading to the formation of tritiated gamma-aminobutyric acid (GABA), alanine, malic acid and citric acid. The compounds which were not labelled by tritiated water in heavily irradiated seeds but which were labelled in non-irradiated seeds were the following: lactic acid, glutamine, sucrose, glutamic acid, aspartic acid and sugar phosphates.

Difficulty was experienced in enabling sufficient C-14 substrate to enter the seeds for studying the metabolism of these compounds. The seeds were therefore ground in liquid air prior to the C14-tracer studies. The metabolism of this powder was examined using tritiated water. Over short time intervals this metabolism was similar to that of whole seeds except for the heavy labelling of lipid material which was shown to be ninhydrin positive. At longer time intervals this powder did not develop the complex patterns typical of whole seeds. The metabolites lactic acid, glutamine, sucrose and sugar phosphates were not labelled during 24 hours imbibition in tritiated water, of the Liquid Air Powder (LAP) but were labelled on imbibition of whole seeds.

Imbibition of the LAP of both irradiated and non-irradiated seeds with the C-14 substrates gave similar distributions of radioactivity. Aspartate-U-C14 was not rapidly metabolised within 4 hours. In non-irradiated seeds traces of a ninhydrin positive compound (U1) postulated to be succinylarginine were found after 4 hours. Incubation with

GABA-1-C14 led to labelled flavonoids within one hour and to labelled alanine and an unknown (U2) within 4 hours. The metabolism of glutamate-U-C14 rapidly led to the formation of GABA at short time intervals and at longer time intervals (4 hours) to labelled GABA, alanine, and two unknowns called "YPN" and U3. In the irradiated seeds labelled lipid was also formed. The unknowns U2 and U3 are postulated to be the same ninhydrin positive compound, possibly a peptide.

The transaminases, glutamate-oxaloacetate and glutamate-pyruvate were found to be active in enzyme extractions of dry seeds. Evidence showing a reduction in the activity of transaminases with time of imbibition is also reported. Paper chromatographic analyses of seed metabolism, including a novel method developed for a rapid enzyme assay are included. These results were similar to those obtained using C-14 substrates. In addition conversion of GABA and glutamate to an unknown called UVGB was found. The metabolism of aspartate led to alanine and of alanine, to GABA.

The limited metabolism displayed by heavily irradiated seeds immediately after irradiation did not lead to the formation of a seedling for those Sinapis alba seeds which had received a dose of 2.98 Mrep or more. The germination of a very large range of seed species was examined after receiving chronic doses of irradiation. Only parsnip seeds would not germinate after receiving a dose of 0.5 Mrads. Most seeds showed similar responses to that for mustard seeds.

One group of irradiated mustard seeds were left in a drawer in the laboratory. These seeds showed complete recovery in both the ability to germinate and in their labelling patterns when imbibed with tritiated water after less than 6 months storage. A similar set of seeds stored over silica gel did not display any recovery. A large experiment was undertaken in which seeds of many species were examined after various

periods of storage at fixed or room humidities. Some recovery was observed although not of the same order as that found earlier. Storage at very high humidities led to loss of the ability to germinate. The effects of various solutions were also examined and once again a limited amount of recovery was observed. It is therefore postulated that some other factor, not a fixed relative humidity, was responsible for the dramatic recovery of the first group of mustard seeds. This could be a short period at high relative humidities followed by a longer period at low humidities or possibly some gas in the atmosphere of the laboratory at that time.

Plants were grown from the fully recovered mustard seeds. The greater weight and height of these plants compared to those from non-irradiated seeds were shown to be statistically significant.

1. THE GERMINATION OF SEEDS

1-1 THE PROBLEM

The seed plants or spermatophytes are distinguished from other plants by their mode of reproduction. This group forms the dominant vegetation over most of the land surfaces of the earth and are readily found even in the most inhospitable regions such as tundra, deserts or salt marshes.[1]

The common factor shown by the ancestral forms of present-day cultivated plants is their "weediness", i.e. the ability to colonize open or disturbed habitats with bare soil and the inability to withstand a high level of competition from other plants. It is thought that plants with weedy tendencies colonized kitchen middens and rubbish heaps in the vicinity of man's dwellings.[5] The womenfolk would gather the seeds with large food reserves from here as well as from their natural habitats. Gradually these plants would become established in the vicinity of the dwellings, partly by natural colonization and partly from seeds dropped there by man himself. These plants would be regularly harvested and would no longer be gathered from their natural habitats. Mutations for increased yield and palatability would be selected unconsciously. The actual process of sowing seeds almost certainly came very late, after a high level of social and cultural organization in the primitive agriculturists had taken place. When man began to retain the seeds and to place them in the soil again at the right time in prepared fields or gardens he would consciously select favourable mutations.

The keeping quality of seeds accounts for their special importance in the history of the human race. Grains were favoured by the earliest farmers as they not only gave greater return for the

effort involved but they could also be stored with ease for considerable periods. Corn and rice, in particular, were stored and traded, imported and exported. Under primitive conditions such activity was impossible for vegetative structures such as cabbages and potatoes.

The New World was the source of many of our most important domesticated plants, e.g. potato, tomato and maize. Three fifths of the world's agricultural wealth today, is estimated to be derived from plants unknown to Europe before Columbus.[6]

In a country such as New Zealand which relies so heavily on an agricultural economy the cultivation of plants is extremely important. We are, in effect, entirely dependent on the "grass" of our fields and hence, on the seeds from which the plants were obtained.

Very little is known of the biochemistry of seed germination. It is of interest not only from the point of view of "How do seeds germinate?" but also from a consideration of basic control of cell metabolism. In seeds we have an excellent system in which the processes of cell elongation are separated from the processes of cell division and in which biochemical control of enzyme activation and inactivation must be rigorous. The seed, in order that it might survive, contains sufficient reserves to sustain a rapidly growing seedling for several weeks. Hence seeds are an extremely interesting topic for examination, for a full understanding of what happens within a seed when it germinates could greatly enlarge our knowledge of all biochemical systems, including the human body.

1-2 SEED MORPHOLOGY

The non-vascular plants, i.e. those which do not produce specialised internal tissues for the internal conveyance of materials; such as the algae, fungi, and Bryophytes; have, as their typical reproductive unit, a single detached cell known as a spore. The Gymnosperms and Angiosperms reproduce by means of seeds. Although vegetative propagation is important in many species and predominates in some, it is doubtful that any wild angiosperm has entirely given up the production of seed. In all short lived species and in the great majority of perennials as well, the seed is an indispensable means of survival and spread.

It is an obvious biological necessity that a seed needs to be compact, not easily damaged by external forces and that it should have good keeping qualities. To achieve this in part, the water content of an air dry seed is 10-15% and at such values the level of metabolism occurring within the seed is extremely low.

Every normal seed contains an embryo and in fact, sometimes more than one. A high proportion of orange pips contain multiple embryos, one being formed sexually and the remainder by vegetative budding of the nucellus.[7] It is the embryo which, on germination, develops into the seeding. A seed also contains, in addition to the embryo, reserve substances which sustain the seedling in the early stages of growth before it becomes self-supporting.

In many seeds the reserve substances are contained within the embryo itself, and hence the embryo makes up the greater part of the total volume of the seed. There are, however, almost as many varieties of seeds, in which the reserve materials are contained within an "endosperm". An endosperm is present in every developing angiosperm seed but is often absorbed by the embryo before the seed itself has ripened, and the seed is called "non-endospermic". In an "endospermic

seed" the endosperm survives, at least until the time of germination. The embryo, in such seeds usually is less, and often very much less, than half of the total bulk. Few seeds have any significant reserves beyond those contained in the embryo and endosperm but some, e.g. stitchwort do contain a nutritive perisperm which is a parental tissue, deriving from the nucellus of the ovule.

The embryo and its reserves are always enclosed in a protective covering. This coating is of highly specialised tissue, often with several very different cell layers each of which is presumed to have its own special function. The cell walls are thickened and heavily impregnated with materials like cutin and suberin. Cutin is a complex mixture of oxidation and condensation products of fatty acids. In plants or seeds cutin protects against mechanical injury and also functions to prevent excessive water loss. Suberin is chemically similar and occurs most commonly in cork cells, rendering them impervious to water.

Many structures commonly known as seeds are in fact one-seeded fruits. The protective layer of a true seed is called the "testa", whereas one-seeded fruits have a covering layer which is called the "pericarp". Radishes and bean are true seeds but lettuce or lawn grasses are in fact one-seeded fruits, each consisting of a seed (with its testa) enclosed in a pericarp. In the cases where the pericarp remains attached to the seed it takes over some of the protective functions which are elsewhere performed by the testa.

The protective coating, even where it is not particularly hard or thick, is usually very resistant to attack by chemical reagents or decay. In many cases it is highly impervious to water but it cannot remain indefinitely so as the seed needs to absorb water before it can germinate. Many seeds can absorb water through only a small part of the surface

or only after the coating has been broken down by slow processes of softening and decay.

The structures of representative species of seeds are illustrated in figure 1-2.1.

1-3 SEED DORMANCY

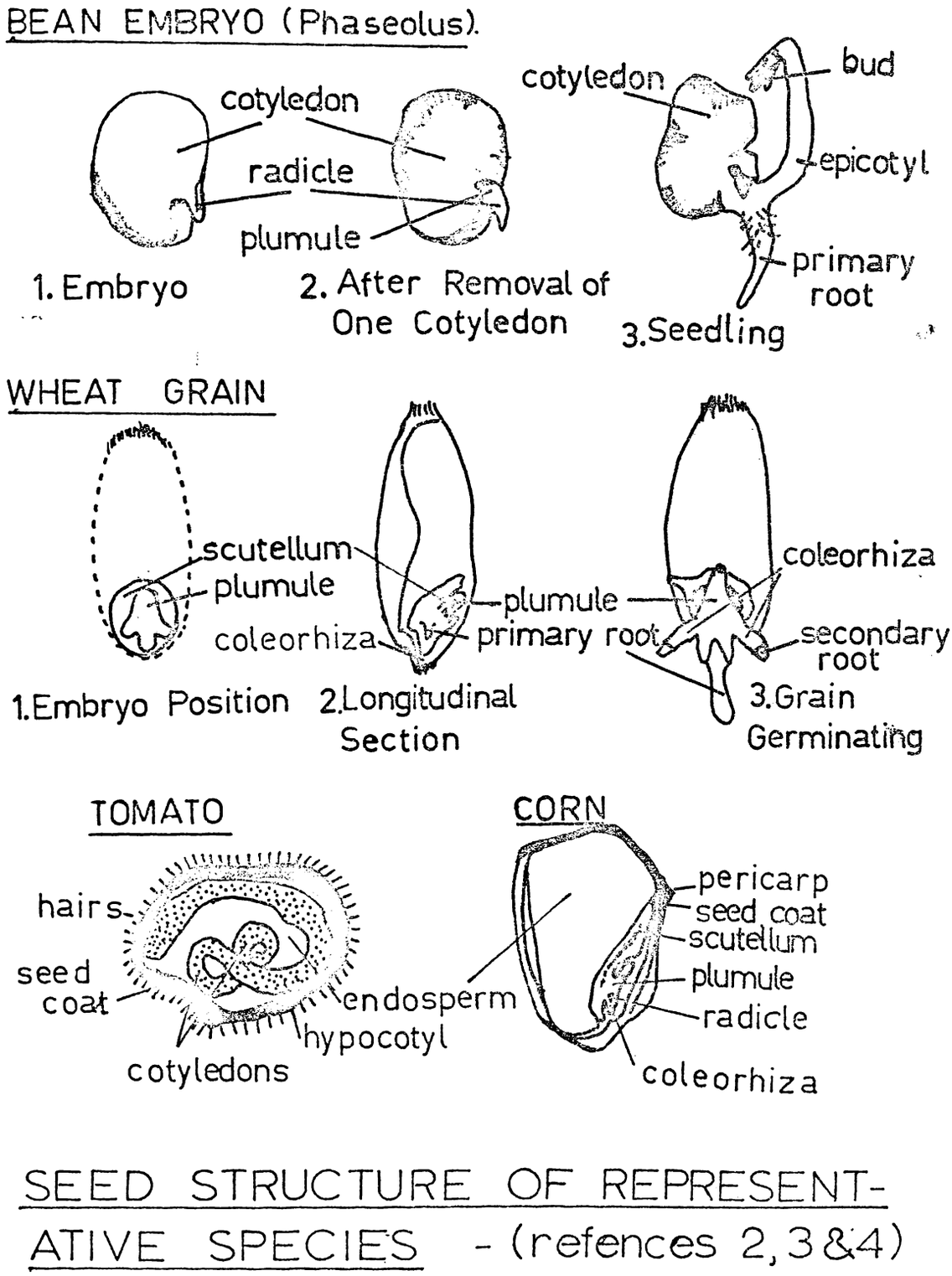
1-3.0 Introduction

Periods of rest or dormancy are not unique with seeds; in fact they are common in plants, animals and microorganisms. In this condition, organisms can withstand environmental upsets of prolonged duration. Among animals such as insects and bears, the period of hibernation may be an important ecological adaptation which enables these organisms to survive extreme temperatures and restricted food supplies. Microbial spores can survive boiling, submergence in concentrated acid, and the extremes of cold and vacuum which can be developed by man. The resistance of seeds to unfavourable environmental conditions makes possible the storage of grains. Buds of plants, which normally remain dormant only during winter may on occasion remain latent during most of the life of the plant and develop shoots under conditions which are little understood.

Dormancy is widespread in nature and it is likely that there are parallel mechanisms in these organisms by which dormancy may be explained.

1-3.1 Seed Ripening

There are two types of dormancy for seeds,[8] one of which is retained only as long as the seed remains dry, whereas the other is independent of moisture.



SEED STRUCTURE OF REPRESENTATIVE SPECIES - (refences 2, 3 & 4)

FIG. 1-2.1

Seed ripening is associated with the loss of the second type of dormancy, although as a result of man's selection of seeds that germinate promptly, desiccation remains the main block to renewed growth of the seeds of many but not all crop plants. Seeds of most noncultivated plants as a result of natural selection, have special blocks to germination. The main blocks to germination that are known to occur, are effects associated with covering layers, presence or absence of inhibitors or promoters, temperature requirements differing from those of growth, light and dark. When the seed ripens under natural conditions these blocks are eliminated by various methods including time, temperature changes, leaching, decay of surrounding fruit tissue and by the action of light. After-ripening may be defined as any changes which occur in seeds during storage leading to an improvement in germination.

The inability of many nonafter-ripened seeds to germinate in certain temperature ranges in which the wholly after-ripened seeds do, has been shown to be highly dependent on the presence of intact structures[8] such as fruit, seed coats and endosperm, which limit the supply of oxygen to the embryo. These structures may also be impermeable to water and other dissolved gases, in particular carbon dioxide. The mechanical strength of the coats of some seeds is believed to be sufficient to restrain the enlargement of the embryo. Dormancy can also be associated with the presence of chemical inhibitors actually in the seed coat itself.

Breaking or removing the seed coat frequently improves the germination percentage. Germination of many grass seeds is improved by cutting, breaking, acid-treating, or removing certain seed coverings.[8] In grasses the seed coat is derived from both pericarp and remnants of the integument, and it is not known which part has an inhibitory influence.

The inner seed coat of Cucurbita pepo is more permeable to gases than the outer, but it nevertheless functions as the rate-limiting layer as the outer membrane is perforated by the micropyle.[9] The inner coat contains a layer of living cells, the death of which increases its permeability. It is worth noting that improved germination has often been obtained by using an atmosphere enriched in oxygen.[8]

It has been shown[4] that nonafter-ripened cereal caryopses can germinate at 10°C. During the after-ripening period the caryopses gradually become able to germinate at increasingly higher temperatures. The gradual widening of germination temperature ranges through an increase in the maximum temperature permissible for germination at relatively constant minimum temperature has been shown to be characteristic of cereal grains and seeds belonging to the family Rosaceae.[10,11]

Many seeds, e.g. Amaranthus retroflexus and Thlaspi arvense will only germinate at high temperatures before after-ripening but upon storage gradually acquire the ability to germinate at lower temperatures.[12,13,14,15]

These two types of widening of temperature ranges for seed germination originate from a close adaptation either to a hot and arid or a cold unfavourable season. In the third type which has originated from adaptation to two unfavourable seasons, a hot and arid summer and a cold winter, the widening of temperature range takes place through an increase in maximum temperature as well as through a decrease of minimum temperature. This has been shown to be characteristic of many seeds and is the more common form of temperature range adjustment.[16]

Many seeds have to undergo a period of low temperatures before they will germinate. This presumably corresponds to winter in the natural habitat. Such dormancy is commonly broken by 3 days moist storage at 2-3°C.

A light requirement exists for many seeds. Such seeds generally either do not germinate at any given temperature in darkness or, less commonly the temperature range in darkness is much more narrow than in light. For example, freshly harvested Lactuca seeds of certain varieties germinate in darkness only in a narrow temperature range below 18°C but in light they can germinate at 18-25°C.[17] Evenari and Neumann[18] found that removal of coats from lettuce seeds eliminated their light requirement for germination, and Kugler[19] working with Arabidopsis thaliana, also found that the light requirement was lost if the coats were removed or even pricked. Evenari concluded that the inner-most membrane of the lettuce seed was the effective one and that the beneficial effects observed when the pericarp alone was removed were due to accidental damage to the inner membrane.

The seeds of higher plants which require light for germination normally require red light and are inhibited by near-infrared (735mu). Bellis, Fagus, Taraxacum, lettuce and tobacco seeds all respond to red light of wavelength of 660 mu and are inhibited by near infrared.[7] It has since been shown that some dark germinating seeds which are insensitive to light nevertheless depend upon a phytochrome which is in the seeds as P_{730} (i.e. the phytochrome which promotes germination of light requiring seeds). Light sensitivity of these seeds can only be demonstrated by using light rich in far-red. This causes conversion of the P_{730} to P_{660} which does not permit germination.[20]

Light with a very high far-red component does exist in natural habitats. Chlorophyll absorbs maximally at approximately 660mu and exhibits negligible absorbance beyond 710mu. Hence light which has been filtered through overhanging green leaves contains virtually no red light. Such light prevents the germination of lettuce seeds.[20] Hence once again, the light requirement is probably a highly specialized

adaptation of the seeds, to prevent germination in undesirable conditions. Seeds under forest canopies, buried underneath leaves or in thick stands of plants are essentially in far-red light.

Chemicals can replace light in the cases of certain seeds, including lettuce,[21] Arabidopis thialana and Bidens radiata.[22] In these seeds gibberellins are the effective factor. Very few species do not respond to these compounds. In many cases dormancy is broken, in others the rate of germination or total percent germination is enhanced. Gibberellin-like compounds have been found in Phaseolus seeds as well as many other species.[23] There is some evidence that the amount of gibberellin-like substances increase in dormant seeds during after-ripening.

Two types of germination promoters have been widely recognized namely, nitrates and materials having an -SH function such as cysteine, glutathione and thiourea. Thiourea is particularly effective in promoting germination of some light requiring or temperature inhibited seeds such as lettuce.[24] Thiourea is used at very high concentrations (about 0.5%) and severely inhibits subsequent growth unless removed after imbibition. The presence of a C=S group in thiourea with possible isomerism to C-SH causes this compound to be grouped with glutathione and cysteine, which are effective at low concentrations.

Hydroxylamine strongly promoted germination of tobacco seeds[25] at 0.001M but was inhibitory in action at 0.005M. Some response was obtained with amino acids, alanine and glutamic acid being particularly effective. Citric, α-ketoglutaric, fumaric, and malic acids, which are compounds of the Citric Acid Cycle, all gave some promotion of germination. This was also true for malonic acid, which is generally considered to compete with succinic acid.

Cyanide, azide and carbon monoxide which are normally metabolic inhibitors interfering with terminal oxidation will break the dormancy of several species of seeds.[26] Other species such as malonate, fluoride and iodoacetate also acted as promoters, though to a smaller extent chloramphenicol which is an inhibitor of protein synthesis in bacterial chloroplasts or mitochondrial systems and which at relatively high concentrations may also directly affect respiration, can break the dormancy of lettuce and barley seeds.[27]

Response of seeds as a consequence of direct promotion cannot be readily differentiated from response attributable to poisoning of an inhibitor. Inhibitors have been shown to play an important part in dormancy. In some desert plants, chemical compounds are present which prevent germination of the seeds until these compounds have been washed out by sufficiently heavy rain. Hence, until conditions are suitable for the future growth of the plants formed, the seeds cannot germinate. Water soluble inhibitors have been shown to be present in other seed species, e.g. beet.[20]

Abscisic acid was isolated as a result of investigations of bud dormancy in plants. Since then, it has been discovered in the coat, endosperms, and embryo of many seeds.[28,29] The dormancy of several species of Rosa seems to be correlated with their abscisic acid content. There are serious objections to the possibility that abscisic acid may be a universally important endogenous inhibitor of germination. Gibberellic acid fails to overcome the effect of abscisic acid in test systems, although gibberellins are extremely effective in breaking dormancy of many seeds. Abscisic acid can inhibit the synthesis of all nucleic acid fractions and can inhibit the synthesis of the enzyme α-amylase in barley endosperm. It is possible that control of nucleic acid synthesis is the basis of the dormancy of seeds.

One of the first endogenous inhibitors to be discovered in seeds or fruits was parasorbic acid which was separated from fruits of Sorbus aucuparia.[8] It is the β-unsaturated lactone hexene-2-olid 1,5. Another naturally occurring β-lactone is coumarin which markedly inhibits root growth at concentrations of 7×10^{-6}M.

Evenari[30] listed the natural germination inhibitors that have been identified as ammonia, hydrogen cyanide, ethylene, essential oils including both aldehydes (citral, cinnamaldehyde, salicylaldehyde, benzaldehyde) and mustard oils (allyl- and β-phenethylisothiocyanates), alkaloids (cocaine, caffein, physostigmin, nicotine), unsaturated lactones (coumarin, protoanemonia, parasorbic acid) and unsaturated acids (cinnamic, caffeic, ferulic). Phthalids and dehydracetic acid can be added to this list.

Germination inhibitors have been isolated from over 100 species of plants. In more than 60 of the species the inhibitor was found in seeds or fruits, and in 22 species seeds were specifically mentioned as the source.[30] The inhibitor may be present in all parts of the seed. The naked embryos of iris seeds grow readily but are inhibited by the presence of even a small piece of endosperm. The inhibitory substance in seeds of Cucurbita was found to be present in both the seed coat and the embryo.[31] Germination inhibitors were found to occur in the extracts of beet seed balls. Stout and Tolman[32] ascribed the effect to the action of ammonia liberated by nitrogenous compounds in the pericarp and other structures of the seed.

Kugler[19] extracted from the seed coat and embryo of seeds of Sinapis alba a fraction that fluoresced in ultra-violet light and that inhibited germination of Sinapis and other seeds. From chromatographic analysis it was assumed that amino acids and related compounds rather than oils were the important inhibitors.

Germination inhibitors have been found in most structures of the seed, in fruit and in other plant parts not structurally associated with seeds, but which come in contact with them in the soil after the seeds are shed.[30]

Figure 1-3.1 illustrates some of the most effective germination inhibitors and promoters. Salts of heavy metals such as copper and mercury, although not illustrated, are also extremely effective inhibitors.

Inhibitors applied at low concentrations generally promote the germination of both seeds[30] and spores as well as cell elongation.[33]

Growth regulating compounds such as indole-3-acetic acid, 2,4 dichlorophenoxyacetic acid and maleic hydrazide are relatively inactive as inhibitors of seed germination. Measurable effects are usually obtained only at concentrations greater than 10^{-3}M, while effects in growth tests are often maximal at 10^{-5}M or less.[8]

The control of dormancy and its breaking is possibly achieved by the lowering of the content of an inhibitor to a level which permits germination. The second hypothesis is that an increase occurs, in the level of promoters which overcome the inhibitory effects. Other factors such as the permeability of the seed coat may, however, be more important, and have to be overcome before germination can occur.

1-3.2 Compounds Present in Dormant Seeds

1-3.2(a) Introduction

The chemical composition of seeds shows variability between species and even varieties. The compounds found can be divided roughly into two groups which are (i) the normal constituents which are likely to occur in every plant tissue and (ii) the storage materials which will be used by the germinating seed to provide compounds necessary for growth

until the young seedling formed becomes self-supporting.

Seeds can be divided into those whose main storage material is carbohydrate and those whose main storage material is lipid. Seeds containing proteins can belong to either group. The occurrence of large quantities of lipids differentiates seeds from all other plant tissues except certain fruits, since lipids do not usually occur in large amounts in plant tissues.

The constituents of seeds are determined genetically but the relative concentrations of these constituents may vary according to environmental factors such as climate, temperature or mineral nutrition.

The variations displayed by seeds, in their chemical composition are shown in table 1-3.2(a).1.[4]

Very few seeds are known in which the predominant storage material is protein, the main exception being soybeans. Table 1-3.2(a).2 shows the chemical composition of soybeans.

1-3.2(b) Carbohydrates

The two chief storage carbohydrates are starch and hemicelluloses. The former is found in all the food grains and in legumes, while hemicelluloses, both pentosan and hexosan, occur in the endosperm or cotyledons of various species, e.g. soybean. In addition many other carbohydrates occur in seeds but not necessarily as storage materials.

It is generally accepted that the granules from most plant species contain a mixture of two polysaccharides. The major component is normally amylopectin and usually amounts to 75-85% of most starches. It has a branched structure in which chains containing an average of about 20 to 25 α - (1$\rightarrow$4) - linked glucose residues are interlinked by α- (1$\rightarrow$6) glucosidic linkages to form a bush-like structure.

Table 1-3.2(a).1

CHEMICAL COMPOSITION OF SEEDS[4]

Main Storage Compound	Seed Species	% of Air-dry Seeds			
		Carbohydrates		Protein	Fats
		Starch	Sugar		
Carbo-hydrates	Zea mays	50-70	1-4	10.0	5
	Pisum sativum	30-40	4-6	20.0	2
	Acer saccharinum	42	20	27.5	4
	Triticum	60-75		13.3	2.0
	Fagopyrum esculentum	72.0		10.0	2.0
	Chenopodium quinoa	48.0		19.0	5.0
	Aesculus hippocastanum	68.0		7.0	5.0
	Castanea vesca	42.0		4.0	3.0
	Quercus pendunculata	47.0		3.0	3.0
Lipid	Linum usitatissimum	23.0		23.0	34.0
	Brassica rapa	25.0		20.0	34.0
	Papaver somniferum	19.0		20.0	41.0
	Cannabis sativa	21.0		18.0	33.0
	Amygdalus communis	8.0		24.0	53.0
	Aleurites moluccana	5.0		21.0	62
	Arachis hypogea	8-21	4-12	20-30	40-50
	Helianthus annus	0	2	25.0	45-50
	Ricinus communis	0	0	18.0	64

Table 1-3.2(a).2

CHEMICAL COMPOSITION OF SOYBEANS[34]

Major Constituents	% Dry Wt.	Mineral Constituents	% Dry Wt.	Vitamins	ug/gm
Ash	4.6	Magnesium	0.22	Thiamine	17.5
Fat	18.0	Sulphur	0.41	Riboflavin	3.6
Fibre	3.5	Chlorine	0.024	Pyridoxine	11.8
Protein	40.0	Iodine	trace	Nicotinic Acid	21.4
Pentosans	4.4	Sodium	0.34	Pantothenic Acid	21.5
Sugars	7.0	Manganese	0.0028	Inositol	2291.0
Starch-like substances	5.6	Zinc	0.0022	Biotin	0.8
		Aluminium	0.0007		
Phosphorus	0.63	Copper	0.0012		
Potassium	1.67	Iron	0.0097		
Calcium	0.26				

Amylose, normally the minor component of starch, is an essentially linear polymer of glucose containing more than 99% of α-(1$\longrightarrow$4)-glucosidic linkages. The structures of these compounds are illustrated in Fig. 1-3.2(b).1. Amylose and amylopectin differ in many physical properties, particularly in molecular size and solubility.

In addition to amylose and amylopectin certain starches contain a small proportion (5-10%) of a third component. In wheat starch this material is a short-chain amylopectin, while in some maize starch the polysaccharide is intermediate between amylose and amylopectic.

The amylose content of most starches is 15-20%. However certain varieties of maize (e.g. "Amylomaize") and pea starches may contain 50-75% of amylose, while waxy-cereal starches such as in most types of maize and sorghum contain less than 1%.[35] Maize, in addition to the components already mentioned, also contains another polysaccharide called "phytoglycogen" - which has the molecular structure of glycogen.

The structure of glycogen, normally thought of as being the nutrient polysaccharide of animal tissues only, is similar to that of amylopectin in that it is a branched molecule. Its chain length, however, is usually shorter (10-20 glucose units) and hence it is even more highly branched than amylopectin.

The polysaccharides present in seeds, apart from starch, include pure cellulose and various types of hemicellulose. Both water soluble and water insoluble-alkali soluble hemicelluloses have been found. The latter class can be obtained from the husks and endosperms of cereal grains. These husk hemicelluloses are characterized by a high xylan content and the presence of uronic acid residues. The alkali soluble endospermc hemicelluloses are characterized by having a lower pentosan content and fewer uronic acid residues. The water soluble hemicelluloses in the endosperm are termed "amylan".[36] A β-linked glucose polymer has

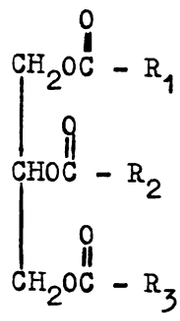
been obtained from both oats and barley. It consists of unbranched chains of β-linked glucopyranose units having approximately equal numbers of (1 $\longrightarrow$ 3) - and (1 $\longrightarrow$ 4) β-linkages. Other water soluble hemicelluloses obtained from cereals include pentosans, such as the araboxylan from wheat, which had a chain of (1 $\longrightarrow$ 4) β -linked xylose units with side chains of single arabo-furanosyl units joined by (1 $\longrightarrow$ 3) and (1 $\longrightarrow$ 2) bonds to the xylose chain. Other water soluble hemicelluloses present include mannans and galactans[36]. The polyuronides of the seed coats are possibly connected with seed dispersal and water uptake during germination.

Pectins are a normal constituent of plant cells and hence of seeds. Glucose, fructose, sucrose, raffinose and stachyose occur in greater or smaller amounts in most seeds. In Sinapis alba seeds sucrose, stachyose and raffinose are present in small quantities.[37] Sucrose is uniformly distributed in the dry embryo of maize and other cereals but reducing sugars, e.g. glucose could not be detected.[38] Appreciable amounts of sucrose (30 mg/gm) occur in Grand Rapid lettuce seeds.[8]

Many secondary plant products are present in seeds in the form of glycosides. The oil of bitter almond contains the glycoside amygdalin, which on hydrolysis yields two moles of glucose and one mole each of benzaldehyde and hydrogen cyanide. Nucleic acids, nucleotides and nucleosides also found in seeds, contain a sugar oxy- or deoxy-ribose linked by a glycosidic bond to a nitrogenous base.

1-3.2(c) Lipids

Many seeds are economically important as a rich source of lipid material. The lipids are normally present in the form of the glycerides of fatty acids.



Where R_1, R_2, R_3 represent the alkyl chain of the fatty acid

Structure of a Triglyceride

The groups R_1, R_2 and R_3 may be the same or different. Lipid forms the most characteristic seed reserve material and is normally stored in a liquid form as oil globules. Fats have a low oxygen content, and hence, a higher calorific value. Using fats for the reserve material more calories can be stored in a given bulk and weight than is the case when using starch. It is significant that many plants which accumulate starch in their vegetative storage organs form oily seeds containing only tiny quantities of starch.

In general in higher plants the C-16 saturated and the C-18 unsaturated fatty acids are the most common. A very large number of seeds contain these as their major fatty acids. Many other seeds in addition to one or more of these acids have as major components one or more distinctive acids, either saturated or unsaturated. The structures of some of the fatty acids occurring in seed lipids are illustrated

It is possible to group most seed fats according to their predominating component acids. It is normally found that seed fats of plants in the same botanical family fall in the same group and often show great similarity in their composition. The three major groups in this classification are those in which palmitic, oleic, linoleic and/or linolenic acids are major components. These groups are:

I Those in which linoleic and linolenic acids predominate

II Those in which oleic and linoleic acids predominate

III Those in which oleic and palmitic acids predominate.

In all groups the composition of the fats is remarkably stable which implies that the control mechanisms are inherently genetic in character.

There are, however, a number of fats in which linoleic acid is prominent, which also contain specific saturated acids, and there are others in which linoleic or linolenic is abundant in the seed fat of one species, whilst in another related species a quite different unsaturated acid replaces these almost entirely. The most common examples of the latter are: (i) the prominence of the elaeostearic glycerides in the seeds of Aleurites fordii and montana whilst in other species of Aleurites polyethenoid unsaturation is confined to linoleic and linolenic glycerides; and (ii) the abundance of ricinoleic glycerides in the seeds of Ricinus species. The percentage composition of certain seed fats which contain unusual fatty acids as one of the major components is illustrated in Table 1-3.2(c).1. As seed fats become less unsaturated and palmitic acid makes its appearance in larger quantities there is often an increase in the stearic acid content as well.

It has been shown that a given plant species, capable of existence in different climates, produces when grown in a cold climate more unsaturated (linoleic and linolenic) acids in its seed fat than when it is grown in a warmer climate. Seeds in whose fats the higher saturated acids predominate will be those of tropical or subtropical growth, as the seed fats must be fluid at the temperature of the living plant.[39]

Table 1-3.2(c).1

The Unusual Fatty Acid Composition of Certain Seed Fats[40]

Species	% Composition of Total Fat					
	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Others (unusual)
Hydnocarpus wightiana	-	2	-	6	-	Hydnocarpic 49 Chaulmoogric 27 Gorlic 12
Petroselinum sativum	-	3	-	15	6	Petroselinic 76
Ricinus communis	-	-	2	7	3	Ricinoleic 87
Picramnia lindeniana	22	33	3	22	-	Tariric 20
Veronia anthelmintics	-	3	1.5	2	9	Epoxyoleic 78

Other lipid materials found in seeds are the esters of higher alcohols i.e. waxes. In general the dry matter in pasture grasses contains 4-6% of lipids of which 1.5-4% consists of waxes (chiefly n-hexacosanol). However they do not appear as major components of many seed fats. The seeds of the sub-tropical North American shrub, Simmondsia californica which belongs to the family Buxaceae are exceptional.[39] Glycerides are completely absent and the seed fat is composed of a mixture of wax esters of higher unsaturated alcohols with higher unsaturated fatty acids.

Seeds also contain phosphatides but the amounts are usually very small, frequently only 0.1-0.2% of that of the glycerides. These seed phosphatides have not been studied extensively. The few results

available suggest that linoleic acid is often the most prominent fatty acid in these compounds; but any specific acids of the corresponding seed glycerides are also found, usually in relatively smaller quantities than in the glycerides.[39] Seed phosphatides may resemble animal phosphatides in possessing an increased proportion of saturated acids as compared to the corresponding glycerides, the increase being in palmitic acid in seeds. Also some seed phosphatides contain very minor amounts of unsaturated C_{20} acids which are absent from the glycerides.

1-3.2(d) Free Amino Acids and Closely Related Compounds

Since the advent of paper chromatography there has been a great increase in the number of amino and imino acids recognized as seed constituents. It is, however, not possible to make many generalisations regarding their distribution. The protein amino acids are normally present in the free amino acid pools but in relative amounts that vary greatly between species. For many of the other acids the distribution within and between families shows no apparent pattern. Some acids do, however, seem to be especially characteristic of certain plant families, e.g. citrulline for the Curcurbitae.

AMINO ACID	SEED	REFS.
Alanine	Wheat, pea, *Saraca indica*, *Lupinus* *Phlox paniculata*, *Baikiaea plurijuga*	41,42
Arginine	*Lupinus*, *Lathyrus* spp., *Vicia* spp. Wheat, *Saraca indica*, pea.	41,42,43
Aspartic acid	*Lupinus*, pea, *Baikiaea plurijuga* *Saraca indica*, *Phlox paniculata*, Wheat	42,41
Cysteine	*Lupinus*, Wheat	42,44,41
Glutamic acid	*Lupinus*, pea, *Baikiaea plurijuga*, *Saraca indica*, *Phlox paniculata*, Water melon	42,45
Glycine	Pea, *Saraca indica*, *Phlox paniculata*, Wheat	44,42,41
Histidine	*Lupinus*, pea.	44,41,45
Leucine	Pea, *Phlox paniculata*	44,42,41
Isoleucine	Wheat	
Lysine	*Lupinus*, Pea and Wheat	44,41
Methionine	Pea	41
Phenylalanine	Wheat	41
Proline	Maize, Wheat, *Saraca indica*	8, 41, 42
Serine	*Lupinus*, Pea, Wheat, *Saraca indica* *Phlox paniculata*	41,42,44
Threonine	Pea, Wheat, *Saraca indica*, *Phlox paniculata*	41,42,44
Tryptophan	wheat	41
Tyrosine	Lupin, Pea, Wheat	41,44
Valine	Pea, Wheat, *Saraca indica*, *Phlox paniculata*	41,42,44
Glutamine	Pea, Wheat	41,44
Asparagine	Pea, *Saraca indica*, Wheat	41,44
GABA	Mustard, Pea, Hubbard Squash, *Saraca indica*	338,42,41
Alpha-amino-butyric acid	*Phlox paniculata*, Wheat	41

AMINO ACID	SEED	REFS.
Beta-alanine	Legumes, *Saraca indica*	42,44
Gamma-methylene glutamic acid	" "	42,41
Gamma-methylene glutamine	Pea, *Saraca indica*	46,42
Gamma-hydroxy-glutamic acid	*Phlox paniculata*, *Phlox decusata*	47,42
Alpha-amine-adipic acid	Pea, Corn	47,46
S-(2-carboxy-1-methylethyl)-1-cysteine	*Acacia millefolia*, *Acacia willardiana*	48
Homoserine	Legumes, Pea	44,46
Dihydroxyphenyl-alanine	*Dolichos*	46
Pipecolic acid	Coconut, Beans, Wheat, *Baikiaea plurijuga* and *Saraca indica*	41,42,46
Baikiain	*Baikiaea plurijuga* and Pea	42,46
Homoarginine	*Lathyrus*	48
Canavinine	*Canavalia*, Leguminosae (Papillionoideae only) *Vicia* spp., *Lathyrus* spp.	43
Lathyrine	*Lathyrus* spp.	43,46
Gamma-hydroxy-homoarginine	*Lathyrus*, *Vicia*	43
Gamma-hydroxy-arginine	*Vicia*	43
Citrulline	Water Melon	45
Agmatine	*Ricinus*	43
Putrescine	Rye, Barley	46
Acetylhomo-serine	Pea	42
Alpha-(methylene cyclopropyl) glycine	*Litchi chinensis*	48
Beta-pyrazol-1-yl-alanine	Water melon, Marrow, Melon, Cucumber *Ecballium elaterium*, *Bryonia dioica* and *Echinocystis lobata*	45,48

A survey of amino acids in seeds cannot be complete as progress in this field is so rapid. The following list of amino acids is however, representative of the great variety of these compounds now known to be present in seeds.

1-3.2(e) Protein and Peptides

(i) γ-Glutamyl peptides

The first γ-glutamyl dipeptide was found in legume seeds in 1958. Since then several more have been discovered in seeds. Except for glutathione and glutamine there is no evidence for the existence of γ-glutamyl compounds in animal tissues.

The γ-glutamyl dipeptides which have so far been discovered in seeds, listed in Table 1-3.2(e), make an appreciable contribution to the total non-protein amino nitrogen fraction. For example γ-glutamyl-S-methylcysteine accounts for over 40% of the free amino nitrogen in kidney beans.[50] At the same time the content of glutamic acid is less than 10% and that of methylcysteine far less than this.

Table 1-3.2(e)

THE γ-GLUTAMYL PEPTIDES OF SEEDS[50]

Peptide	Source
γ-glutamyl-phenylalanine	Soybean seed
γ-glutamyl-tyrosine	Soybean seed
γ-glutamyl-leucine	Lime bean seed)
" "	Kidney bean seed)
γ-glutamyl-methionine	Kidney bean seed
γ-glutamyl-S-methylcysteine	Kidney bean seed
" "	Lima bean seed
γ-glutamylaminopropionitrile	Sweet pea seed
Glutamine	Most seeds

(ii) Proteins

The seed proteins consist of both metabolically inactive (the storage proteins), and metabolically active (e.g. the enzymes and nucleoprotein), groups. Metabolically active protein belongs to the albumin or globulin proteins. In wheat these two groups account for no more than 15% of the total protein. The distribution between the metabolically active and inactive groups is similar for most cereals.

Seed storage proteins generally have a high nitrogen content, high proline content and are often low in their content of lysine, tryptophan and methionine.[4] Some amino acids, e.g. hydroxyproline are found frequently in protein but rarely in the free state. The protein of barley contains high amounts of glutamine.[51] In Lupinus seeds the major amino acid in the protein is arginine which contains large quantities of nitrogen.[44] The seed storage protein is stored in an insoluble form in granules. Before it can be utilised it must be converted into soluble compounds.

The proof of existence of active enzymes in the mature seed is difficult as most enzyme analysis methods involve hydration or alteration of the tissue. However, the enzymes reported as being in the dry seed are probably, at least, preformed in the seed andnot synthesised during germination but instead merely activated.

Those enzymes which have been reported include glutamic acid decarboxylase which has been shown to be active at low moisture contents[52] of greater than 25%. Although this is higher than the normal water content of dry grain it is insufficient for germination to occur. At moisture values above 15% the transaminases, glutamate oxaloacetate and glutamate pyruvate transaminases are active.[52]

The activity of various enzymes in wheat were determined by Firenzuoli et al[53]. For malate dehydrogenase which was found to be the most active enzyme an activity of 1 umole of substrate/minute/mg of protein is quoted. Phosphoglycerate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, fructosediphosphate aldolase and phosphogluconate dehydrogenase were also reported to be active. Enzymes which were thought to be active but had an activity of less than 10^{-2} umoles of substrate/minute/mg protein were pyruvate kinase and isocitrate dehydrogenase.[53] Isocitrate lyase is almost undetectable in the mature castor bean endosperm.[38]

Proteins were separated from mature seeds of Sinapis alba, Brassica campestris, Brassica nigra and Brassica oleracea by electrophoresis. Bands exhibiting β-galactosidase, β-glucosidase and esterase activity were shown to be present.[54]

The amylases have been studied extensively. It is now accepted that β-amylase is preformed in cereal grains and that α-amylase must be synthesized during germination.[38,55] In dry maize (Zea mays) β-amylase was detected in the endosperm only and had an activity of 0.06 mg maltose/minute/endosperm.[55]

Seeds tend to contain large quantities of phytin which is the calcium and magnesium salt of inositol-hexaphosphoric acid. Many seeds have been reported to contain an acid phosphatase activity which is specific for phytin.[56] This activity is distributed throughout all seed organs. In wheat seeds 80% of the total activity is found in the endosperm and the remainder of the activity is in the scutellum and the germ.[57]

In the mature seed of castor bean the ability of the 105,000 g supernatant to support protein synthesis "in vitro" is low[58] but in the mature wheat embryo this low endogenous activity can be accelerated 100-fold by the addition of polyuridylic acid,[59] which acts as a synthetic m-RNA.

An aminoacylase which hydrolyses N-acetyl amino acids has been demonstrated in rape seed. In addition this enzyme which was purified 150-fold hydrolyzed certain dipeptides and chloroacetyl-L-tyrosine.[60] The same authors have shown that amino-acylases are widely distributed in the seeds of higher plants.[61]

Ribonuclease activity has been detected in the dry seed of maize. Its activity was observed to fall during the first 24 hours of germination.[62]

1-3.2(f) Organic and Keto Acids

In dormant seeds most of the organic acids are present as salts or bound to sugars through glucoside linkages. The only organic acids which have been shown to occur in all seeds are citric and malic acids. The amount of these sometimes exceeds several percent of the dry weight. Compounds called "sugar acids" have been detected as a major component of seeds of Leguminosae. These acids are empirical isomers of simple sugars and are thought to arise as products of the primary oxidation of the sugars.[63]

The quantities of organic acids which have been detected in many seeds are striking as the cells of the seeds are devoid of vacuoles in which the major part of organic acids are normally found. The bulk of the organic acids are localised in the storage tissue, the cotyledons or endosperm, but the percent concentration in the embryo can be higher as for example in cereals where the % concentration of citric acid in the embryo is twice that of the endosperm.[63]

Of the seeds examined so far the Leguminosae contain the greatest concentration of organic acids. This may be up to 12% of the dry seed weight. The amount of citric acid present in a seed appears to be related to its type of storage material. Those seeds which contain larger amounts of storage protein also contain larger amounts of citric acid. This is shown in Table 1-3.2(f).1.

Table 1-3.2(f).1[63]

THE DISTRIBUTION OF MALIC AND CITRIC ACID IN SEEDS

Seed	Species	Citric Acid	Malic Acid
		mg/100 gm Dry Wt.	
Starchy (cereals)	Barley	70	
	Oats	62	
	Rye	69	
	Wheat	12-78	14-59
Oily	Rape	198	
	Sunflower	66-160	180
	Flax	90-207	40
	Hemp	90	60
Protein (Leguminosae)	Broad bean	408-659	47-132
	Pea	290-803	67
	Bean	1220	270

The concentration of citric acid in the Broad bean is 1.8 mg per seed and that of malic acid is 0.6 mg per seed.[65] Gobis[64] has postulated that the high concentration of citric acid is associated with synthesis of protein in the seeds. The content of malic acid does not vary as greatly between species not does it appear to be associated with the

type of storage material.

Other aliphatic acids which have been found in seeds include oxalate, tartarate, aconitate (in cereals), malonate (in Leguminosae), acetate (mainly oily seeds), lactate and various keto acids. The cyclic and aromatic acids which have been shown present include quinic, benzoic, cinnamic, salicylic, caffeic, hydroxy cinnamic,[63] p-coumaric[66] and chlorogenic acids.[63] In most seeds these acids occur in much lower quantities than do citric and malic acid. However some seeds have an unusually large amount of one or two of these acids. In wheat the predominent keto acid is glyoxylic acid.[41] Table 1-3.2(f).2 shows the distribution of keto acids in the wheat germ and in the whole seed. These values can be compared to those much higher values obtained for amino acids in the wheat seed, e.g. glutamic acid with a concentration of 1570 ug/gm in the germ and of 45 ugm/gm in the whole seed.

Table 1-3.2(f).2

THE KETO ACIDS IN WHEAT SEEDS[41]

	Germ ug/100gm	Whole Seed ug/100gm
Glyoxylic acid	165	17
α-Ketoglutaric acid	70	1
Pyruvic acid	40	5
Oxaloacetic acid	5	1
Hydroxy pyruvic acid	4	1
α-Keto β-hydroxy butyric acid	1	1
α-Keto γ-hydroxy butyric acid	1	1
Succinic semialdehyde	1	1

1-3.2(g) Flavonoids

The flavonoids all contain a diarylpropane unit (C_6-C_3-C_6).

The literature on the occurrence of flavonoids in seeds is not vast. They are, however, known to occur widely often as the corresponding glycoside although few have been identified.

The flavonoids of Theobroma cacao beans were studied and found to consist of Quercetin, anthocyanidin hydrochloride, leucocyanidin L_1, leucocyanidin L_2, 3-β-D-galactosidyl cyanidin, 3-α-L-arabinosidyl cyanidin and epicatechin.[66]

Seeds of Arachis species contain 5,7,4'-trihydroxy-3'-methoxy catechin (arachidose) as the glucoside (arachidoside).[47]

The seed coats of many seeds contain anthocyanis or leucoanthocyanins. The seed coat of Phaseolus vulgaris contains leuco-delphinidin and leuco-pelargonidin as well as delphinidin, petunidin and malvidin as the corresponding glycosides plus at least two flavonol glycosides.[47]

1-3.2(h) Nucleic Acids and Related Compounds

The nucleic acids occur partly in their free form and partly in the form of nucleoproteins. The ratio of RNA to DNA in many seeds is approximately 10:1. During seed maturation the loss of water is accompanied by a very marked decrease in the total RNA of the endosperm of Castor Bean.[68] In the mature Castor Bean seed the RNA content is 0.27 mg per endosperm and the DNA content is 13-14 ug/seed.[69] This RNA of the castor bean endosperm consists of "heavy" ribosomal RNA 73 ugm, "light" ribosomal RNA 35 ugms, and s-RNA 42 ugms.[70]

Brown[71,72] has studied the nucleoside and nucleotide content of various seeds. The results obtained for mature pea seeds are shown in Tables 1-3.2(h).1 and 1-3.2(h).2. Similar results were obtained for mature seeds of Lupinus luteus and Phaseolus vulgaris. The coenzymes NAD and NADP were present mainly in the reduced forms in these seeds. The nucleotide complement of mature pea seeds is more typical of a system predominantly committed to energy production, whereas for a seedling it is that of a system mainly concerned with biosynthesis and chemical transformations.

Acetyl CoA could not be detected in pea seeds but acetyl-3'-dephospho-CoA was present at a concentration of 1.98 umoles/10 gms.[71] This is likely to be a storage form of acetyl CoA. Uric acid was not detected in pea seeds but 23 mgs/100 gms of seed were found in seeds of Vicia faba.[71] No free purine or pyrimidine bases were detected in these three species.

Table 1-3.2(h).1

The Nucleotide Content of Mature Pea Seeds.[72]

Nucleotide	Content (umoles/10 gm)
NAD	0.01
AMP	4.47
GMP	0.13
NADP	0.03
IMP	0.15
UMP	0.57
ADP-ribose	0.39
ADP	2.18
UDP-glucose	0.79
UDP-galactose	
Adenine nucleotides	0.14
ATP	0.86
$NADH_2$	0.40
$NADPH_2$	0.14

Table 1-3.2(h).2

Nucleoside Content of Mature Pea Seeds[71]

Nucleoside	Content (umoles/10gm)	Total corresponding nucleotides (umoles/10gm)
Adenosine	1.52	7.51
Uridine	1.98	1.36
Guanosine	0.67	0.13
Xanthosine	0.91	None detected

In dry cotton seeds the free nucleotides were present in the form of nucleoside monophosphates. The nucleotides were accumulated in the embryos of the seed, in higher amounts than in the cotyledons.[73]

1-3.2(i) Other Compounds Present in Seeds

In addition to the compounds already mentioned seeds contain a large number of other substances.

All seeds contain an ample store of mineral elements as shown for soybeans in Table 1-3.2(a).2. Inorganic phosphorus is present in seeds in very small quantities. Phosphorus is normally stored in the form of phytin which is the calcium and magnesium salt of inositol hexaphosphate. In cotton seed embryos the phytin represents 80% of the total phosphorus.[57] On germination inorganic phosphorus can be released by enzymatic hydrolysis.

The mineral content of the testa is usually greater than that of the remaining part of the seed. The embryo has a higher content of inorganic substances than the reserves of the endosperm. Analysis of seed ash has shown that the amount of potassium varies from 12 to

50% and the amount of sodium is less than 2%. The magnitude of the chloride ion concentration is normally of the same order as that of sodium. Iron and magnesium have been present in all seeds examined. Aluminium is also widely distributed in seed species. Cacao seeds have been shown to contain copper. Traces of lead have been detected in the seeds of Randia dumetorum. Certain pericarp tissues contain considerable quantities of silicon.[74]

The mineral composition of seeds is essentially similar to that of the plant and usually comprises all the essential and minor elements although the form of storage may differ.

Chlorophyll is absent in most seeds although it has been found in the seeds of gymnosperms. Protochlorophyll is, however, found in seeds of the Cucurbitaceae. A protein-protochlorophyll complex has been obtained from the inner seed coats of Cucurbita pepo and was resolved into two different pigments. This protochlorophyll was found to be concentrated in discrete particles of approximately 1.7 microns in diameter in the inner seed coat.[75]

Phytosterols occur in a number of seeds. Stigmasterol has been isolated from the calabar bean (Physostigma venenosum) and soybean. Others that have been found in seeds include sitosterol (soybean), brassicasterol (rapeseed, Brassica rapa) and digitoxigenin from seeds of Digitalis purpurea.[47]

In all cases where seeds have been examined vitamins have been found. The presence of these vitamins is particularly important in those seeds which are used for food or fodder. For such seeds detailed information on the vitamin content is available and attempts are continuously being made to raise the vitamin content by selective breeding. Table 1-3.2(i).1 gives figures for some seed vitamin contents.

Tocopherols are present in the oil of many seeds. All of the known vitamins have been shown to occur in at least some seeds.

Table 1-3.2(i).1

The Vitamin Content of Some Seeds[4]

Seed	Mg/100 gm dry weight				I.U.
	Thiamin	Riboflavin	Nicotinic acid	Ascorbic acid	Vit.A
Wheat	0.45	0.13	5.4	0	0
Rice	0.33	0.05	4.6	0	0
Barley	0.46	0.12	5.5	0	0
Maize	0.45	0.11	2.0	0	450
Soybean	1.03	0.30	2.1	0	140
Broadbean	0.54	0.29	2.3	4	100
Peas	0.72	0.15	2.4	4	100
Sunflower	0.12	0.10	1.4	0	30

The growth substance content of various seeds has been examined. The occurrence of indolylacetic acid in a number of seeds such as rice,[76] has been demonstrated. Other indole derivatives have also been discovered in seeds, for example indolepyruvic acid in Zea mays.[77] The latter substance occurs at a concentration of 0.4 mg/kgm and was shown to exhibit growth control. Abscissic acid has been demonstrated in many immature seeds and in seed coats.[78] Gibberellic acid and gibberellin-like substances have been found in seeds of Pisum sativum,[79] Phaseolus multiflorus,[80] Canavalia gladiata,[81] lettuce and in runner beans as well as the seeds of many other plants.[4] The possible importance of these compounds in the control of seed dormancy has already been discussed.

Carotene and various other carotenoids have also been detected in seeds. Lutein (3,3'-dihydroxy-α-carotene $C_{40}H_{56}O_2$) and lycapene

($C_{40}H_{56}$) have both been detected in many seeds, for example in the seeds of tomato.[82]

1-3.4 The Metabolism of Dormant Seeds

The main difficulty in studying the metabolism of dormant seeds lies in the fact that dry seeds possibly contain inactive forms of enzymes which are effectively activated by the method of investigation. The results obtained using methods which have not interferred with seeds in any way are, however, not ambiguous.

The respiration of mature dormant seeds has been studied extensively. As the seeds are maturing the rate of oxygen uptake decreases to the very low almost undetectable values characteristic of the ripened dry seed. Removal of the seed coat of the castor bean seed has little or no influence on the respiration rate, thus suggesting that diffusion through this is not a limiting factor for gas exchanges. The respiratory quotient (RQ) has a value close to 0.7 or even lower.[70]

Sturani and Cocucci[69] have shown that the ribosomal apparatus of unripe castor bean seeds disappears almost completely at maturity hence it is unlikely that these dormant seeds possess the ability to synthesize proteins. In peanut seeds,[83] however, ribosomes appear to be present even in the mature seed and the main control of protein synthesis was the lack of synthesis of m-RNA. The amino acid incorporating system obtained from ungerminated wheat embryos was practically inactive in the incorporation of amino acids into protein. However, when supplemented with synthetic messenger in the form of polynucleotides high polymerisation of amino acids into polypeptides was observed.[59] These results support the hypothesis that in many seeds prior to imbibition all the components involved in protein synthesis are present in the embryo with the exception of active messenger RNA.

On storage of mature pea seeds for 12 months the viability fell by 8% but there was no apparent change in the nucleotide pattern. Air-dried peas expire 3 ml of carbon dioxide per kilogram in 24 hours. Brown[72] has calculated that in terms of respiratory enzymes 10 gms of these seeds would be expected to produce between 1.3 and 13.0 umoles of ATP/day, depending on the relative extents of aerobic and anaerobic respiration. However even after storage for 12 months the ATP content of 10 gm samples of pea seeds was found to be unchanged. He postulates that ATP formation was accompanied by ATP utilisation. The RNA content of the mature dry castor bean endosperm is 0.27 mg/endosperm. If these seeds are stored for a short period the amount of RNA decreases slowly to a value between 0.18 and 0.20 mg/endosperm.[69]

Enzymatic decarboxylation and transamination of glutamic acid have been studied in wheat grains[52] at various water contents. Moisture levels as low as 18% activated both enzyme systems whose activity increased rapidly with moisture content up to that required for germination of the grain. Transamination of α-ketoglutarate (α-KG) with alanine occurred at moisture levels as low as 15%. At higher levels transamination was followed by rapid decarboxylation of glutamate. Glutamic acid decarboxylase was found to be located almost entirely in the embryo. The respiration of most cereals has been shown to exhibit a marked increase at water contents above 14 to 15%[4] probably due to the action of decarboxylases. For flax seeds the "critical value" for the water content is only 11%. This is thought to be due to the oils in the seed causing the water content of the hygroscopic substances of the seed to be higher than that of the hydrophobic substances.

The changes in chemical composition of dry mustard seeds were studied[84] over a period of five years' storage. The composition of the oil changed markedly. After two years the acid number increased and the

iodine number decreased as did the content of vitamin E. The seeds retained 95% viability after five years' storage. Maize seed stored at high relative humidity showed a marked decrease in non-reducing sugars.[59] Small quantities of lactic acid have been shown to accumulate during storage of the dry castor bean.[63]

It has been reported that slow oxidation of sulfhydryl groups occurs on storage in the dry pea seed. This could be an important factor in the loss of viability on storage.[85]

Seeds were exposed in a closed vessel to the vapour of tritiated water.[86] After one day and twenty days no tritiated compounds were observed. However, after ten months' storage labelled lipid and solid residue were observed. This pattern of labelling was totally different to that observed during germination.

In seeds which are truly dormant either due to a chemical inhibitor or some other factor, metabolism must occur such that the effect of the inhibition is overcome. In seeds for which a light requirement has been demonstrated a pigment named phytochrome is involved. The form which absorbs at 660 mu (P_r) is considered to be the inactive form. When P_r absorbs a quantum of light it is converted to the 730 mu absorbing form (P_{fr}). The latter, which is the active form of the pigment, may be transformed back to P_r either by absorption of a quantum of light near 730 mu or by a slower, nonphotochemical temperature sensitive process which occurs in the dark. It is thought that the active form is associated with the synthesis of gibberellins, which are germination promoting substances.

It is worth noting that many of the methods used to break dormancy must be undertaken on seeds which have absorbed water beyond a critical value. Light is ineffective on light requiring lettuce seeds if only provided when the seeds are dry. Likewise stratification at low

temperatures must be carried out on soaked seeds. A minimum amount of water must be supplied and the seed must have begun respiring at an appreciable rate in order for chilling to be effective.

At low moisture levels seeds must exhibit some metabolism although of a minimal nature. At higher water levels the decarboxylases and transaminases appear to cause the increase observed in carbon dioxide production. When the water content of a mature seed reaches a critical value, usually approximately 30%, germination begins.

1-4 PREVIOUS KNOWLEDGE OF GERMINATING SEEDS

1-4.0 Introduction

A concise definition of germination is given by Mayer[87]:- "Germination is defined as that group of processes which cause the sudden transformation of the dry seed into the young seedling". Evenari[30] has divided the process of germination into four distinct phases: (i) imbibition phase (ii) activation phase (iii) phase of mitosis and (iv) radicle protrusion phase.

Unfortunately many authors tend to regard the seedling as a "germinating seed" although in this phase it is behaving more like a plant than undergoing the initiation and activation of enzymic reactions that is typical of a seed before emergence of the radicle.

1-4.1 The Physiology of Germinating Seeds

In the dry seeds of maize[88] and of bean[89] all the tissues are shrunken, the cell vacuoles are small, the nucleus is irregular and the cell contents are plasmolyzed. With the absorption of water the cells become turgid. This process is often very rapid as shown by the doubling

of the weight of many seeds within a few hours of the beginning of imbibition.

In most cases initiation of the growth of the radicle is mainly by cell enlargement which begins after 12 hours imbibition. Cell division may occasionally accompany enlargement, but more often it follows. Germination can, however, proceed in the complete absence of cell division.[20] Elongation of the cells of the coleorhiza of maize[88] can be observed about 20 hours after the beginning of imbibition. Cell division is first observed in the root tip at approximately the time it breaks through the coleorhiza. Cells of the scutellum enlarge greatly without dividing and the nuclei become very prominent.

No cell division was detected in the radicle of Zea mays[90] during the first 24 hours of imbibition but structural changes showed the reestablishment of a developmental pattern. These changes included mitochondrial differentiation, the appearance of vacuoles, the appearance of a complex system of endoplasmic reticulum and the association of ribosomes to form polysome-like structures. No changes were observed in the fine structure of the cotyledons of soybeans before germination.[91] The cells were tightly filled with protein bodies and lipid droplets but these did not coalesce or expand until after germination. The cotyledons were rich in ribosomes but lacked an endoplasmic reticulum.

Mitochondria have been detected in dry seeds of maize and bean. At the start of germination these elongated into rods. Mitochondria were segregated from the scutellum of germinating maize seeds and associated with these particles were enzymes which hydrolysed maltose and starch.[8] In an investigation of the cotyledons of Cucurbita maxima no mitochondrial activity was observed until the radicle had protruded.

The condition of the stem apex or plumule at the time of germination varies greatly. In some of the larger seeds with substantial

reserves the plumule is well developed with several of its leaves clearly visible. It is, however, more usual for the growth of the plumule to be delayed so that the shoot at the time of germination consists only of an undifferentiated apical meristem.

In the great majority of cases the radicle is the first part of the embryo to start active growth. It behaves in a typically root-like manner as soon as it emerges from the seed. The cotyledons are much more variable in their functions. In most non-endospermic seeds the main reserves are in the cotyledons. In endospermic seeds the digestion and absorption of the endosperm reserve is ordinarily carried out by the cotyledons. Besides these functions of storage and absorption the cotyledons often, also, act as photosynthetic organs, although in a minority of species the cotyledons never escape from the seed coat. In the epigeal form of germination the cotyledons emerge and are photosynthetic organs, but in the hypogeal form the cotyledons remain within the seed coats and at best make a trivial contribution to the photosynthetic activity.[7]

1-4.2 Respiration

As seeds take up water there is generally a marked increase in their gas exchange. There is, however, an immediate release of carbon dioxide on wetting most seeds and this has been attributed to both a physical process[92] involving liberation of gas which is absorbed in the seeds and to decarboxylase activity.[93]

The uptake of oxygen slowly rises during the initial period of water uptake, as the seed becomes hydrated reaches a steady state and then increases sharply when cell growth commences.[20]

In the early stages of germination the R.Q (P_{CO_2}/P_{O_2}) of seeds commonly falls below unity e.g. Zea mays, minimum of 0.75; sunflower of

0.55, and for castor bean a minimum of 0.30-0.35.[7] These low values are only temporary for seedlings normally have an R.Q of approximately 1.0. For wheat seeds the R.Q on the first day is 1.0, falling to 0.7 on the second day and rising again after germination. Seeds which store a large amount of fat are, however, characterized by a prolonged period at a low value for the R.Q even after germination. A theoretical R.Q can be calculated for the oxidation of the various foodstuffs e.g. for carbohydrates it is 1.0, fats 0.7 and for proteins 0.8. These values do not, however, make allowance for the activities of enzymes such as the decarboxylases for which the release of carbon dioxide is independent of oxygen uptake.

Furthermore, the R.Q depends on the extent to which there is genuine respiration and to what extent fermentative processes occur. In seeds with very compact tissues fermentation usually occurs initially and only when oxygen penetrates into the tissue does respiration proper begin. In such cases there will be an initial marked increase in carbon dioxide output and only a slight oxygen uptake, giving a high R.Q even though the substrate broken down might be a carbohydrate.

An increase in temperature normally also increases the rate of respiration. It has been shown that this effect depends on the presence or absence of the testa, e.g. in peas whose testa had been removed an increase in temperature raised the oxygen uptake much more than in intact pea seeds.[4] Temperature can only effect respiration if the oxygen is not limited in its diffusion into the tissue.

1-4.3 Protein Metabolism

As already stated all seeds contain a certain amount of protein. Proteases, enzymes which break protein down to its constituent amino acids, are activated in the early period of germination[70,94] and hence

solubilize the storage protein. In lupine seeds which lack the ability to germinate the activity of the proteases actually decreases during germination in contrast to the increased activity of viable seeds.[95] Part of the amino acids formed enter various respiratory and carbon cycles, with ammonia being stored by the formation of amides, the main ones being glutamine and asparagine. With few exceptions, the proteolytic enzymes of seeds and plants have rather low hydrolytic activities[94] e.g. in pea seeds the stores of reserve protein are not depleted until fifteen days after the beginning of imbibition as compared to the few hours taken to digest protein in the digestive tracts of animals.

The bulk of these amino acids are utilised in the formation of new proteins, most of which in the early stages of germination are enzymes or nucleoprotein. As mentioned earlier most of the system necessary for protein synthesis is present in dry seeds but in some seeds m-RNA is not present and in others the polyribosome system is disassociated.

As water is taken up the protein synthesizing apparatus of wheat embryos becomes functional. This is thought to be due, in part, to "activation" of the ribosomes which when obtained from dry seeds exhibit extremely slow incorporation of amino acids into protein. The activity of these can, however, reach the same level as those from imbibed seeds if polyuridic acid, which acts as a synthetic m-RNA is added.[96] It is suggested that during imbibition a pre-formed m-RNA combines with ribosomes to form polysomes. In peas an increase in membrane-bound ribosomes accompanies radicle emergence.[97]

The second type of control of protein synthesis is that in which the level of free ribosomes is extremely low. In castor bean endosperm both polysomes and ribosomes increase rapidly during germination.[58] The total RNA content increased, at first slowly and then rapidly, and this change was shown to occur in the ribosomal RNA.

Protein synthesis is necessary for the germination of most seeds. Some enzymes are present in the dry seed, either in an active or inactive form (which is activated on hydration) but other enzymes are synthesized during the early period of germination. The elongation of the coleoptile of Zea mays is accompanied by considerable protein synthesis. Most of this protein is formed from the soluble nitrogen compounds present in the mature seed.[67] Protein synthesis is a conspicuous feature of cells which are undergoing cell division.

Protein synthesis has been shown to occur in the endosperms, cotyledons and embryos of seeds. In lettuce the endosperm was the most active.[98] The synthesis of enzymes in many seeds, e.g. barley and squash, appears to be controlled by the embryo. Removal of the embryonic axis prior to incubation caused a strong inhibition of the development of respiration and of the release of amino acids from storage proteins. The rises in activity of some enzymes were also markedly inhibited.[99] It is also thought that the presence of certain metabolites can exhibit control at the level of transcription of m-RNA.[70]

1-4.4 Metabolism of Nucleic Acids and Nucleotides

It is generally assumed that the protein synthesis which occurs in germinating seeds is associated with synthesis of RNA. In peanut cotyledons there is a considerable increase in the RNA content during the early period of germination.[67] During germination of wheat and oat seeds the amount of RNA and DNA in the endosperm decreased, while it increased in the embryo, the total amount of each, however, remaining approximately constant on a per seed basis.[4] In Vicia sativa the RNA content of the cotyledons fell from the first day of germination. This was accompanied by large increases in the RNA content of the embryo.

The increases occurring between 24 and 48 hours were correlated with cell division.[100]

5-Amino-4-imidazolecarboxyamide-2-C^{14} has been shown to be an efficient precursor of purine in wheat embryos and the enzyme inosine-5-phosphate dehydrogenase has been demonstrated in pea seeds.[47] In dry cotton seeds the free nucleotides are present in the form of nucleoside monophosphates and they were accumulated in higher amounts in the embryo than in the cotyledons. During the early stages of germination the total amount of free nucleotides decreased by 50% and their degree of phosphorylation increased.[73] An enzyme which phosphorylates cytidine, deoxycytidine, deoxyuridine, uridine and deoxythymidine has been shown to be present during the germination of corn seeds.[101] These results imply that the seeds discussed are capable of synthesizing nucleotides during germination. In the cotyledons of germinating squash seeds only small amounts of C^{14} thymidine were incorporated into nucleotides. Dormant seeds did not show any incorporation.[102] It therefore seems probable that the enzymes necessary for the synthesis of nucleic acids are activated early in germination and that they occur in the embryo, not in the endosperm or cotyledons.

Ribonucleases (RNases), enzymes which hydrolyse ribonucleic acid to form nucleotides, have been studied. An active ribonuclease was detected in dry maize seeds. At the beginning of germination its activity decreased and soon began to increase. The highest specific activity was found in the endosperm and the lowest in the embryo. The embryo was shown to exert an influence on the RNase activity of the endosperm. Different products were formed by the actions of the RNases in the various tissues. Oligonucleotides containing up to 16 nucleotides were formed in the endosperm; oligonucleotides of smaller sizes are formed in the scutellum and in the embryo trinucleotides

predominate.[103] In the cotyledons of Pisum arvense RNase activity also increases during germination but it was found to convert the RNA completely to mononucleotides.[105]

Some of the important cofactors for enzyme reactions are nucleotides and would be expected to be extremely important during germination. Trigonelline is present in large quantities in the seeds of many plants. During germination[49] the N-methyl group is removed forming nicotinic acid which is then converted by a series of reactions to NAD. In germinating seeds of rice and wheat the total nicotinic acid increased with time and reached a maximum value 72 hours after the beginning of imbibition.[104] The total amount of the nucleotides NAD and NADP in both oxidised and reduced forms increased with time during germination. The amount of NAD in wheat and rice reached a maximum at 24 hours. The depletion which followed was accompanied by an accumulation of NADH.

During the first period of imbibition (0-16 hrs) of pea seeds a 250% increase in ATP content occurred. A parallel fall was observed in the content of AMP but no detectable change in the ADP content was found. A 93% increase in xanthosine and a 39% fall in adenosine content was also noted. During the last phase of germination leading to the emergence of the radical, there was a general fall in the amounts of free nucleotides. The contents of AMP, ADP and ATP decreased by 73%, 48% and 52% respectively. The amount of acetyl-3'-dephosphocoenzyme A fell by 53%. However the ratio of (NADP + NADPH)/(NAD + NADH) increased. A 52% decrease in the uridine content was observed but the amounts of other nucleosides remained approximately constant. The concentrations of UDP-glucose and UDP-galactose did not change but a six-fold increase in UDP-fructose was found. At no stage were any free purine or pyrimidine bases detected.[106]

The energy metabolism of lettuce seeds during germination has been studied.[107] Synthesis of nucleotides began after 15 minutes' imbibition and at this time energy rich phosphates began to appear. No ATP was detected in the dry seeds but within one hour ATP represented 90% of the total of ATP, AMP and ADP. The authors postulate that the energy rich phosphorus bond is turned over at a rate between 1 and 4 times per minute from the thirtieth minute of imbibition up to the emergence of the radicals. Synthesis of ATP must be an important feature of the germination of seeds. Most biochemical processes are energy requiring and especially the processes expected to be important during germination; namely, active transport across membranes, cell division and cell expansion. In fact germinating seeds do not retain all their energy in a biologically active form, much is lost as heat. If seeds are allowed to germinate in a vacuum flask a considerable rise in temperature occurs.[38]

Cell division would require the synthesis of both RNA and DNA. Little work appears to have been carried out on the DNA content of germinating seeds but it can be safely assumed to increase in at least those seeds for which cell division is necessary for germination. Synthesis of RNA and DNA would be associated with synthesis of nucleoproteins. These proteins differ to other proteins, in as much as they are extremely rich in basic nitrogen fractions and hence nucleic acid synthesis would need to be accompanied by the metabolism of basic amino acids.[67]

1-4.5 The Metabolism of Peptides

Many seeds contain large quantities of γ-glutamyl peptides. The ordinary proteases and peptidases do not act on γ-glutamyl peptide bonds. The biosynthesis of these compounds may be analogous to the first step of

gluthione synthesis wherein γ-glutamyl-cysteine is formed.

$$\text{glutamic acid} + \text{cysteine} + \text{ATP} \rightleftharpoons \gamma\text{-glutamylcysteine} + \text{ADP} + P_i$$

However attempts to demonstrate analogous systems have been unsuccessful.[50] These compounds could, however, arise from relatively non-specific transpeptidation reactions in which glutathione acts as the donor of the γ-glutamyl group. Many of the γ-glutamyl peptides found in seeds contain a nonprotein amino acid residue as would be expected from the relatively higher concentrations of these compounds in the seeds. These compounds could have an important role in the storage of nitrogen and sulphur. The observed changes in the concentration of γ-glutamyl β-pyrazol-1-ylalanine during germination of cucumber seeds agree with this hypothesis.[108]

During germination of kidney bean the γ-glutamyl peptides appear to be transported from the cotyledons to other tissues. Dipeptides may act as transport forms of nitrogen, sulphur, glutamic acid, or the non-glutamic portion. It is also possible that the peptide form protects the glutamic acid from being metabolised in the conductive tissue before it reaches the active metabolic regions. In germinating kidney bean seeds a net loss of γ-glutamylmethylcysteine occurred. This was thought to be due to the action of a transpeptidase exchanging the methylcysteine residue with another amino acid or amine.[50]

Glutathione, which is the tripeptide γ-glutamyl cysteinylglycine is important in biological systems for its ability to reduce disulphide groups, being itself simultaneously oxidised. The glutathione content of cells rises just before cell division, and is usually high in actively growing cells. The glutathione content of most seeds, and in particular of the embryos, rises during germination.[38] During the early stages of germination of pea seeds the glutathione content increases, rapidly

reaching a maximum value.[109] The glutathione content of the embryos could be an important factor involved in the activation of enzymes during the early stages of germination.

1-4.6 Amino Acids and Amides

Due to the action of proteases during the initial imbibition phase the amounts of free amino acids rise. Some of these amino acids are incorporated into protein and some are metabolised by the seed. The free amino acid content of seeds is usually low, although substantial amounts of nonprotein amino acids do occur. During the initial stages of germination it is possible that the nitrogen stored in these compounds becomes available for the formation of more vital nitrogenous compounds.

The changes in the concentrations of amino acids during germination in wheat grains has been studied.[41] Wheat germ, dampened to 18% moisture content at room temperature for 24 hours, showed a marked decrease in the amount of glutamic acid present. This decreased from a value of over 1500 ug/g dry weight to a value just above 500 ug/g. There was little effect on the amount of aspartate, the amounts of alanine and glycine increased slightly and the amount of GABA showed a moderate increase. At 40% moisture content alanine, GABA and glycine increased sharply in the germ, whereas aspartate and glutamate decreased strongly. The changes in concentration were followed at time intervals, for 24 hours. Glutamic acid decreased steadily reaching a value of less than 100 ug/g dry weight at 24 hours (from an initial value of over 1500 ug/g). The loss of glutamate was accompanied by a spectacular immediate increase in GABA. The concentration of aspartate decreases rapidly for the first hour, rises slightly to the third hour and then slowly decreases over the next 21 hours. The amount of glycine

increased continually, the increase being most rapid for the first 3 hours. Wheat seeds contain high concentrations of glyoxylic acid and this decreases rapidly on germination. Hence this increase in glycine is likely to arise from transamination of glyoxylic acid. Alanine increases steadily throughout the period examined. An increase in the amounts of other protein amino acids, except for a drop in proline, were also observed. These would probably arise from the reserve proteins.

Under a carbon dioxide atmosphere,[41] the large increases in alanine, GABA and glycine were not observed. The drop in the amounts of glutamate and aspartate were even more drastic than in air, and occurred within 1 hour. Exposing the germ to strictly anaerobic conditions led to rapid evolution of H_2S. This could be an important factor in the death of seeds under such conditions.

Using whole wheat grains the seeds were studied during and after germination. The amounts of alanine and glutamate increased rapidly from 0 to 24 hours imbibition, the amounts of glycine and GABA increased only slowly and the amount of aspartate decreased. Serine, threonine, tryptophan, valine, leucine, tyrosine, lysine and ethanolamine nearly doubled in the first 24 hours. The amount of glutamine increased tenfold during the first 24 hours but the amount of asparagine decreased in the first day. Arginine did not change.

The total changes in amino acids can, however, merely indicate their possible metabolism as the rates of turnover differ between seeds. During germination of bean[50] a decrease occurs in the amount of free glycine whereas in lupines[50] and wheat an increase occurs. A dramatic loss of proline similar to that observed in wheat occurs in maize seeds.[110]

The amides are thought to be important as a storage form of nitrogen. The enzyme glutamine synthetase which catalyses the reaction shown has been highly purified from pea seeds.[47]

$$\text{glutamate} + \text{ATP} + NH_3 \xrightleftharpoons{Mg^{2+}} \text{glutamine} + \text{ADP} + P_i$$

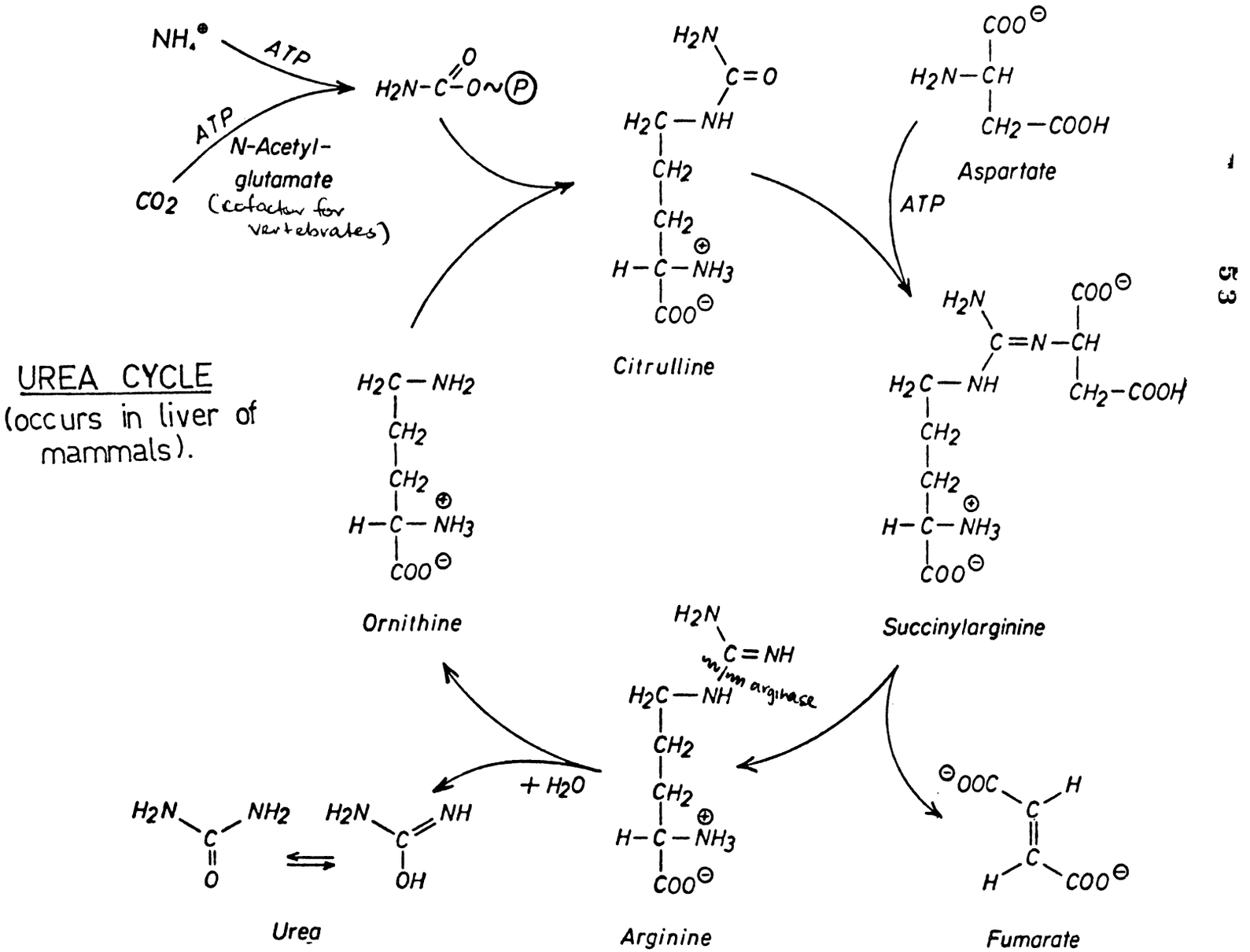
The requirement for Mg^{2+} can be replaced by other ions such as Co^{2+} and Mn^{2+}. The synthesis of asparagine has been shown in wheat germ. The reaction is similar to that for glutamine, ATP and Mg^{2+} are required but the conditions are sufficiently different to explain the previous failure to demonstrate the action of asparagine synthetase in plants.[111]

Germination of peanut seeds is accompanied by massive production of γ-methyleneglutamic acid. In these seeds, γ-methylene glutamine is the form used for nitrogen storage and transport.[112] If the embryo of a seed is removed from the endosperm it is deprived of carbohydrate. The breakdown of protein to amino acids which are then deaminated leads to a very high level of ammonia [113] which acts as a metabolic poison. If however, glucose is supplied to the excised embryo, amides are formed. It has been shown that amide formation only occurs when carbohydrate is supplied and respiration is active. Otherwise free ammonia is formed, eventually leading to the death of the seed. In some plants glutamine alone is formed, in some only asparagine, and in others both compounds. Some seeds, as mentioned earlier, also utilize unusual compounds for nitrogen storage and transport.

There is very little evidence for the existence of the urea cycle (Fig. 1-4.6.1) in plants, although interconversions of the amino acids involved can occur. Some of the reactions do appear to be important during periods of intense metabolic activity such as during germination or fruiting, and are probably associated with nitrogen storage or transport. The enzyme arginosuccinase which catalyses the reaction:-

$$\text{arginosuccinate} \rightleftharpoons \text{arginine} + \text{fumarate}$$

has been demonstrated in jack bean seeds. The ungerminated embryo of

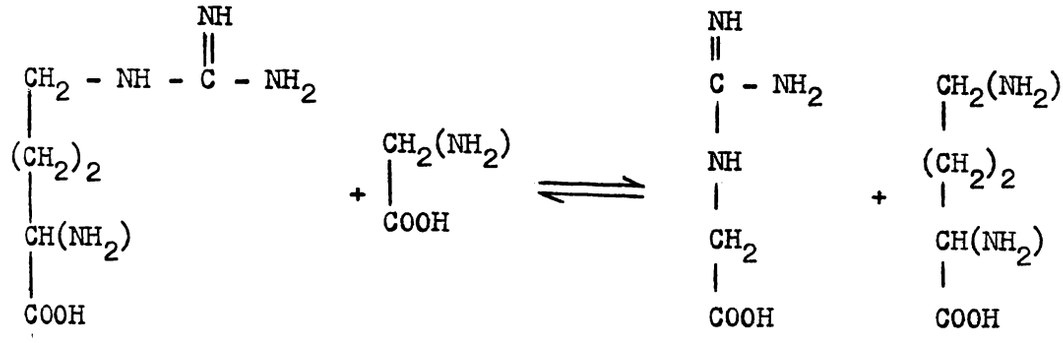


UREA CYCLE

(occurs in liver of mammals).

these seeds contains very large quantities of canavanine. Within a few hours of soaking this virtually disappeared from the embryo and remained at this very low concentration until after germination. During this low period the amount of asparagine increased dramatically.[114] Canavanine can replace arginine as a substrate for arginosuccinase.[115]

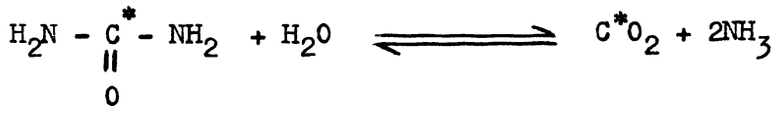
Arginine, itself, and possibly canavanine can participate in a transamidation reaction with glycine.



Arginine + $CH_2(NH_2)COOH$ ⇌ Guanidoacetic acid + Ornithine

This reaction has been demonstrated in seeds which contain large quantities of arginine, such as pea seeds.[47] Methylation of guanidoacetic acid yields creatine and the breakdown of this compound can lead to the important metabolite carbamyl phosphate.[49]

When discs of Vicia faba cotyledons were incubated with C^{14}-arginine labelled urea was formed. The activity of the urea increased to a maximum and then decreased with time. The rate of production of labelled carbon dioxide increased with time.[116]

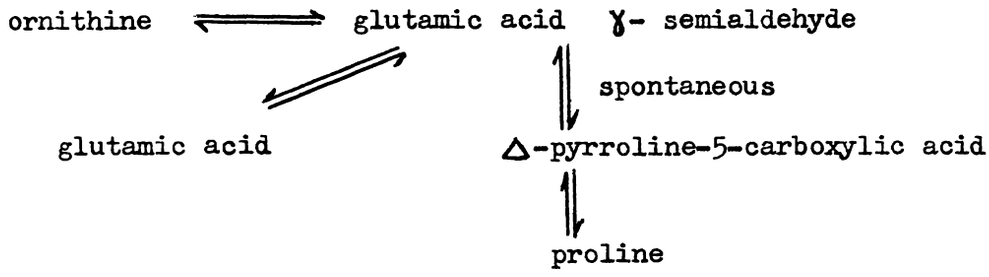


$H_2N - C^*(=O) - NH_2 + H_2O \rightleftharpoons C^*O_2 + 2NH_3$

The enzyme urease which catalyses the above reaction has been detected in a number of seeds,[47] including jack bean.

Discs of pea cotyledons[116] when exposed to u-C^{14}-arginine labelled, within 5 minutes, the amino acids ornithine and proline for which the

activity increased with time, passed through a maximum and then decreased to a low, steady state activity at 3 hours. Glutamate was lightly labelled after 15 minutes and its activity did not increase with time. Arginosuccinic acid became lightly labelled after 15 minutes and this increased with time. Citrulline was heavily labelled after 3 hours and then decreased slowly. These results show that ornithine can be converted to proline more readily then to glutamic acid.



The transaminases, dehydrogenases and decarboxylases of germinating seeds have been studied. The most important amino acid dehydrogenase is the enzyme glutamic acid dehydrogenase. It catalyses the reaction:-

$$\text{L-glutamic acid} + NAD^+ + H_2O \rightleftharpoons \alpha\text{-KG} + NADH + H^+ + NH_3$$

Other L-amino acids can also be oxidised by this enzyme, although at a much lower rate. This reaction is readily reversible and hence could be a method of converting α-ketoglutaric acid (α-KG) to glutamate. It has been shown to be present in dry barley seeds and to increase on germination.[117]

In wheat seeds the enzyme, glutamate-oxaloacetate transaminase (GOT) is present in the dry seeds. Its activity increased during the two days of germination and then fell slightly.[53]

Glutamate pyruvate transaminase (GPT) was found to be activated at moisture levels as low as 15% in wheat seeds.[52] In a study[118] of

germinating wax bean, pea, corn, squash, oat, pumpkin and barley seeds it was found that the activity of the transaminases GOT and GPT increased with time during germination. The reactions catalyzed by these enzymes are:-

GOT glutamate + oxaloacetate $\rightleftharpoons$ aspartate + α-KG

GPT glutamate + pyruvate $\rightleftharpoons$ alanine + α-KG

The major amino acid decarboxylase found in seeds is glutamic acid decarboxylase, leading to the formation of γ-amino butyric acid (GABA). At moisture levels of 25% and above, in wheat seeds, glutamic acid is rapidly converted to GABA.[52] The activity of this enzyme decreases 2-3 fold in lupine seeds at the approximate time of germination but this occurs in both viable and nonviable seeds.[95] In squash seeds glutamic decarboxylase is inhibited by carboxylic acids.[119]

Uniformly labelled glutamic acid was incubated with green grain (Phaseolus sp) seeds during germination. After 72 hours 95% of the activity was in the carbon dioxide evolved. Aspartic, asparagine, glutamic and glutamine all contained a small amount of activity and arginine and proline contained considerably less.[120]

GABA is found in high concentrations in many seeds and is rapidly formed from glutamic acid during the early phases of germination. In the potato 60% of the GABA present disappeared during active protein synthesis and did not reappear in protein hydrolysates.[67] The investigation of its metabolism has been undertaken using seedlings more often than not. As mentioned earlier the activity of glutamic decarboxylase decreases with the formation of the seedling and hence GABA has possibly lost its importance at this stage. These reactions shown could, therefore, be merely ways of removing excess GABA formed during germination and converting it into more useful compounds for the seedlings. In wheat and barley

seedlings GABA has been shown to transaminate with oxaloacetate and pyruvate.[121] The transamination of GABA and α-KG has been shown to occur in peanut, barley, wheat and lupine seedlings.[122] When C^{14}-GABA was supplied to carrot root extract labelled glutamate and glutamine were formed rapidly.[123] After a short time several other nitrogenous compounds were also labelled. With pea discs and extracts of peanut mitochondria C^{14}-GABA led to the formation of labelled organic and amino acids. The authors interpreted this as demonstrating formation of succinic acid followed by further metabolism involving the tricarboxylic acid cycle.[122]

The amino acids of the endosperm and the embryo during germination of the castor bean seed have been examined.[124] In the embryo glutamate and aspartate were virtually absent. Glycine and alanine were present at very low concentrations in comparison to the amounts in the protein of the endosperm. Glutamine comprised 40% of the amino acids in the embryo. These values reflect extensive metabolism of the hydrolysis products of the endosperm protein. The authors applied C^{14}-labelled amino acids to intact excised endosperms to follow their utilisation. Aspartate, glutamate, alanine, glycine, serine, leucine and GABA were converted to sugar to varying extents. Sucrose was the principal product with roughly equal amounts of C^{14} in its glucose and fructose moieties. Smaller but equal amounts of radioactivity were detected in free fructose and glucose. Proline, arginine, valine and phenylalanine were not appreciably converted to sugars and appear to be transported to the embryo intact.

More than 50% of the added aspartate was metabolised during the first hour and there was a large incorporation of C^{14} into organic acids. After this time, the activity of the acids declined and a linear increase in the activity of the sugars occurred, accompanied by a slow release of $C^{14}O_2$. At 2 hours 90% of the activity of the organic acids was in malate,

approximately 10% in fumarate with succinate and citrate virtually unlabelled. There was no conversion of aspartate to asparagine, although added glutamate was partially converted to glutamine. Use of serine-3-C^{14} gave labelled sugars, showing that serine is not converted via glycine and glyoxylate, but probably through the formation of hydroxypyruvate. A small portion of added proline was converted to glutamine and glutamate. The small conversion to sucrose shown was probably due to further metabolism of the glutamate formed. The results of these experiments are illustrated in Table 1-4.6.1.

Table 1-4.6.1

Utilization of Labelled Amino Acids by the Endosperm of Castor Bean Seeds[124]

Amino Acid Added	% of C^{14} Recovered after 12 hrs			
	Amino Acids	CO_2	Organic Acids	Sugars
Alanine-U-C^{14}	25	9	3	64
Aspartate-U-C^{14}	30	6	30	30
Glycine-U-C^{14}	55	12	7	30
Serine-3-C^{14}	48	0	15	30
Glutamate-U-C^{14}	30	2	6	22
GABA-U-C^{14}	58	3	13	22
Glutamine-U-C^{14}	72	8	7	13
Leucine-U-C^{14}	84	2	3	12
Proline-U-C^{14}	90	0	3	7
Arginine-U-C^{14}	93	0	2	5
Phenylalanine-U-C^{14}	96	0	2	2
Valine-U-C^{14}	96	0	2	2

From these results it is probable that in castor bean seeds the carbon from the glucogenic amino acids is transported to the embryo as sugar whereas the other amino acids are transported intact, the nitrogen of the glucogenic amino acids being transported in the form of glutamine.

1-4.7 The Metabolism of Carbohydrates

The term "cellulase" has been applied to impure enzymes catalysing not only the hydrolysis of cellulose (to cellodextrins, cellobiose and glucose) but also the hydrolysis of other β-glycosides. The enzyme β-glucosidase occurs notably in almonds although it is present in many other seeds as well. Ground defatted almonds were extracted with 25% saturated ammonium sulphate solution and the enzyme was precipitated by adding further ammonium sulphate until 50% saturation was reached.[36] A similar enzyme was isolated from barley seeds and found to be readily inactivated by heat.[36]

A β-glucosidase specific for cellobiose, called cellobiase, has been found in many plant seeds.[36] On germination the cellobiase activity of barley increased,[36,117] reached a maximum at the seventh to eighth day (in the seedling), and then declined sharply. The optimum pH value was 4.0 to 4.5 and the optimum temperature 37°C.

The α-(1⟶4)-glucosidic linkages of starch may be degraded by either hydrolytic or transferring enzymes. The former group is represented by the amylases. Three types of amylase are known but of these only two are of importance in plants or seeds. The enzyme, α-amylase, catalyses an essentially random hydrolysis of the starch components leading to the production of a mixture of oligosaccharides which are then slowly degraded to a mixture of maltose, α-dextrins, and small quantities of glucose. The α-dextrins are branched oligosaccharides and contain the original α-(1⟶6)-interchain linkages of the starch.

β-Amylase catalyses a stepwise hydrolysis of alternate linkages in an amylose-type molecule. Enzyme action is arrested by the presence of other types of linkages (e.g. inter-chain linkages) so that with amylopectin only the exterior chains are degraded and the products are maltose and a β-dextrin which corresponds to the interior of the original polysaccharide. The extent of conversion into maltose is therefore related to the lengths of the outer chains.

It is generally accepted that β-amylase is activated during the earliest period of germination in cereals and other seeds containing starch.[125] This enzyme has been shown to occur in only the endosperm of maize grains.[55] The formation of α-amylase in barley was shown to occur in the endosperm; but only after four days or more of imbibition. The synthesis of this enzyme in both maize[55] and barley[126,127] is controlled by gibberellic acid released from the embryo.

Neither these amylases nor the phosphorylase of the Glycolitic Pathway can hydrolyse (1$\longrightarrow$6)-interchain linkages. Two α-(1$\longrightarrow$6)-glycosidases have been found to be synthesized in germinating broad bean and barley.[35] One of these attacked amylopectin but had no activity on oligosaccharide α-dextrins, while the second carbohydrase showed the reverse specificity.

Phosphorylase catalyses the reversible transfer of α-glucosyl units to and from the non-reducing end of an amylose-type molecule or the outer chain of an amylopectin as shown below.

$$(G)_n + m(G.1.P) \rightleftharpoons (G)_{n+m} + mHOP$$

Chain of α-(1$\longrightarrow$4)-linked glucose residues — glucose-1-phosphate — inorganic phosphate

Phosphorylase was first demonstrated in extracts of pea seeds by Hanes in 1940[128] and the reaction was shown to be reversible. Small

amounts of starch however do need to be present for rapid synthesis of oligosaccharides by this enzyme. The chain length is then increased. Phosphorylases are present in both dry and germinating seeds.[4]

The precise course of starch breakdown in any given seed will be determined by the relative amounts of these enzymes present, as well as by the types of starch in the endosperm. The maltose formed by the action of β-amylase, rarely accumulates in seeds. It is further broken down, by the action of maltase, to glucose.

The glycolytic breakdown of glucose and glycogen, as illustrated effects the conversion of hexose phosphates to pyruvic acid and lactate. This pathway is an anaerobic process capable of producing only limited quantities of ATP. The several irreversible steps in the degradative pathway are effected in the direction of synthesis by other enzymes. This pathway is also important in organisms for the supply of precursors of necessary carbohydrates such as hexosamines, pentoses and uronic acids.

During germination of the castor bean a rapid increase in the concentrations of all glycotic intermediates was observed.[70,129] The activity of the enzyme hexokinase, which converts glucose to glucose-6-phosphate was observed to increase early in the germination of castor bean[130] and of summer barley.[117] In barley seeds its activity rapidly reached a peak, then gradually disappeared and appeared to be completely absent in the seedling.[117] The conversion of glucose-1-phosphate to glucose-6-phosphate is catalysed by the enzyme phosphoglucomutase. This enzyme was also observed to rapidly increase in activity in both germinating castor bean seeds[130] and summer barley.[117] In the latter case it disappeared during the later stages of germination and was not present in the young seedling.[117] Phosphohexoisomerase, the enzyme for

the conversion of glucose-6-phosphate to fructose-6-phosphate increased in activity during the first 24 hours of germination of the castor bean.[130]

The conversion of fructose-6-phosphate to fructose-1,6-diphosphate, catalysed by the enzyme phosphofructokinase is irreversible. For glucogenesis to occur (i.e. the reverse of glycolysis) the enzyme fructose diphosphatase which catalyses the reverse reaction must be present. This enzyme has been shown to be present in mature seeds[70] and, during the germination of castor bean seeds, to reach very high levels of activity.[130]

Aldolase, necessary for the splitting of fructose-1,6-diphosphate to dihydroxyacetone phosphate and 3-phosphoglyceraldehyde, has been detected during the germination of many seeds.[70,130] Its activity increased during the first 24 hours of imbibition in the seeds examined. In wheat seeds the enzymes of the glycolysis cycle generally decrease after germination but this is most marked for aldolase which had virtually disappeared within 48 hours of the beginning of imbibition.[53] In summer barley seeds aldolase completely disappears immediately preceding germination but it later reappears in the young seedling.[117]

The enzyme for the conversion of 1,3-diphosphoglycerate to 3-phospho-glycerate is phosphoglycerate kinase. This reaction involves the production of ATP and hence in the direction of glycolysis is considered to be important. The activity of this enzyme was observed to increase for the first 48 hrs during germination of wheat and then to decrease markedly.[53] A cell free extract of pea seeds was able to convert 3-phosphoglycerate formed in this reaction to 3-phosphohydroxypyruvic acid and via 3-phosphoserine to the amino acid serine. If other seeds are also capable of undergoing a similar reaction the interconversion of carbohydrates and amino acids is readily possible.[131] Pyruvate kinase

converts phosphoenol pyruvate to pyruvic acid. This kinase is a transphosphorylase which requires magnesium and a monovalent ion such as potassium or rubidium. Provided that these ions are present the reaction may be reversed but the equilibrium strongly favours the formation of pyruvate. Pyruvate kinase increases during germination of wheat but rapidly disappears after the 48th hour of imbibition.[53] Lactic acid can be formed from pyruvate by the action of the enzyme lactic dehydrogenase. The formation of lactic acid during germination has been shown in a large number of seeds.[113]

The presence in the incubation medium of free sugars, at concentrations 10-100 mM, has been shown to enhance by approximately 200% the rate of increase of these enzymes of the glycolysis cycle during germination of the castor bean. In the presence of sugars, massive starch accumulation and a marked increase in the amount of hexose-6-phosphate was observed.[129]

Another important pathway of carbohydrate metabolism is the pentose phosphate shunt which begins and terminates with intermediates of the glycolysis pathway. It normally has a crucial role in most cells but makes a relatively minor contribution to the net oxidation of carbohydrate. Carbon dioxide can be continually produced from Carbon 1 of hexose phosphate by repeated turns of the cycle.

The evidence for the existence of this cycle in seeds is scant,[4] although convincing evidence has been shown for germinating mung bean.[132] The presence of all the enzymes necessary for the oxidation of glucose-6-phosphate to ribulose phosphate and the further conversion of the latter, as shown in Figs. 1-4.7.2 and 3, were demonstrated.

The first step involves the conversion of glucose-6-phosphate to glucono-δ-lactone-6-PO_4. The enzyme necessary has been demonstrated in a

Pentose Phosphate Metabolism

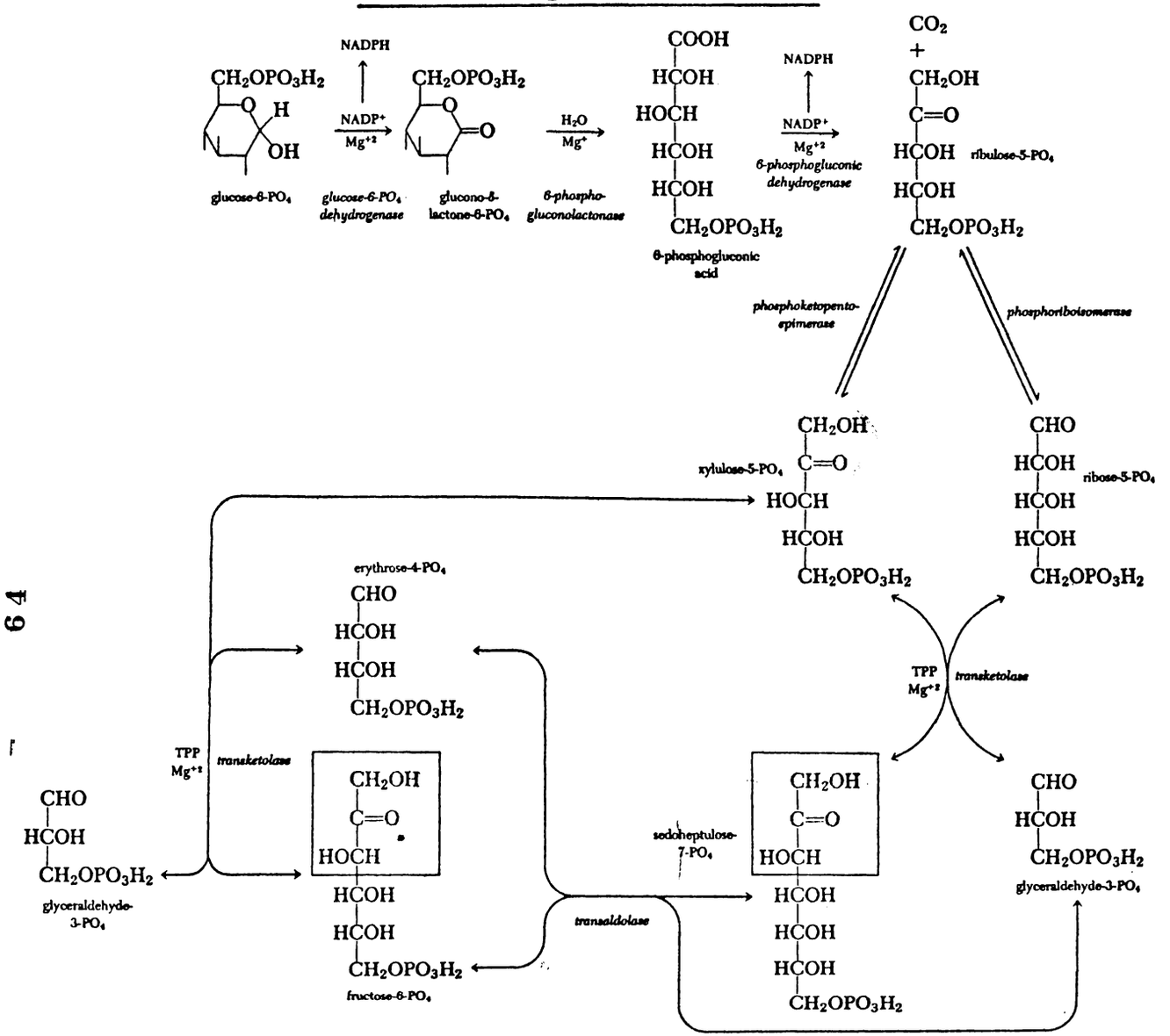


Fig. 1-4.7.2

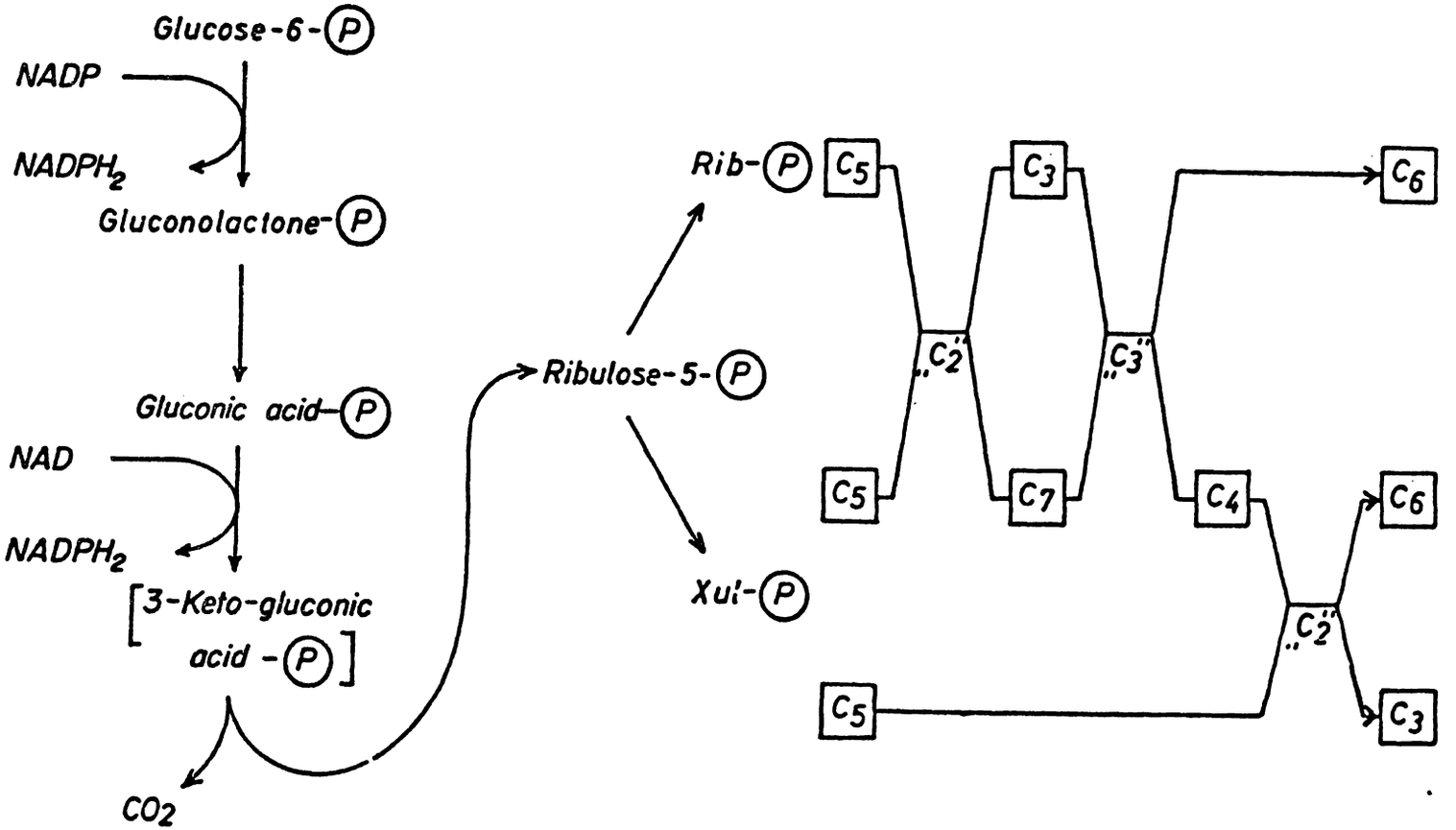


Fig. 1-4.7.3

PENTOSE PHOSPHATE METABOLISM

number of seeds, including lettuce,[4] wheat,[53] barley[117] and castor bean.[130] In general the activity of this enzyme increases during the early stages of germination and decreases during the later stages. In summer barley it was observed to disappear during germination and did not reappear in the young seedling.[117]

The enzyme for the third step, the conversion of 6-phosphogluconic acid to ribulose-5-phosphate has been shown to be present in the same seeds and to show a similar increase during germination, followed by a decrease thereafter. The formation in the next step of ribose-5-phosphate would be expected to be extremely important in germinating seeds. It is the starting point for the synthesis of nucleotides and hence for nucleic acids. The formation of glycine from ribose-5-phosphate has been demonstrated in spinach.[47] It is possible that most seeds contain only the enzymes necessary for the formation of ribose-5-phosphate and not those enzymes involved in the later part of the cycle.

In principle the glycosidases, including those discussed earlier and invertase, are also able to promote synthesis. However, synthesis from the monosaccharides is severely limited by the positive free energy of the reaction but when disaccharides are available longer chains can be formed by transglycosidation, e.g. the formation of polyfructoside from sucrose. A side product of this reaction is glucose. Monosaccharides must first be activated usually by combination with the nucleitide, uridine triphosphate (UTP). The synthesis of sucrose in plants is illustrated . In the germination of cereal seeds the mechanism involves, initially, the phosphorylation of glucose in position 6, in the presence of ATP. Part of the glucose-6-phosphate formed, is converted to fructose-6-phosphate and part to glucose-1-phosphate. The synthesis of sucrose then occurs as shown below:

$$GIP + UTP \rightleftharpoons UDPG + PP_i$$

$$UDPG + F6P \rightleftharpoons \text{sucrose-phosphate} + UDP$$

$$\text{sucrose-phosphate} \longrightarrow \text{sucrose} + P_i$$

An alternative mechanism for the synthesis of polysaccharides uses ADP as the activating group. Adenosine triphosphate reacts with glucose to form adenosine diphosphate glucose. The enzyme necessary, ADPG pyrophosphorylase is located in the endosperm of wheat, and is also present in maize, oats and barley.[35] This pathway is also used by germinating rice seeds.[35] The activity of the enzyme pyrophosphorylase increases during the early period of germination of the castor bean seed.[129]

The changes in the amounts of carbohydrates in germinating seeds have been studied, usually at 24 hour time intervals. Such studies have limited use in determining the metabolism of seeds as a substance could be actively synthesised and metabolised at the same rate and hence would show a steady state concentration.

The changes in carbohydrates, occurring during germination of wheat seeds, are shown in Table 1-4.7.1. Both total sugar and reducing sugar increase between day one and day two.

Table 1-4.7.1

Changes in the Composition of Wheat Seeds During Germination[4]
(Data in mg per 100 seeds)

Day	Dry Weight	Reducing sugars	Total sugar	Dextrin	Starch
0	2685	0	53.9	43.5	1781
1	2708	0	44.7	82.0	1621.3
2	2593	22.8	137.2	111.0	1079.0
3	2544	83.1	121.4	81.3	1343.5

In many cereal seeds starch appears in the cells of the radicle after only 3 hours imbibition in light. This is well before the beginning of cell elongation which does not occur until 18 hours.[8]

Appreciable amounts of sucrose occur in lettuce seeds. During the first 24 hours of imbibition this decreases.[8] In white mustard seeds (Sinapis alba) a decrease in the quantity of sucrose contained in the cotyledons from 0.16 to 0.12 mg per seed occurred during the first 24 hours.[133] Sucrose but not glucose is present in the embryo of dry maize seeds. Glucose was first detected when the embryo began to elongate.[38] During the germination of spring barley the contents of glucose and fructose did not alter during the first two days. After this period the quantity of glucose rose more rapidly than that of fructose.[117]

Germinating green grain seeds fed U-C^{14}-glucose rapidly converted this to C^{14}-glutamic acid.[120] Non-dormant and dormant seeds of Avena fatua (wild oat) metabolized C^{14} maltose in different ways. In nondormant seeds C^{14}-maltose administered to the endosperm was readily converted to sucrose in the scutellum and translocated to the embryo whereas, in dormant seeds, little sucrose was synthesized but glucose and maltose accumulated in the endosperm.[189]

The amino sugars are characterized by the replacement of a hydroxyl group of the sugar by an amino group. The amino sugars of greatest importance are glucosamine and galactosamine which are widely distributed in nature in the form of polymers, and muco-substances. Chitin, which is the main polysaccharide of the hyphal walls of most fungi, is a polymer of N-acetyl glucosamine. The pathway of glucosamine metabolism has been studied in germinating mung bean seeds (Phaseolus aureus). D-glucosamine was converted using ATP to D-glucosamine-6-phosphate which then reacts with Acetyl CoA to yield N-acetyl-D-

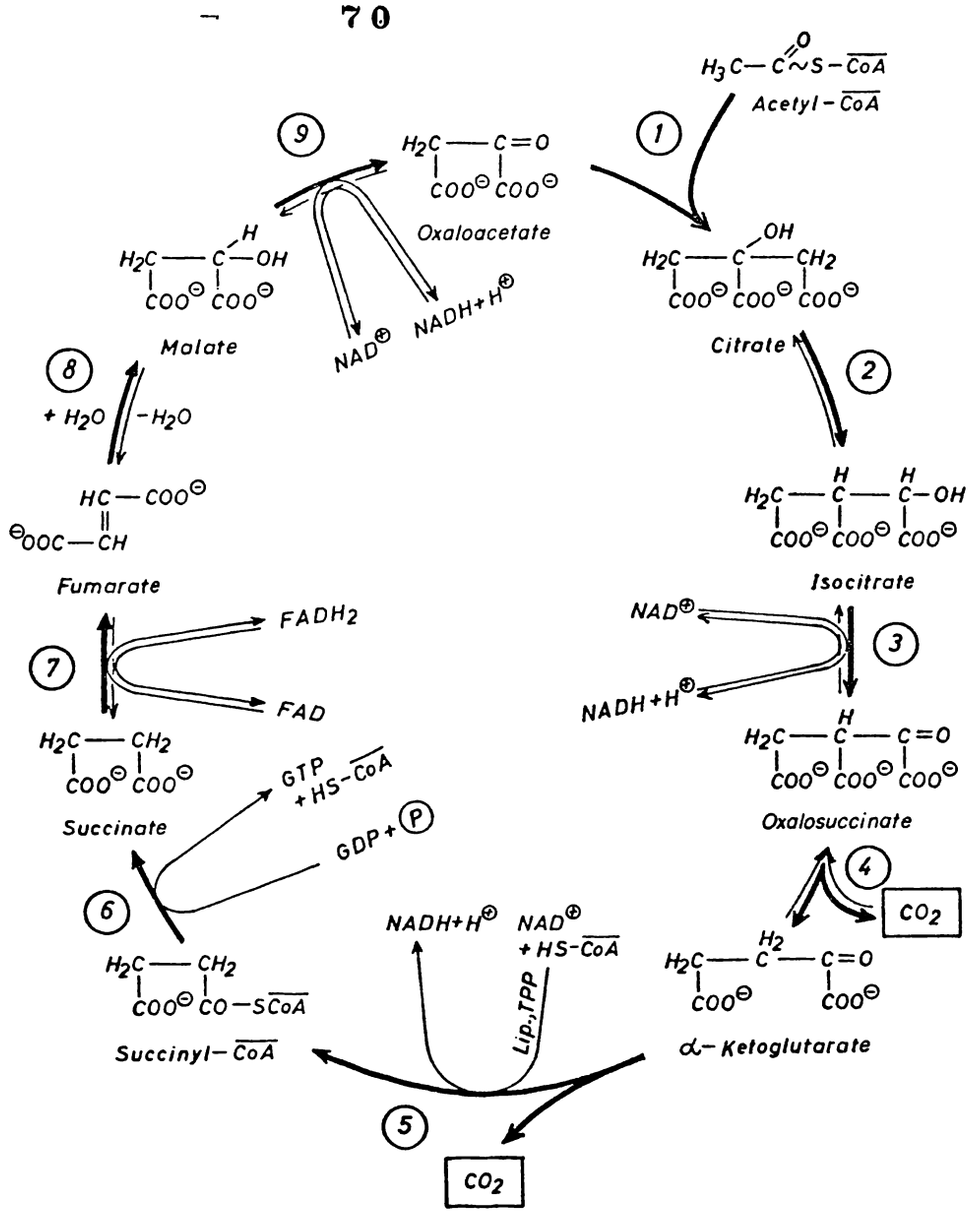
glucosamine-6-phosphate. Glucosamine-6-phosphate is also in equilibrium with fructose-6-phosphate and ammonia.[134]

1-4.8 The Metabolism of the Organic Acids

The tricarboxylic acid cycle (TCA cycle) which operates under aerobic conditions enables the complete oxidation of pyruvic acid to carbon dioxide and water (Fig. 1-4.8.1), with a large release of energy locked in the form of ATP. The criteria proposed for[4] evidence of its functioning are (i) proof of the existence of the complete cycle, (ii) proof that the cycle can be entered at any point, (iii) proof that any substance which is an intermediary of the cycle may be used as a substrate. Based on this criteria there is little evidence for the operation of the TCA cycle in germinating seeds.[4]

The presence of the enzyme isocitric dehydrogenase has been demonstrated in germinating seeds. It catalyses the conversion of isocitrate to oxalosuccinate. It was absent in the cotyledons of dry seeds of Vigna sesquipedalis but appeared soon after the beginning of imbibition.[8] In wheat, it was also found to be absent in dry seeds but appeared during imbibition, increased for the first 48 hours and then remained constant.[53]

Malate dehydrogenase, which causes the conversion of malate to oxaloacetate has also been studied. This enzyme does, however, also occur as part of the glyoxylate cycle. In dry wheat seeds this was found to be the most active enzyme. The activity increased during the first 48 hours and then dropped slightly.[53] During germination of castor bean the activity increased rapidly during the first 24 hours. It was not inhibited by puromycin or actinomycin, implying that a zymogen was being activated rather than de nova synthesis of the enzyme. In wheat, the ratio of malate dehydrogenase to GOT remained constant throughout the



TRICARBOXYLIC ACID CYCLE

Fig. 1-4.8.1

first few days of germination of wheat. This reinforced the hypothesis that the association of these 2 enzymes which are difficult to separate occurred in the same molecular structure.[53] Isoenzymes of malate dehydrogenase have been found in corn scutellum, germinating pea, mung bean, soybean, cotton, pumpkin, radish, cabbage, corn, oat and wheat seeds.[135]

Biological oxidations occur in water solutions, usually between pH 6 and 8. In its simplest term, physiological oxidation deals with the removal of hydrogen from the substrate and the combining of it with molecular oxygen to form water or hydrogen peroxide. It is doubtful whether any living cells carry out this process in such a simple direct fashion. The oxidation process in tissues is often a long and complex chain phenomenon. Four different types of reaction systems are involved in cellular oxidations. These are (i) the dehydrogenases (ii) the hydrogen transport system or respiratory chain (iii) oxidase enzymes which activate the oxygen so that it will quickly oxidize the hydrogen supplied by the hydrogen transport system and (iv) the peroxidases which destroy any peroxide formed.

At certain stages in the electron transport chain, some of the energy produced by oxidation is trapped and stored in the form of high-energy phosphates (ATP) for use in metabolic processes. This is termed "oxidative phosphorylation" and so far experimental evidence limits this process to the mitochondria. One molecule of ATP can arise for each electron (or hydrogen) transferred between $NADH_2$ and flavoprotein, flavoprotein and cytochrome, and cytochrome c and oxygen. Hence 3 molecules of ATP are formed per atom of oxygen. This chain is shown in Fig. 1-4.8.2.

The first step, the oxidation of succinic acid to fumarate, is catalysed by the enzyme succinate dehydrogenase. In germinating castor

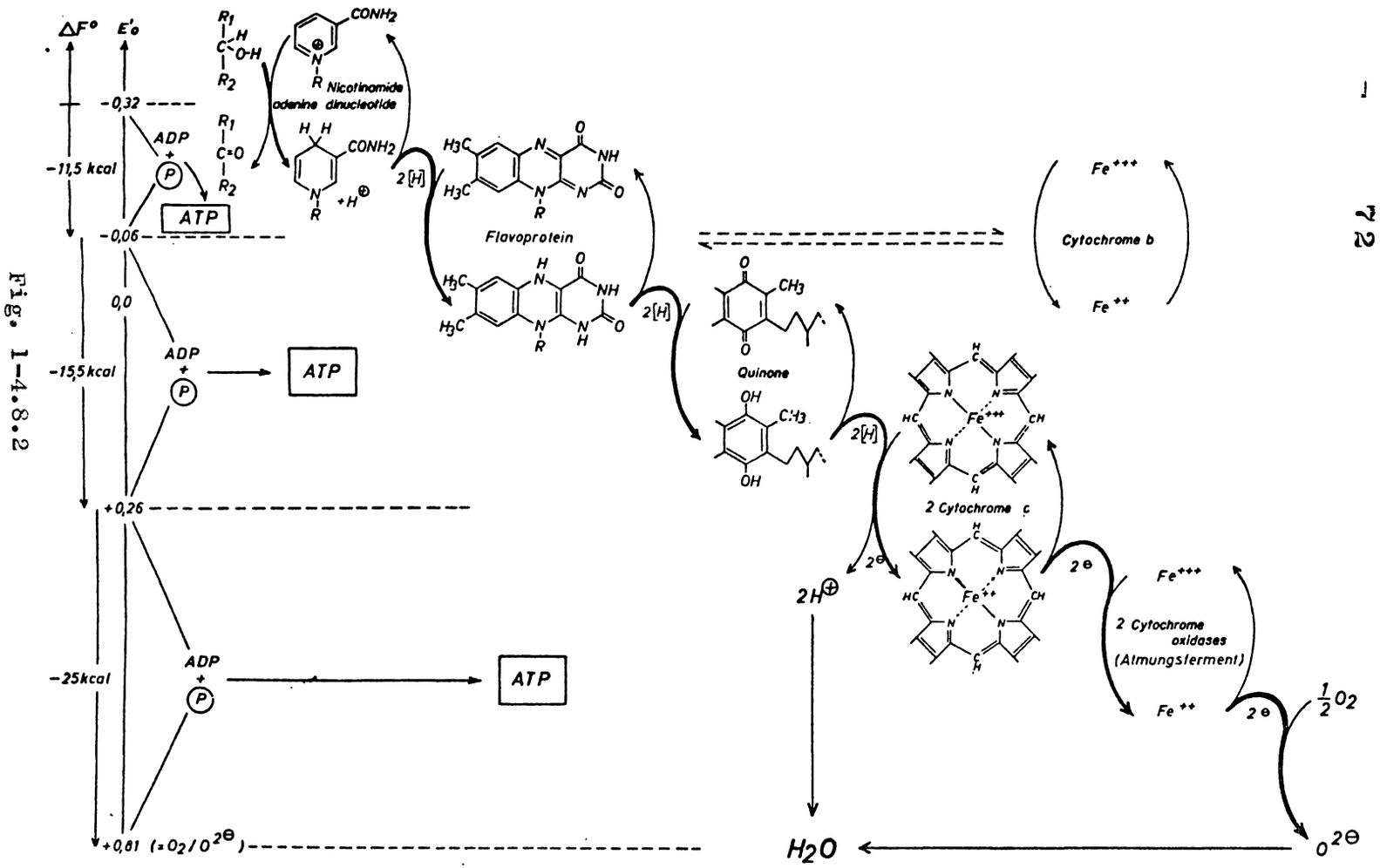


Fig. 1-4.8.2

Sequence of redox systems in the respiratory chain

RESPIRATORY CHAIN

bean seeds the activity was found to increase rapidly during the first 24 hours. It was not inhibited by either puromycin or actinomycin and hence was probably formed from a zymogen.[136] The embryo of wheat was also found to contain this enzyme after 70 or 80 hours' imbibition.[137] In the cotyledons of squash, (Cucurbita maxima), which took 3 days to germinate, a slight increase in activity from day 0 to day 1, a more rapid increase to a maximum at 3 to 3.5 days, and a rapid decrease in activity as the cotyledons became green leaves, were observed.[138]

The enzyme for the addition of oxygen to ferrocytochrome c, forming ferricytochrome c plus water is called cytochrome oxidase. Its activity increased rapidly during the first 24 hours of imbibition of castor bean and this was thought to be due to the activation of a zymogen.[136] Cytochrome oxidase was detected in the embryo of wheat after 70 to 80 hours' imbibition.[137] In squash cotyledons a slight decrease was observed from day 0 to day 1, followed by a gradual rise in activity to a maximum at 3 to 3.5 days. As the cotyledons became green leaves the activity decreased.[138]

The enzyme formic dehydrogenase (EC 1.2.2.1) which catalyses the reaction -

$$\text{formate} + \text{ferricytochrome } b_1 \rightleftharpoons CO_2 + \text{ferrocytochrome } b_1$$

has been detected in several seeds. Its activity decreases during germination.[8]

An important pathway by which pyruvate may be metabolised is via the glyoxylate cycle, illustrated in Fig. 1-4.8.3. This cycle contains several reactions in common with the TCA cycle. The important feature of this cycle is the condensation of acetyl CoA formed from pyruvic acid,

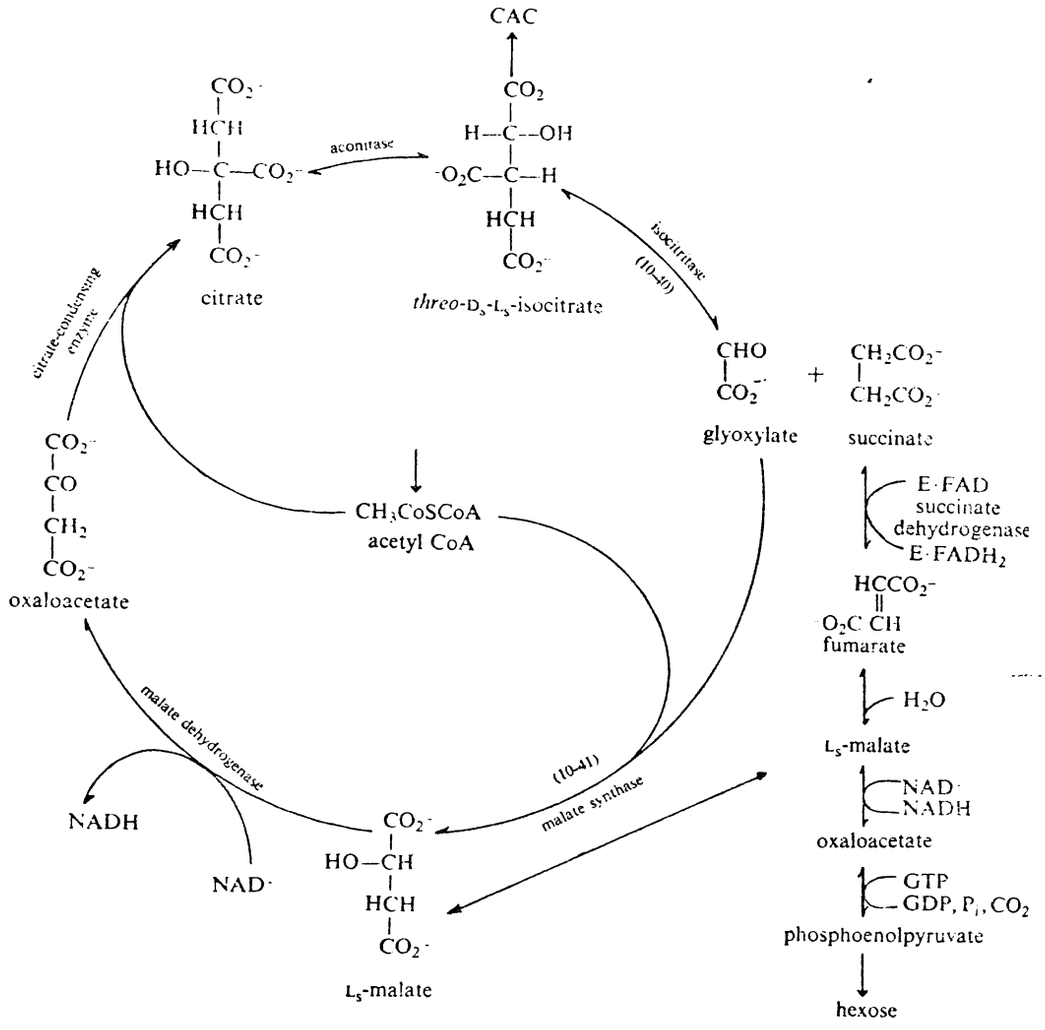


FIGURE 1-4.8.3 The Glyoxylate Cycles.

Acetyl CoA + oxaloacetate + H_2O	→ citrate + CoASH	$\Delta G^{0\prime} = -7.5$ kcal/mole
Citrate	⇄ D_s-*threo*isocitrate	$\Delta G^{0\prime} \simeq 0$
D_s-*threo*isocitrate	⇄ succinate + glyoxylate	$\Delta G^{0\prime} = 2.11$ kcal/mole (10-40)
Acetyl CoA + glyoxylate + H_2O	→ malate + CoASH	$\Delta G^{0\prime} = -10.63$ kcal/mole (10-41)
Malate + NAD^+	⇄ oxaloacetate + NADH + H^+	$\Delta G^{0\prime} = 6.69$ kcal/mole
NADH + ½O_2 + H^+ + [3ADP + 3P_i]	→ NAD^+ + H_2O + [3ATP]	$\Delta G^{0\prime} = -54.4$ [or ~ -29] kcal/mole
2 Acetyl CoA + ½O_2 + [3ADP + 3P_i]	→ succinate + 2CoASH + [3ATP]	
Succinate + ½O_2 + 2P_i + 2ATP	→ fumarate + H_2O + 2ATP	
Fumarate + H_2O	⇄ malate	
Malate + NAD^+	⇄ oxaloacetate + NADH + H^+	
Oxaloacetate + ATP	⇄ phosphoenolpyruvate + ADP + CO_2	
Phosphoenolpyruvate + NADH + ATP	→ ½ hexose + NAD^+ + ADP + 2P_i	(10-42)
Net: 2 acetyl CoA + O_2 + H_2O	→ ½ hexose + CO_2 + 2CoASH	
or, from acetate, 2 acetate + O_2 + H_2O	→ ½ hexose + CO_2 + 2H_2O	

THE GLYOXYLATE CYCLE

with glyoxylic acid to form malate. The glyoxylate arises from isocitric acid with the concomitant formation of succinic acid by the action of isocitrase.

The activity of isocitrase has been investigated in a number of seeds. It was not found in dry castor bean seeds[139] but the activity increased rapidly, in both the endosperm and cotyledons during germination.[70] Evidence of the de nova synthesis of isocitrase during germination has been found in peanut cotyledons[140] and castor bean seeds[141]. In the case of peanut[140] it was shown to be synthesized after the onset of germination from a pool of amino acids which did not arise directly from the hydrolysis of storage protein. In germinating squash seeds removal of the embrionic axis prior to imbibition caused a strong inhibition of the synthesis of isocitrase.[99] The presence of glucose in the incubation media for castor bean seeds inhibits the rise in activity of isocitrase by 86%.[142] Similar results were obtained using squash[141] seeds.

Malate synthetase causes the condensation of glyoxylate and acetyl CoA, forming malic acid. Malate synthetase is present in dry castor bean seeds. A slight increase in activity from its original level was observed during germination.[139] The authors investigated several seeds and plants, and concluded that germinating fatty seeds were the superior source of the enzyme. In peanut seeds malate synthetase was formed by de nova synthesis in a manner similar to isocitrase, during germination.

Evidence for the occurrence of malate dehydrogenase has been discussed under the enzymes of the TCA cycle. In general it was present in many dry seeds and its activity was found to rapidly increase during the early stages of germination by activation of a zymogen.

Glyoxysomes which are distinctive particles similar to mitochondria although heavier, have recently been discovered in plants. These contain the enzymes isocitrase, malate synthetase, citrate synthetase and malate dehydrogenase but the enzymes fumarase, NADH oxidase, and succinic dehydrogenase were found to be completely absent. Purified mitochondria from these plants were shown to lack malate synthetase and isocitrase.[143,144] Several authors have reported an increase in the number of mitochondria in germinating seeds after 24 hours' imbibition. However it is possible that they observed either glyoxysomes or a mixture of glyoxysomes and mitochondria, and hence past work on the number of mitochondria in germinating seeds is inconclusive.

An intensive study of the changes in concentrates of organic acids in germinating wheat grains has been undertaken.[41] Wetting the germ to 18% moisture content caused rapid decreases in the keto-acids glyoxylic, alpha-ketoglutaric, and oxaloacetic. The amounts of glyoxylic and alpha-ketoglutaric acids decreased to approximately half of the original concentration in only 3 hours. Oxaloacetic acid could not be detected at all after 10 hours. With increased temperature the disappearance of these acids was more rapid.

At 39-40% moisture content alpha-ketoglutaric acid almost disappeared within 3 hours and was barely detectable after 27 hours. Oxaloacetic acid disappeared completely within 3 hours after a barely noticeable maximum. The higher moixture level had little additional effect on glyoxylic acid. During the first 9 hours it decreased rapidly but this was followed by a slight increase. If the germ is kept under a carbon dioxide atmosphere the drop in alpha-ketoglutarate, oxaloacetate and the corresponding amino acids is even more drastic than in air or under a nitrogen atmosphere. Exposing the germ to strictly anaerobic conditions is also accompanied by the rapid evolution of hydrogen sulfide.

A continuous increase in glyoxylate under a carbon dioxide atmosphere, in contrast to the final decrease under nitrogen, was also observed.

During the germination of whole seeds the concentrations of alpha-ketoglutaric acid, pyruvic acid, glyoxylic acid and oxaloacetic acid remained at constant very low values for the first 24 hours. Their next analysis at 72 hours, after germination, showed a very marked increase to maximum values for alpha-ketoglutaric acid and pyruvic acid. Slight increases in glyoxylic and oxaloacetic acid were observed in the same period.

When sufficient water was added to the grains to produce a final content of 25%, all keto acids decreased after exhibiting a small initial maximum. The rate of decrease observed was greatly accelerated under a nitrogen atmosphere.

Citric acid has been found in most dry seeds examined[65] and is thought to be important during the early stages of germination. Citrate can serve as a source of reducing power and also as an activator for Acetyl CoA carboxylase, both of which are essential for fatty acid synthesis. It can also be a source of acetyl groups as shown in the glyoxylate cycle. In animal cells the cleavage of citrate does not seem to be essential as there are many other sources of acetyl groups.[145]

During germination of broadbean seeds the amount of citrate decreased sharply during the first 24 hours but the amount of malate was virtually unchanged. This decrease continued, under light, until post-germination. In seeds germinating in the dark a transient increase in both malic and citric acid was observed from the 3rd to the 5th day. Germination occurred after approximately 5 days. It was shown that the citrate was being metabolised in the germ and that malate was partly transferred from the cotyledons to the embryo.[65] The drop in citric acid in the embryo during the first 2 days left only 50% of the original amount present. It is possible that certain quantities of citric acid

are utilized by germinating seeds for the synthesis of fatty acids contained in the membranes of newly formed cells. This decline in citrate during the initial period of germination cannot be explained in terms of the TCA cycle. The conditions prevailing in the seeds do not favour aerobic reactions as until the radicle penetrates the seed coat the uptake of oxygen is extremely limited. After protrusion it shows a marked increase.[146] Activation of the TCA cycle is known to be delayed until after sufficient time has passed necessary for the swelling and attainment of full activity by mitochondria.[147]

The ability of mitochondria prepared from seeds to oxidize tricarboxylic acid cycle intermediates has been shown for peas, peanuts, mung beans, lettuce, castor beans and others.[4] However in both "Progress", the light insensitive variety, and "Grand Rapids", the light sensitive variety of lettuce the oxidative activity of the mitochondria remained extremely low until after germination had occurred. The ability of "Grand Rapid" seedlings to oxidize the tricarboxylic cycle substrates developed gradually, the succinic oxidase system being the first to appear.[190] The linkage of oxidation to phosphorylation is experimentally more difficult to show. The P/O ratios obtained are usually lower than those obtained for animal material.[4] The behaviour of mitochondria in vivo does not necessarily reflect the behaviour of the entire cell. The ability of isolated mitochondria to oxidise a certain substrate depends very largely on the method of isolation. It is also possible that the conditions under which the activity is examined differ completely from those in the cell such that <u>in vivo</u> it is inhibited.

Slices of castor bean endosperm incubated with pyruvate-1-C^{14} rapidly led to the formation of labelled carbon dioxide and unlabelled acetyl CoA. When either 2-C^{14} or 3-C^{14} pyruvate was used, labelled carbon dioxide was released slowly and labelled citrate and malate formed rapidly.

After a period amino acids related to the TCA cycle were discovered to be labelled and eventually labelled protein was formed.[148]

Germinating pea seeds were exposed to an atmosphere containing $C^{14}O_2$. Rapid labelling of malic and citric acid occurred. Thirtyfive to forty percent of the label in malate occurred in the C1 position and in citric acid 70-75% was in the C6 position. These results show that at least two major carboxylations had occurred in the germinating peas.[149] Lettuce seeds incubated with $NaHC^{14}O_3$ labelled malate, citrate, aspartate, glutamate, glutamine, glycine, serine, alanine and asparagine within 90 mins. Analysis of the extracts after 3 hours imbibition showed that approximately 33% of the label appeared in malic acid and 12 to 15% in each of the following; glutamine, aspartate, citrate and glutamate. Other labelled compounds were asparagine, serine, glycine, succinate, fumarate, glycerate and alanine.[159]

The metabolism, of germinating lettuce and white mustard (Sinapis alba) seeds, has been investigated using tritiated water as a tracer. The results obtained for mustard are shown in Table 1-4.8.[173]

Table 1-4.8

Compounds Labelled During Imbibition of Sinapis alba Seeds in Tritiated Water [173, 315]

Compound	Time (minutes)								
	5	10	15	30	60	90	120	180	1440
GABA	+	+	+	+	+	+	+	+	+
Aspartic Acid	+	+	+	+	+	+	+	+	+
Glutamic Acid		+	+	+	+	+	+	+	+
Alanine		+	+	+	+	+	+	+	+
Malic Acid			+	+	+	+	+	+	+
Citric Acid			+	+	+	+	+	+	+
Sucrose				+	+	+	+	+	+
Lipid								+	+

Note: The "fructose?" reported in this paper has since been shown to be sucrose.

The amino acids GABA, aspartate, glutamate and alanine were labelled within 10 minutes of imbibition. After a further 5 mins. citric and malic acid became labelled and within 30 mins. of imbibition in tritiated water sucrose was also labelled. These results show the initial importance of amino acid metabolism followed at a slightly later stage by organic acid metabolism.

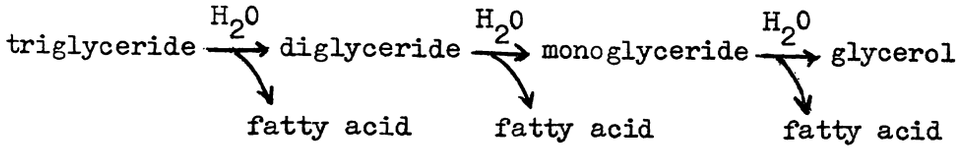
The glycollate metabolism of the endosperm of Ricinus communis (castor bean) has been examined by feeding micromolar quantities of glycollate—2-C^{14}. It was found that the label was readily incorporated into glyoxylate, glycine, serine and carbon dioxide. Only small amounts were incorporated into sugars.[150] In the castor bean endosperm malate is considered to be a precursor of sugar.[151] When incubated with acetate-1-C^{14} or acetate-2-C^{14} labelled sucrose is formed rapidly. Incubation with succinate-1-C^{14}, succinate-2-C^{14} and malate-C^{14} also led to labelled sugars.[151]

The amino acids which can be converted to one of the C_4-dicarboxylic acids or to pyruvate are called "glucogenic amino acids" as in the presence of the TCA cycle or the glyoxylate cycle and glycolysis, they can be converted to sugars. Only three carbon atoms of the amino acid are used in the synthesis of glucose. Active C_1 fragments can be produced from the amino acids, serine, glycine and histidine as well as from pyruvic and citric acids.

For the formation of sugars via a reversal of glycolysis oxaloacetic acid can be converted directly to phosphoenol pyruvate liberating carbon dioxide. The cofactor inosine triphosphate (ITP) is necessary. This overcomes the unfavourable equilibria between pyruvate and phosphoenol pyruvate.

1-4.9 The Metabolism of Lipids

The first step in the degradation of fats is their hydrolysis to fatty acids and glycerol by the action of lipases. Lipases are rather non-specific esterases and their mode of action may be represented as follows:



The rate at which glycerides are hydrolysed, in general, increases with the number of fatty acid residues on the glyceride, with the chain length and with the degree of unsaturation of the fatty acid. The breakdown products of the hydrolysis of lipids do not accumulate in the seeds.

The fatty acid may be broken down by the process of β-oxidation, resulting in the cleavage of two carbon units in the form of an acetyl group which can enter the various metabolic systems. This reaction requires both CoA and ATP but the latter only for the initial activation of the fatty acid before it enters the β-oxidation cycle. The removal of each acetate fragment via β-oxidation produces one molecule of $NADH_2$ and one molecule of reduced FAD. Oxidation of each mole of reduced NAD through the respiratory chain yields three moles of ATP and oxidation of each mole of reduced FAD yields two moles of ATP. Hence with the formation of each mole of acetyl CoA, five moles of ATP are formed.

An alternative method of degradation is by α-oxidation in which the fatty acid is peroxidatively decarboxylated and carbon dioxide formed. The long chain aldehyde formed is oxidised to the corresponding acid by a reaction linked to NAD and can then undergo α-oxidation again. The combined action of the two enzymes necessary results in

a stepwise decarboxylation of acids with chain length between C_{15} and C_{18} to acids of chain length C_{14} as only fats from C_{14} to C_{18} serve as substrates for the first enzyme in this scheme, fatty acid peroxidase. The requirement for hydrogen peroxide is satisfied by a peroxide generating system. Free H_2O_2 is not active. In crude cell fractions it is sufficient to add glycollic acid or any of a number of L-α-hydroxy acids, since glycollic acid oxidase is present in the crude preparations.[47] This enzyme (EC 1.1.3.1) also oxidises L-lactate and hence, this could be a possible use for the large quantities of lactic acid formed during germination of seeds.

$$R - \underset{\underset{H}{|}}{\overset{\overset{OH}{|}}{C}} - COOH \xrightarrow[\text{oxidase}]{\text{glycollic}} R - \overset{\overset{O}{\|}}{C} - COOH + H_2O_2$$

Little is known of the mechanisms of oxidation of the unsaturated fatty acids. However, α-oxidation of oleic acid would produce a fatty acid containing one less carbon atom and this compound could be degraded directly by β-oxidation. Oleic acid, itself, cannot undergo β-oxidation past the double bond unless the enzyme, enoyl hydrase, converts the β, γ double bond in the derivative formed to an α, β bond as required for fatty acid oxidation.

A soluble lipase has been isolated from wheat germ. The enzyme did not contain -SH groups at the active centre.[152] Isoenzymes of the fatty acid peroxidase required for α-oxidation have also been found in wheat germ.[153]

An acid lipase has been found in the endosperms of dry castor bean seeds. It was thought that a neutral lipase appeared during germination but attempts by the same authors to repeat the experiment

failed.[154] During ripening of castor bean seeds an alkaline lipase (pH 8.5 to 10.5) has been found but this is absent in ripened seeds.[155] Much research has been carried out on the lipid metabolism of castor bean. Some lipid has been shown to be converted to sucrose, via the glyoxylate cycle within the first few hours of imbibition. However, lipid does not completely disappear from the endosperm until 7 to 10 days from the onset of germination, at which time the seedling has been formed.[70] When non-green cotyledons from germinating castor bean seeds were incubated with glycerol-1,3-C^{14} some activity appeared in carbon dioxide and 3 to 4 times as much in sucrose. When this labelled sucrose was investigated the glucose and fructose moieties were found to contain equal amounts of activity. The pattern obtained implied that the glycerol moiety had been incorporated intact into the sugars.[47] Castor bean seeds incubated with acetate-C^{14} metabolised over 60% of this within 2 hours. The organic acids contained the major part of the incorporated activity during the early periods but in a short time the sugars became labelled. The results obtained showed that α-KG, the only compound not belonging to both the TCA cycle and the glyoxylate cycle, was bypassed during the formation of sucrose.[151]

The conversion of glycerol-1,3-C^{14} to labelled carbon dioxide has been shown in germinating peanut seeds. The conversion required the presence of mitochondria, ATP, an acid of the TCA cycle, thiamine pyrophosphate, magnesium and NAD. A number of labelled organic acids were also found. An α-glycerophosphate dependent reduction of cytochrome c was also observed. This was due to the reaction:

$$\alpha\text{-glycerophosphate} \xrightleftharpoons{2H} \text{dihydroxyacetone phosphate}$$

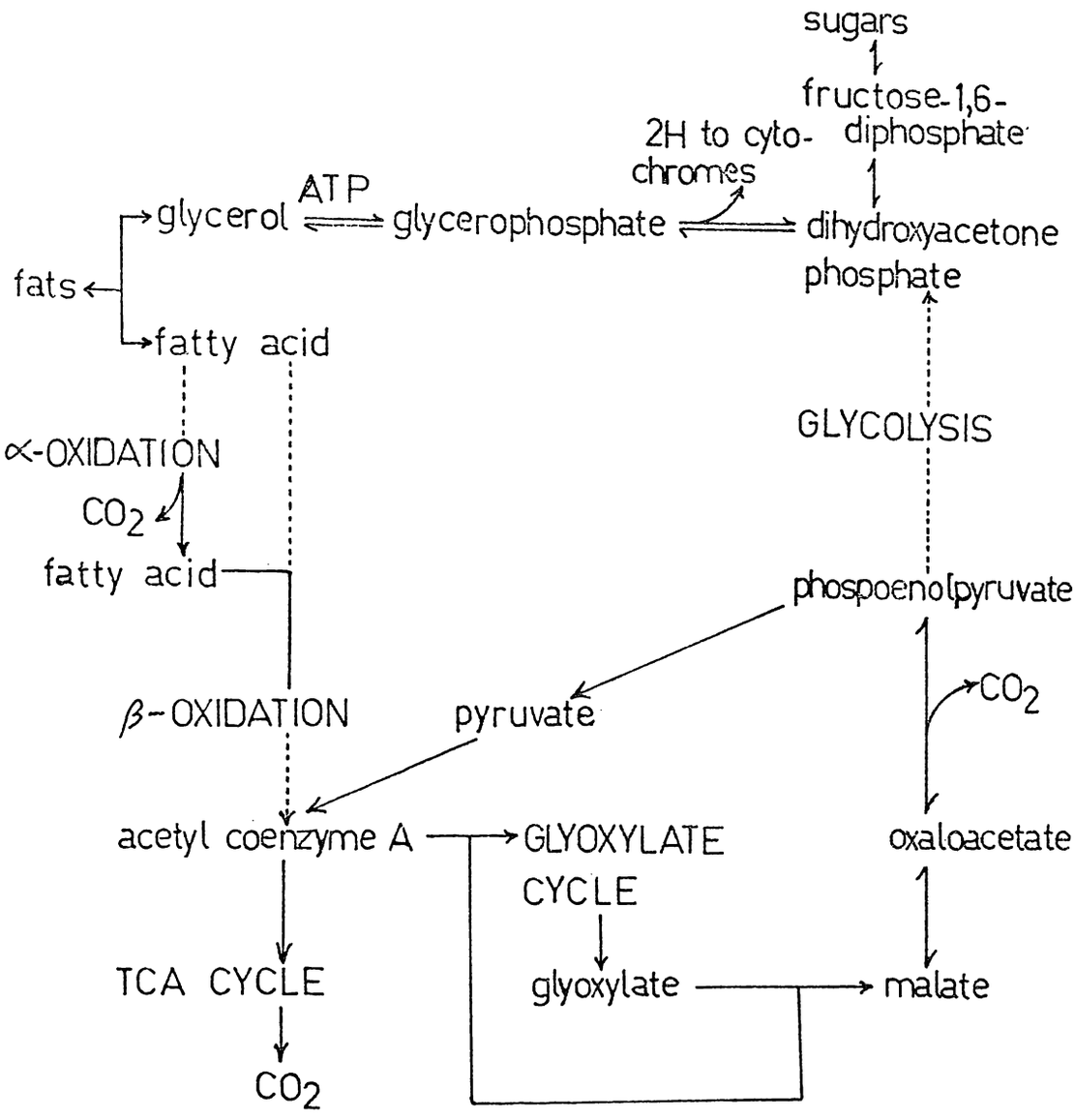
being linked to the cytochrome system. The equilibrium was found to greatly favour the production of dihydroxyacetone phosphate.[156]

Fatty acids labelled in the carboxyl group containing 2, 3, 4, 5, 8, 12, 16, 17 and 18 carbon atoms when incubated with peanut cotyledon, were found to form labelled carbon dioxide. The use of palmitic acid labelled at C_2, C_3, C_{11} and C_{14} formed labelled carbon dioxide readily, thus showing extensive degradation of the long chain. These oxidations required ATP, CoA, a TCA cycle acid, NAD, NADP, glutathione and Mn^{2+}.[157] The long chain fatty aldehyde dehydrogenase necessary for α-oxidation of fatty acids has been found in the mitochondria and microsomal fractions of the cotyledons from germinating peanut seeds.[47]

An extract obtained from an acetone powder of germinating pea seeds contained the enzyme, fatty acid peroxidase.[47] Cell free extracts of the cotyledons of germinating pea seeds can convert palmitic-1-C^{14} to $C^{14}O_2$ without the need for cofactors.[155] These results show the presence of an α-oxidation scheme for fatty acids.

The lipases of Douglas Fir seeds have been examined.[158] In the dry seed an acidic and a neutral lipase were found with similar activities. During the early stages of germination the activity of the acid lipase increased 7-fold and that of the neutral lipase, 4-fold.

During the early periods of germination the lipases were found in the embryo and after a considerable time-lag lipase activity of the endosperm increased. Fats disappeared from the embryo during the initial stages of germination but in the endosperm a store of fats remained until after germination, thus helping supplement the food resources obtained by photosynthesis in the young seedling. An outline of the major metabolic pathways of fat catabolism as thought to occur in germinating seeds is shown in Fig. 1-4.9.1.



MAJOR METABOLIC PATHWAYS OF FAT CATABOLISM

FIG. 1-4.9.1

1-4.10 The Metabolism of Phosphorus

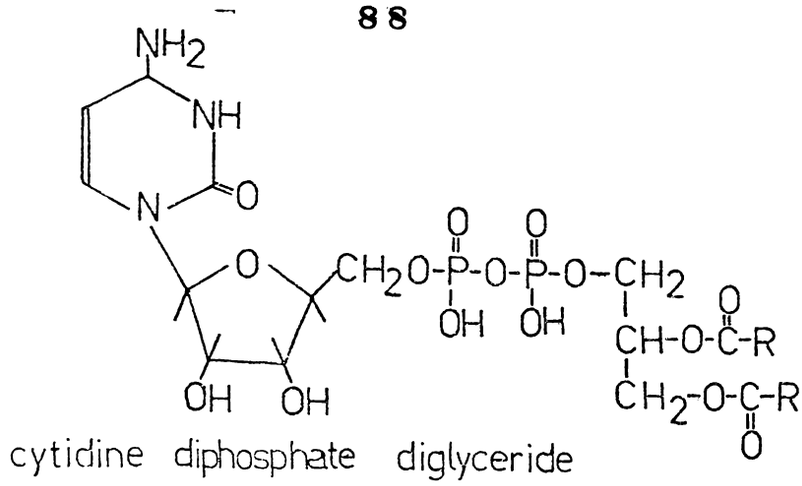
Phosphate plays an extremely important role in a variety of reactions in seeds. The formation of nucleic acids, nucleotides, phospholipids and of compounds in glycolysis, requires phosphate. Phosphorus is found in seeds mainly in the organic form. Very little seems to be present as inorganic orthophosphate among the phosphorus containing organic compounds which occur in seeds are the nucleic acids, nucleotides, nucleosides, phospholipids, sugar phosphates and phytin. The calcium and magnesium content of phytin is variable, e.g. in wheat 12% Ca and 15% Mg, while in oat 8.3% Ca, 15% Mg and 5.7% Mn. The absolute amount of phytin also varies between species and varieties, but it may constitute up to 80% of the total phosphorus content of the seed. Such large amounts of phytin will probably act as a store of inorganic phosphate which can be released during germination by the action of the enzyme phytase. This enzyme is not entirely specific and may hydrolyse other phosphate esters as well.[4]

The concentration of phytin in wheat embryos separated from the seed after 2 hours' soaking was 6 umole/100 embryos. This amount decreased during germination reaching half the initial concentration within the first 24 hours. The activity of the acid phytase present in wheat embryos increased rapidly for the first 4 hours and then more slowly. Between the 24th and 72nd hour its activity doubled. The rapid increase within the first 4 hours was due to activation of a zymogen. The increase in inorganic phosphate which occurred during this period accounted for only part of the disappearance of phytin and, hence, must have been rapidly converted to organic phosphorus. Addition of inorganic phosphorus to the incubation media was shown to inhibit the increase in activity of phylase.[56,57]

In castor bean a similar reaction has been shown to occur. Some phytin is mobilised during the first few hours of germination but phytin does not disappear completely[130] from the endosperm before germination has occurred.[70] An acid and an alkaline phosphatase have been found present and the activities of these increase rapidly during the first 24 hours of imbibition.[70]

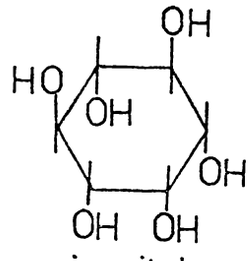
Similar rapid disappearance of phytin from germinating seeds has been observed in oats, peas, lettuce and barley.[4] In cotton seeds all the phytin was contained in the cotyledons whereas in some other seeds, e.g. oat phytin was also present in the embryo from which it disappeared rapidly during germination. Some phosphorus was transported to the embryo during the germination of oat.[4]

Phytase hydrolyses the phosphate ester bonds of inositol hexaphosphate forming orthophophosphate and inositol. Little work has been carried out to determine the fate of the inositol formed although a net loss of this compound has been shown to occur during germination of oat seeds.[172] It would be unprofitable for the seed to store phosphorus in this form unless the inositol, itself, can also be metabolised. In plants inositol phosphatides are common and have been found in the phospholipids of cell membranes.[47] Cytidine-diphosphate-diglyceride reacts with meso-inositol to form the phosphatide 1-phosphatidyl-L-mesoinositol as shown in Fig. 1-4.10. An enzyme, inositol oxygenase, so far found only in kidney, converts inositol to D-glucuronate by the addition of oxygen followed by the loss of water. Labelled inositol fed to rats rapidly gave rise to labelled glucose.[49] Inositol is thought to have a reserve function in the shark similar to that of glycogen in other species.[49]

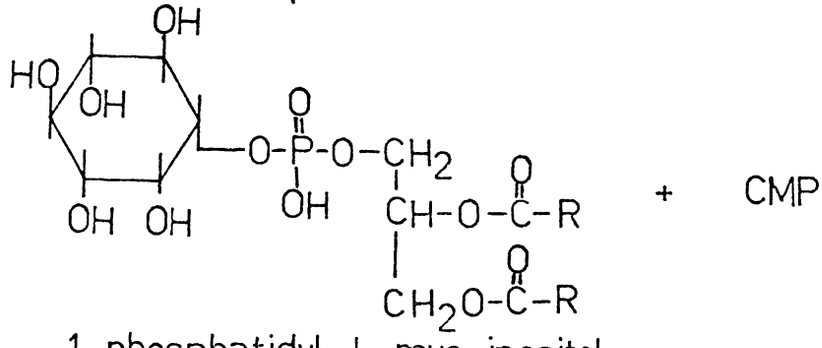


cytidine diphosphate diglyceride

+



inositol



1-phosphatidyl-L-myo-inositol

THE ESTERIFICATION OF INOSITOL

FIG 1-4.10.1

In contrast to the large amount of data available on phytin metabolism, other aspects of phosphorus metabolism are less clear. There does, however, seem to be an increase in phospholipids during germination.[4] When intact lettuce seeds were germinated in $NaH_2P^{32}O_4$ and analysed prior to radicle emergence the only labelled compounds detected were orthophosphate and ethanol-1-phosphate, the latter being an artefact produced during the extraction procedure. In contrast when punctured seeds were used, esterification of P^{32}-orthophosphate was detected within 3 hours after the beginning of imbibition, even though punctured seeds did not germinate more quickly than the controls. Of the P^{32} esters detected considerable activity corresponded to phosphorylcholine and phospholipids. Far-red light exposure in the presence or absence of gibberellic acid, kinetin or thiourea did not seem to alter the metabolism of P^{32}. These results suggest that the coats surrounding the embryo were impermeable to phosphate ions.

1-4.11 The Metabolism of Other Compounds

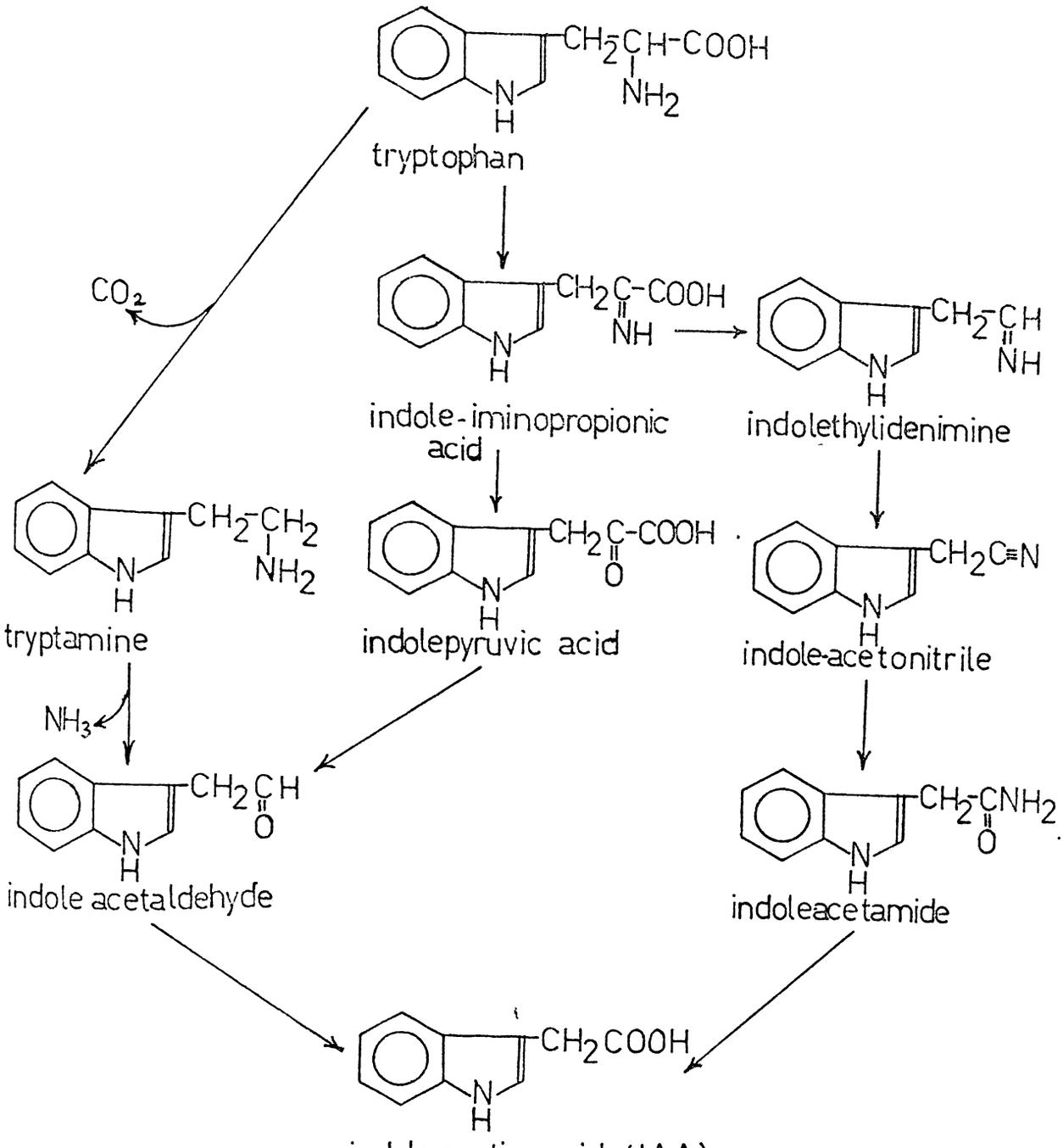
During the early period of germination of many seeds growth hormones are formed from precursors which are released from the storage cells.[38] In maize seeds the content of the auxin, indoleacetic acid (IAA), increased rapidly during the first 36 hours of imbibition, reaching a mean value of 363 ugm/100 seeds at 36 hours. From this period, until that of radicle emergence at about 48 hours a steady decline in content occurred. These changes were shown to be in the endosperm only, very little IAA was found in the embryo or scutellum at any stage of germination. No evidence for the existence of a growth inhibitor was found.[160] Similar experiments on germinating pea seeds showed that at all stages the IAA content was much lower than in maize. In the early

stages of germination it was extremely low and it increased very slowly as germination proceeded. In the earlier sampling times, however, an inhibitor of growth was detected. The concentration of this substance decreased during the early stages of germination and had completely disappeared within 48 hours, the time of radicle emergence.[160] An investigation of the auxins of wheat gave results similar to those obtained for maize. During the first 48 hours the free auxins increased and this was followed, after germination, by a decrease in content.[161] Gibberellic acid, labelled with tritium was incubated with dwarf pea seeds.[162] An acidic biologically active compound was formed, as well as several radioactive but biologically inactive neutral compounds.

Very little work has been done concerning the biosynthesis of IAA in seeds but the pathway could be similar to that for micro-organisms and some plants as shown in Fig. 1-4.11.1. It is of interest that several intermediates of this pathway (e.g. tryptamine) have been found in mature seeds.

The content of ascorbic acid in dry seeds is usually extremely low but this increases rapidly during the early stages of germination.[8] Ascorbic acid was not detected in the dry wheat grain but it increased during germination.[161] Ascorbic acid which is the gamma-lactone of a hexonic acid (i.e. a sugar acid) is formed in some animals from glucuronic acid via gulonic acid. Up to now this compound has been shown only to have a possible role in hydroxyproline formation and to be important in poising the redox potential of cell contents.

Intact lettuce seeds incubated with $Na_2S^{35}O_4$ yielded no labelled compounds prior to radicle protrusion. When punctured seeds were used the S^{35}-sulphate was metabolised within 3 hours of the beginning of imbibition. Numerous spots were found to be labelled but the only



POSSIBLE MECHANISM FOR THE BIOSYNTHESIS OF INDOLEACETIC ACID

FIG 1-4.11.1

compounds identified were cysteine and methionine. The authors concluded that the sulphate had been reduced to sulphydryl.[159]

An alcohol dehydrogenase causing the conversion of an alcohol to an aldehyde has been reported present in summer barley seeds. During germination it was found to disappear but it reappeared later in the young seedling.[117] Catalases which convert hydrogen peroxide to oxygen and water have been widely reported to occur in germinating seeds. Catalase was shown to increase in activity due to its synthesis during the germination of viable lupine seeds but to decrease during imbibition of non-viable lupine seeds.[95] It could possibly function as a means of producing oxygen close to the metabolic centres requiring it.

1-5 The Effects of Ionizing Radiations on Seeds

1-5.0 Introduction

The main aim of the science, Radiobiology, is to understand the steps, beginning with the absorption of energy and ending in death or injury. In order for ionizing radiation to act on a living or non-living system it must be absorbed and it is this absorbed energy which produces changes at the molecular level. All cell constituents including the macromolecules (e.g.DNA) and smaller molecules (e.g. ATP) can be detrimentally altered by such radiation. With very few exceptions the biological effects only become apparent some time after the irradiation. The length of the interval may vary from hours to decades. Studies of radiation effects on simplified systems, consisting of solid or aqueous solutions of organic compounds are essential for an understanding of the very early events in irradiated organisms.

1-5.0(a) Types of Radiation

The principle types of ionizing radiations are the x- and gamma-rays. These are electromagnetic waves of extremely short wavelength. The term gamma-ray is used when the radiations are given off by radioactive substances and the term X-ray when they are produced in special high-voltage equipment. Originally there was a distinction between the wavelengths of these two forms but with recent technical advances X-rays can now be produced with the shorter wavelengths typical of gamma-rays. The nuclei of most artificial radioactive nuclides emit gamma-rays and these are almost always accompanied by emissions of other kinds. Their energies vary considerably, ranging from 2 KeV to 2.76 MeV. The gamma and X-rays are the most penetrating of all radiations.

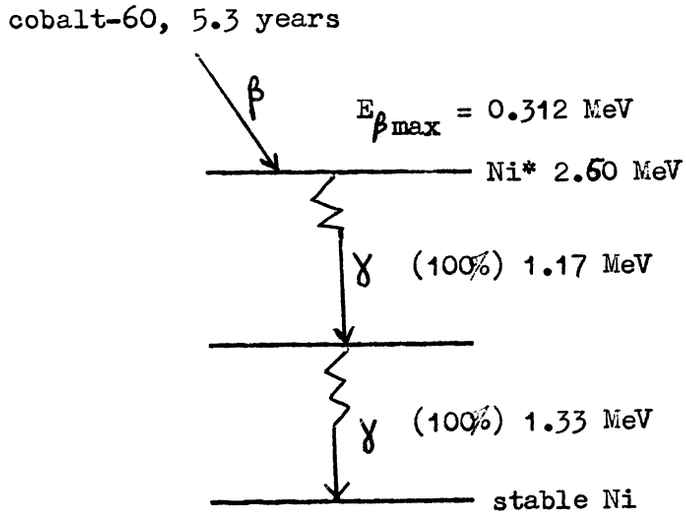
Beta radiation consists of a stream of very fast electrons. The energy of the β particles covers a wide range extending from 18 KeV (H_1^3) to 4.81 MeV (Cl^{38}). Due to their low mass their velocity is very high; between 90 and 98% of the speed of light. Unlike gamma radiation, which has a spectrum of rays with discrete energy values, beta-radiation has a continuous spectrum.

Alpha particles are more characteristic of heavy radioisotopes from Z = 82 upwards. These particles have a charge of +2 and consist of 2 protons and 2 neutrons. Their energy normally lies between 4 and 9 MeV and their velocity at emission is of the order of 2×10^8 cm/sec. The range in air amounts to no more than a few centimetres and alpha particles can be completely absorbed by a thin metal shield or even a sheet of paper.

All of these radiations are referred to as "ionizing radiations" in contradistinction to the longer wavelength radiations of infra-red,

visible or ultraviolet light. The biological effects of ionizing radiations are similar and often identical as they cause the removal of electrons from atoms in the material through which they are passed.

The radiation used for producing non-viable seeds in this thesis consisted of the gamma rays from a cobalt-60 source. The decay scheme[174] of this radioisotope is shown below.



1-5.0(b) Units of Dose

The unit of radioactivity is the "Curie" which corresponds approximately to the activity of a gram of radium. It is the amount of a radioisotope which decays at the rate of 3.7×10^{10} disintegrations per second.

The radiation dose actually received by the irradiated body is described in terms of the ionization produced. The oldest unit used to measure dosage was defined in terms of the ionization produced in air and is called the "roentgen", abbreviated to "r". The roentgen is defined as the quantity of X or gamma radiation producing 1 electrostatic unit of ions of either charge per 0.01293 gms of air which

occupies one ml at 0°C and 760 mm of Hg pressure. Assuming an average energy loss of 32.5 electron-volts per ion pair in breaking a bond an alternative definition is that 1r is the quantity of X- or gamma radiation which loses 83.4 ergs per gram of air. The value of 32.5 eV appears to be constant for all substances,[175] although it is far above the dissociation energy of most bonds. The extra energy appears as kinetic energy of the ions formed and electronic excitation within the ions formed.

To extend this definition to other types of radiation the following units have been used:

1 rep = (roentgen equivalent physical) - that quantity of radiation of any type, producing energy losses of 83.4 ergs/gm in water or tissue.

1 rem - (roentgen equivalent man) - that quantity of radiation of any type producing effects in man equivalent to 1 r of X or gamma radiation. This unit depends on the specific effect in man used as the criterion.

1 reb = (roentgen equivalent biological) - same as 1 rem except animals or plants may be used instead of man.

1 rad = that quantity of radiation of any type producing energy losses of 100 ergs/gm of absorbing material.

The ratio of the rem to the r is often called the relative biological effectiveness (RBE). For X and gamma rays the RBE is equal to unity.

A common quantitative measure of the efficiency of a radiation-chemical effect is the number of molecules destroyed or produced for each 100 eV of energy absorbed. This is called the G-value for the

reaction. The energy deposited per rad in 1 ml of water is about 6.25×10^{13} eV per ml of water solution.

1-5.1 Radiation Chemistry

Radiation chemistry may be defined as the study of the chemical effects produced by the absorption of ionizing radiations.

In dilute aqueous systems most of the radiation energy will be absorbed by the solvent, water, and only a relatively small part by the solute. Therefore in the case of aqueous systems the majority of the primary active species is produced by the action of the radiation on the water and the radiation chemical processes are then due to the chemical reactions of these active species with the substances present in the aqueous system. This is the basis of what is called the "indirect effect" in contradistinction to the "direct" effect to which, in this case, some of the water molecules are subjected.

In water hydrogen atoms and hydroxyl radicals are formed by the action of ionizing radiations.

$$H_2O \rightsquigarrow H + OH^{\bullet}$$

Ionizations can also be caused:

$$H_2O \rightsquigarrow (H_2O)^{+} + e^{-}_{(aq)}$$

$$e^{-}_{(aq)} + H_2O \longrightarrow (H_2O)^{-}$$

These ions formed can be involved in both diassociations and recombinations, the latter especially when a solution of pure water is irradiated.

The disassociations which can occur are as follows:

$$(H_2O)^- \longrightarrow H + OH^-$$
$$(H_2O)^+ \longrightarrow OH\cdot + H^+$$

Both H atoms and OH radicals are very reactive. The hydroxyl radicals are very strong oxidizing agents, i.e. they can readily accept electrons forming hydroxyl ions e.g.

$$Fe^{2+} + OH\cdot \longrightarrow Fe^{3+} + OH^-$$

They can also dehydrogenate a molecule (RH) to form water:

$$RH + OH\cdot \longrightarrow R\cdot + H_2O$$

Hydrogen atoms are generally strong reducing agents as they are capable of donating an electron to form hydrogen ions. However, under certain conditions they can also act as oxidizing agents, as by the dehydrogenation reaction:

$$RH + H \longrightarrow R\cdot + H_2$$

The trapped electrons formed by the ionizations also act as reducing agents and have been shown to react in particular with gases dissolved in water.

$$O_2 + e^-_{aq} \longrightarrow O_2^-$$
$$O_2^- + H^+ \rightleftharpoons HO_2$$
$$2HO_2 \longrightarrow H_2O_2 + O_2$$
$$CO_2 + e^-_{aq} \longrightarrow CO_2^-$$

The hydrogen peroxide formed with oxygen and the CO_2^- radical ion formed with carbon dioxide can both react with any other solutes present. In the case of the latter it can react with organic molecules to form carboxylic acids.

In attempts to understand the biological effects of ionizing radiation many studies have been made of the effects on substances either in the solid state or in aqueous solutions. An examination of synthetic polymers in the dry state showed that ionization of the polymer, the direct effect, led to cross-linking and the scission of bonds along the chain. In dilute aqueous solution the polymer reacts with the free radicals formed by the water but in general this led to exactly the same effect on the polymer, i.e. crosslinking and scission. In both cases small molecules such as H_2 or NH_3 were often eliminated but the change in weight was negligible. On irradiation of polyethylene crosslinking was the predominant result but with polyisobutylene only scission occurred. This was shown to be due to the fact that a carbon atom linked to four other carbon atoms was the weak point in the chain and scission occurred at this point. Polymers containing aromatic rings showed added stability due to the aromaticity of the rings.[175]

When cysteine was irradiated hydrogen peroxide and hydrogen sulphide were the main products. No ammonia, carbon dioxide or carbon monoxide were formed.[175,178] The irradiation of other amino acids or proteins caused the formation of NH_3, CO_2 and CO. The proteins stabilised the free radicals in cysteine residues so that virtually no hydrogen sulphide was eliminated.[175] The <u>in vitro</u> irradiation of proteins does not reveal a very high radiosensitivity. If the change in enzyme activity is measured large doses of radiation are required for effect (see Table 1-5.1.1). Dry powdered ribonuclease and adenyl pyrophosphatase required respectively 3.4×10^7 and 5.5×10^6 rads of γ-rays for inactivation.[179] When soft X-rays were used to irradiate thin films of several enzymes 37% of their activities remained after receiving a dose of 3×10^6 rads.[180]

Complexes of enzyme-substrate or enzyme-inhibitor appeared to be more sensitive than the enzyme on its own. When dried films of these

Table 1-5.1.1

Radiation Effects on Proteins *in Vitro*[82]

Protein	Effect Measured	G value
Albumin	Thiol destruction	3
Pepsin	Production of carbonyl groups	1.2
Cytochrome c	Oxidation	1.62
Glyceraldehyde-3-dehydrogenase	(i) Thiol destruction	0.23
	(ii) Inactivation	0.06
Carboxypeptidase	Inactivation	0.55
D-amino acid oxidase	Inactivation	0.31
Ribonuclease	Inactivation	0.09
Trypsin	Inactivation	0.077
Hexokinase	Inactivation	0.033
Lysozyme	Inactivation	0.03
Catalase	Inactivation	0.009
Alcohol dehydrogenase	Inactivation	0.06
Aldolase	Inactivation	0.03
Chymotrypsin	Inactivation	0.14
Pepsin	Inactivation	0.006
DNAase	Inactivation	0.25

Table 1-5.1.2

Radiation Effects on DNA *in Vitro*[82]

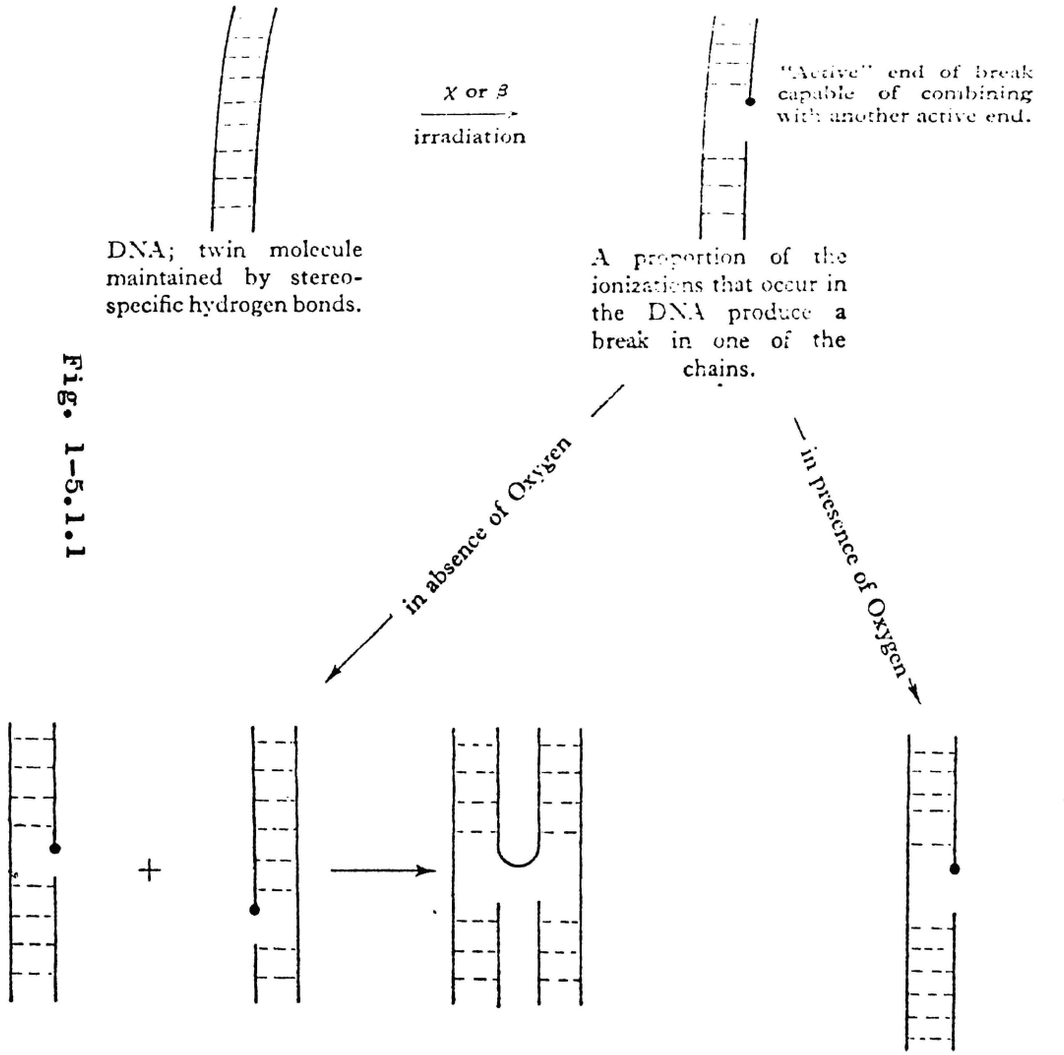
Radiation Effect	G value
Sugar phosphate rupture	0.45
Double strand breaks	0.12
Base alteration	2.0
Cross linking	0.08
Hydroperoxide formation	1.0
Single strand breaks	2 - 10
Decreased molecular weight	0.14
Hydrogen bond breakage	50 - 60
Release of inorganic phosphate	0.09
Release of ammonia	0.47

complexes were irradiated it was shown that one ionization occurring at any point whatsoever in the complex led to inactivation. This is not true for most pure proteins.

Irradiation of DNA can produce marked structural and functional changes. The changes that have been investigated are shown in Table 1-5.1.2 and possible alterations are illustrated in Fig. 1-5.1.1. Every molecule of DNA present in a cell seems to have a unique biological function and so these irradiation effects could be extremely important *in vivo*. DNA containing a greater proportion of the bases guanine and cytosine are less radiosensitive than those containing a greater proportion of adenine and thymine.[181] The polymerization of both ribomononucleotides and deoxyribomononucleotides has been shown to occur on irradiation in aqueous solution using doses up to 18.0×10^4 rads of gamma rays.[182]

All polymeric carbohydrates which have been studied are degraded by excess radiation. Cellulose forms sugars at high doses above 50 Krads. Dextran suffers a reduction in viscosity at 0.3 megarads and gives rise to glucose, gluconic acid, glucuronic acid and other products of low molecular weight.[177] Doses of 5 megarads and above are needed before appreciable amounts of sugars are formed as degradation products of starch.[183,184,185] Such degradation causes a decrease in the pH of the solution.[185]

In vitro unsaturated fatty acids are extremely radiosensitive, being oxidized to form hydroperoxides. Hydroperoxides formed from lipids are more stable both *in vitro* and *in vivo* than hydrogen peroxide. If vegetable oils are irradiated under vacuum or a nitrogen atmosphere peroxides are not formed.[187] Vegetable fats exposed to a dose of 5 megarads under vacuum formed very few radiolytic products. These were essentially the hydrocarbons containing one or two fewer carbon atoms than the original fatty acids.[186]



DNA; twin molecule maintained by stereo-specific hydrogen bonds.

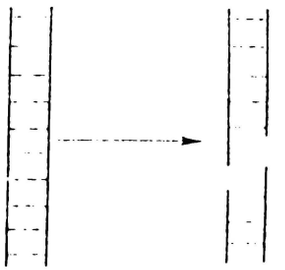
A proportion of the ionizations that occur in the DNA produce a break in one of the chains.

Two molecules join if the active ends can meet. In DNA fibres swollen with water the cross-linking efficiency is higher because the molecules are more mobile.

"Active" end becomes peroxidized and is no longer capable of forming a crosslink. (Rate of diffusion of oxygen into dry DNA is slow and "active" ends persist for many days because lack of movement prevents crosslinking.)

Fig. 1-5.1.1

(b) Main-chain scission



This occurs when there is a break in each of the adjacent chains less than about 5 nucleotide units apart.

This is produced by radiation:

1. Every time a DNA molecule is traversed by an α-particle* (600 eV double break).
2. When a cluster of ionizations (or other high energy event) is formed by sparsely ionizing radiations (850 eV double break).
3. When by chance two isolated breaks come into juxtaposition. From statistical calculation one "double break" will occur for every 70 random single breaks. This mechanism is responsible for main-chain scission by the indirect action of H and OH radicals formed in the water

* Some crosslinks are produced at the same time as main-chain scission by α-rays due to the relatively sparsely ionizing δ-rays.

CHANGES PRODUCED IN THE MACROMOLECULAR STRUCTURE OF DNA BY THE DIRECT ACTION OF IONIZING RADIATIONS

A dose of 4.8×10^5 rads of Co^{60} causes destruction of 70% of Vit. A, 37% of Vit. B_2, 100% of Vit. C, 61% of Vit. E and 40% of carotene pigments.[187] In the cases of conjugated proteins, e.g. hemoprotein the conjugated group appears to play the role of a "sink" for absorbed energy giving conjugated proteins greater stability than the associated protein alone when exposed to ionizing radiations.[188]

The G-values for small molecules tend to be higher than those for the macromolecules e.g. NADH 1.7, EtOH 5.9, Coenzyme A 9.2 and glutathione 10.7.

One cannot readily assume that radiochemical mechanisms functional in dilute or relatively pure solutions are also operative in complex biological systems. Such an approach can, however, suggest molecular events underlying observed radiation effects.

1-5.2 Biochemistry of Ionizing Radiation

1-5.2(a) The Initial Chemical Lesion

When ionizing radiation enters living cells it deposits its energy as discrete bundles of ionization along its track. Damage to the organism may arise from (a) the direct action which is molecular damage occurring in the molecule which absorbed the energy, and (b) the indirect damage arising from the formation from water of highly reactive free radicals which react with other cell constituents.

A dose of 100 rad will kill many mammalian cells but such a dose will only produce approximately 1000 chemical reactions within the volume of a cell nucleus.[191] To a first approximation these reactions will be shared equally amongst all the cell constituents and although 100 rads will inactivate a few molecules of some important enzymes, the fraction

of the total activity of any one enzyme destroyed is extremely small. In a bacteria cell a dose of 1000 rads has been estimated to cause radiochemical change to only 600 out of a total number of 4.7 million protein molecules.[192] Assuming a reasonable degree of proportionality a dose of 1 Mrad would cause approximately 6×10^5 intracellular changes but each molecule is likely to be hit more than once and even such a large number of events would not be easily detectable among the many million molecules in the cell. Hevesy and Forsberg[193] have calculated that a dose of 2×10^6 rads (i.e. 2 Mrad) could result in breaking only 0.003% of the bonds in an organism. They used the value of 25 eV per C-H bond broken but other authors[194] have shown that for DNA in particular 60 eV are needed to cause one break. One Megarad of γ-rays is equivalent to 10^8 ergs/gm of tissue and will give rise to approximately 10^{18} ionizations per cc of tissue. Using this data and the volume of the species it is possible to calculate the likely effect on certain structures.[195,196] These are illustrated in Table 1-5.2.1 for a dose of 1 Megarad.

Table 1-5.2.1

Effect of 1 Megarad of γ-rays on Various Species[195,196]

Species	Volume	No.of Ionizations per unit volume
Enzyme	10^{-19} cc	0.1
Virus	3×10^{-18} cc	3
Bacteria	10^{-12} cc	10^6
Peach cell	1.44×10^6 u^3	1.3×10^{12}
Mitochondrian	4 u^3	7×10^6
Section cell wall (1x2 u)	4 u^3	7×10^6
Galactose molecule	4 A^3	7×10^{-6}

As can be seen in the table, the chance of any particular cell being hit is extremely high, but the probability of any particular enzyme or galactose molecule within that cell being hit is extremely small and in the latter case is negligible. The only type of substance which could in fact be the primary lesion is one for which every molecule is essential to the cell. There are very few, if any, enzymes which fill this role. Every molecule of DNA does, however, appear to have a unique function and hence DNA could be the substance involved in the primary lesion.

The primary lesion need not involve the inactivation of a vital cell constituent. It is possible that radiation produces a new substance which is extremely poisonous or that radiation breaks down barriers within the cell and the resultant disorganization leads to cellular destruction.

The poison theory is generally dismissed and very few experiments have been carried out to test this theory. Certain nutrient media for bacteria cultures are rendered toxic by irradiation but only at doses considerably greater than those required to kill bacteria.[197] The viability[198] and rate of respiration of sea-urchin sperms is reduced when sea water is heavily irradiated, but if the organisms were present during irradiation the effect was much more marked. However, in 1968 the U.S. Food and Drug Administration turned down an American Army application for approval of irradiation sterilization of canned ham. The test data indicated that "a diet of 35% irradiated food was associated with higher infant mortality, adverse effects on body weight and red blood cell counts and an increased incidence of cataracts and tumours."[199]

The permeability of membrane structures, consisting of both lipid and protein, would be expected to be affected by ionization occurring

within its molecule. The permeability of skin is altered within a second or so of irradiation [200,201] and hence would appear to be an immediate effect of irradiation. An immediate change in the permeability to metal ions by plant membranes (Phaseolus aureus) has been shown to occur on irradiation.[202] The enzyme DNA-ase exists in the mitochondria and must cross the nuclear membrane before it can function on its substrate. An increase in the activity of DNA-ase generally occurs on irradiation. Certain proteolytic enzymes, the cathepsins, are released from mitochondria by doses of less than 1000 rads.[191]

Free radicals can be detected, using ESR spectra, in irradiated seeds. The free radicals in the embryo of barley disappear rapidly after irradiation but those in the endosperm show a high degree of stability.[203]

1-5.2(b) Respiration

The respiration of dry dormant barley seeds is not affected by 2,500 rads of X-rays. Doses of 5,000 to 15,000 rads stop, after 4-5 days only, the normal increase in oxygen consumption shown by controls.[204] A decrease in oxygen uptake and an increase in the R.Q. occurred when seeds of corn, wheat, sorghum and radish were irradiated. The authors[205] thought the increase in R.Q. was due to increased activity of glutamic acid decarboxylase. One of the most readily discerned direct effects of radiation on fruits and vegetables is the increased respiration which continues for several days after exposure.[196] The rise in CO_2 evolution was immediate upon exposure to radiation and increased with dose. When the author[196] examined the mitochondrial fraction he noticed a reduction in the amounts of mitochondria which could be isolated from

the fruit tissue. Mitochondria isolated from pears which received 0.25 and 1 Mrad were still capable of metabolizing the TCA cycle substrates, succinic and alpha-ketoglutaric acid.[206] At such doses the probability is infinitesimal that any one mitochondrion may escape an ionization event. Irradiated potato tubers also show an increased rate of respiration and this has been interpreted as being due to an uncoupling of oxidative phosphorylation.

Wheat seeds which had been exposed to 0.8 Mrads of Co^{60} gamma-rays before germination gave small seedlings without cell division or DNA synthesis, but which could fix carbon from carbon dioxide into sugar-phosphates, sucrose, amino acids and aliphatic acids.[217] The incorporation of C^{14}-labelled acetate, alpha-ketoglutarate, citrate and glucose into the pericarp of tomatoes which had received a dose of 0.2 Megarads was examined.[218] The irradiated fruit liberated $C^{14}O_2$ from each of the acids more readily than the non-irradiated control. There was no overall increase in the liberation of $C^{14}O_2$ from glucose.

Within a period after irradiation equal to their normal division cycle bacteria showed no drop in respiration after exposure to 0.6 Mrads. Only 1 in 10,000 of these bacteria continued to grow and multiply, the remainder died after passing through one or more divisions. Amoeba react to ionizing radiations by assuming a spherical shape but their respiration was unchanged by 0.1 Mrad.[207]

1-5.2(c) Carbohydrate Metabolism

Decreased absorption of glucose and other sugars results from total or abdominal irradiation of rats and has been shown to occur after as little as 50 rads.[208] Farrer et al[209] showed that glucose and fructose absorptions 4 hours after a dose of 1410 rads were slightly but significantly reduced, the effect being more marked with xylose

and showing a further fall with all three sugars after 20 hours. A dose of 0.1 Mrads, which caused death within 3½ hours, depleted the glycogen reserves of rat liver.[210]

The steady state concentrations of metabolites in Yoshida ascites cells, which received 1-45 Krad of radiation, have been measured. A slight increase in ADP, a large increase in dihydroxyacetone phosphate and a large increase in fructose-1,6-diphosphate were found. In contrast a decrease in ATP, inorganic phosphate and pyruvate occurred. The concentration of the co-factor NAD was found to be considerably decreased after irradiation.[211]

1-5.2(d) Fat Metabolism

In vitro, unsaturated fatty acids are extremely radiosensitive but the presence of alpha-tocopherol (Vitamin E), which is a powerful anti-oxidant, diminishes the in vivo sensitivity.

The fatty acids of wheat leaves were analysed following doses of 5 to 60Krad.[196] The amount of peroxides increased proportionally with the dose. The peroxide derivatives of fatty acids could be physiologically active as uncouplers of oxidative phosphorylation. Exposure of excised bean hypocotyl segments to a dose of 0.8 Mrad reduced the amount of uncoupling observed using DNP. The authors concluded that irradiation had caused uncoupling of oxidative phosphorylation.[216] Increases in the peroxides of irradiated rat skin,[212] fat of mice[213] and fat of rats[214] have also been observed. These peroxides have been shown to readily oxidize the -SH groups of proteins.[215]

It is thought that ascorbic acid might confer the same anti-oxidant properties in aqueous solutions as does tocopherol in fat. In plants exposed to continuous non-lethal irradiation (5 Krad per day)

the ascorbic acid content falls for the first 2 days and then increases. A relationship between high ascorbic acid content and radioresistance of green plants could exist. Cabbage and gladiolus, which are highly resistant, contain 200-400 mg of ascorbic acid per 100 gm fresh weight whereas Cosmos sulfurens, Nicotiana rustica and Hyoscyamus niger which are radiosensitive contain only 3-100 mg of ascorbic acid. Vicia faba is, however, sensitive to radiation but contains a high proportion of ascorbic acid.[191]

1-5.2(e) Protein and Amino Acid Metabolism

In general, in rats great protein destruction occurs after doses of 400-1000 rads but at the same time the incorporation of amino acids into protein is also enhanced.[191]

One day old seedlings of Cicer arientinum were treated with 450 rads at 30 rads/min from an X-ray source and then kept at 24°C for one hour. An increase in the concentration of alanine was found, the combined content of glutamate and threonine also rose markedly but the concentration of GABA doubled.[219] An increase in GABA (15-16%) has also been shown to occur in rats following irradiation.[220] This increase was not, however, accompanied by an increase in the activity of glutamate decarboxylase.

Disturbances in transamination have been described. The activity of the glutamate-oxaloacetate transaminase was reduced by 95% 4-5 days after irradiation of rats. Pyridoxine, the co-enzyme was not affected in vivo.[191]

1-5.2(f) Sulfhydryl Enzymes and Proteins

In vitro experiments have shown on irradiation the rapid oxidation of the -SH groups of cysteine, inactivation of -SH enzymes and the almost complete recovery of the latter after the addition of cysteine or glutathione.[221,222] Many sulphydryl compounds have been shown to confer protection against irradiation both in vivo and in vitro. Barron[221,222] put forward a theory that the fundamental lesion produced by ionizing radiations in vivo is the oxidation of the -SH groups of the intracellular enzymes and their consequent inactivation. Many workers have shown that sulfhydryl groups are extremely important during mitosis and embryonic development. Haber and Luippold[223] showed that chronically irradiated lettuce seeds could germinate by cell elongation only and not by mitosis as occurred in non-irradiated seeds.

This theory cannot, however, be accepted as a whole. Sulfhydryl compounds will also protect synthetic polymers, not containing -SH groups, in vitro.[175] If an -SH group were oxidised to -S-S- or $-SO_2$ an immediate fall in reduced glutathione and in total -SH groups in the blood would be expected upon irradiation but this does not occur.[191] Gordon[224] has shown that no decrease in the total -SH content of Avena embryos occurs immediately after receiving a lethal dose of radiation. It has been pointed out that if every free radical produced by 700 rads (a lethal dose) of whole-body irradiation of a mouse were used to oxidize only -SH groups (e.g. cysteine) the quantity of cysteine oxidized would be of the order of 25 ug, leaving a much greater quantity unaffected.[225]

Most observations showing a decrease in sulfhydryl groups were made long after irradiation, and the decrease does not precede, but follows the development of anatomical lesions.[191]

1-5.2(g) Enzyme Activities

Increased enzyme activity observed in vivo after irradiation may arise from four different factors. It may be due to (a) destruction of an inhibitor, (b) to the release of an activator, (c) to increased synthesis and (d) to the release of a bound (inactive) enzyme from its link with intracellular structures (nucleus, mitochondria, microsomes or membrane). Decreased activity may result from destruction of the enzyme, the failure of its synthesis, the release of an inhibitor or to the disappearance of an activator.

A stimulating effect on growth has been observed for many seeds and plants after low doses of radiation. These will be discussed more fully in a later section. An increase in mitochondrial cytochrome oxidase but no changes in peroxidase or polyphenyl oxidase after irradiation of potato tubers with 10 Krads.[196] The activity of polyphenyl oxidase in leaf homogenates was unaffected by doses as high as 1 Mrad.[226] The authors suggested that the radiation sensitivity of similar enzyme systems in whole plant systems could be the results of permeability changes rather than direct effects on enzymes.

A marked increase in the activity of phenylalanine ammonia-lyase has been observed in citrus fruit peel after a dose of 0.2 Mrad. The maximum activity occurred one day after irradiation and then decreased to control values in fruit not showing visible radiation damage but remained high in those which did show visible damage.[227] A dose of 3.2 Mrad did not affect the activity of cytochrome oxidase in the potato tuber but the activity of tyrosinase was decreased by 50 percent.[224] When Bacillus subtilis cells or lysates were exposed to 0.14 Mrad there was no measurable effect on the activity of cytochrome oxidase or succinate oxidase.[228]

The biochemical changes during growth of irradiated wheat seeds have been investigated.[229] After 16 hour imbibition no detectable change in enzyme activity was found and the level of free amino acids was unchanged. However, the rate of increase of ∝-amylase and RNAase which occurs after 2-3 days in the controls, was found to decrease with increasing dose. The increase in the activity of 3'-nucleotidase was not so greatly affected.[229] The dehydrogenase activities of Irish grown wheat[230] decreased to about 60% of the control by 1 Mrad and to about 40% of the control by 3 Mrads. The amount of protein increased with increasing dose.[230]

The intracellular yeast catalase is activated by X- or gamma-rays possibly due to the rupture of its link with RNA.[231] Catalase has aroused considerable interest as it is the enzyme necessary for the breakdown of H_2O_2 which is highly toxic and is formed on the irradiation of water. In irradiated wheat seed (80 Krad), however, the activities of catalase, peroxidase and isocitric dehydrogenase decreased on germination.

The extreme radiosensitivity of auxin has been demonstrated in many plant systems.[196,224,233,234] The inhibition of the synthesis of auxin from tryptophan was found to increase exponentially with dose, 10 rads causing 11% inhibition and 25 rads 30%.[234] Quantities of indoleacetaldehyde have been shown to accumulate after irradiation and it is the conversion of this compound to IAA which is blocked by irradiation. Gordon[224] postulated that the magnitude of the irradiation effect implied an interference at the DNA-RNA transcription level.

1-5.2(h) Nucleic Acids

DNA biogenesis shows an immediate sensitivity to low radiation doses. This has been demonstrated by the radioautographic studies of Howard and Pelc[235] on root cells of Vicia faba and also in the more

extensive isotopic work on animals.[191] Although the inhibition of DNA synthesis can be detected in the animal within an hour, the time of the first perceptible plant response has not been precisely established. It is probable that the inhibition occurs very rapidly after irradiation. Synthesis of DNA takes place during the Interphase in meristematic cells. Such cells cannot replicate DNA after irradiation and their mitotic activity is delayed. When Vicia faba root tips are exposed to any sublethal dose (LD_{50} is 400 rads) the proportion of cells in mitosis declines with increasing time after irradiation but later recovers, and may show a compensatory overshoot, until it returns again to the control value. A lethal dose of 1600 rads causes decreasing mitotic activity with time. After 5 or 6 days most of the roots show no mitosis at all. The volume of the cells increases continually until at 5 days post-irradiation the average nuclear volume is twice that in control roots.[236] Such cells are destined to die and do not undergo further mitosis. In germinating lettuce seeds the beginnings of cell division and cell expansion coincide in time but in gamma-irradiated seeds (0.6 Mrad) germination can occur without cell division which occurs 3-4 days later.[223] A similar effect has been observed in the embryos from wheat grain which had received a dose of 0.5 Mrad.[237,217] Post irradiation soaking of seeds in a solution of ATP reduces the amount of mitotic damage.[191]

Irradiated plants showed complete inhibition of the synthesis of t-RNA at doses above 300 rads.[239] One day old seedlings exposed to doses up to 62 Krad of X-rays did not exhibit the rapid increases, during growth, of RNA, DNA, protein and acid soluble nucleotides.[240] Radiation levels of 250 and 500 Krad caused stimulation of nucleic acid synthesis of the cotyledons of peanut seeds when the seeds were germinated immediately after exposure. After storage of the seeds for 2-4 weeks all radiation doses inhibitied nucleic acid formation during germination.[241]

RNA synthesis is less sensitive to ionizing radiations in vivo than is DNA synthesis. Liver, kidney and pancreas all show unaffected biosynthesis of RNA after irradiation but the spleen, appendix, thymus and bone marrow show depressed synthesis.[191]

Total body irradiation (600 rads) increased the total activity of the acid RNAase of the liver, spleen and thymus; and also changed the relative activity of the various fractions isolated from these tissues. A great loss of enzyme activity occurred from the particulate fraction and a corresponding gain occurred in the supernatant. The shift from structure-bound enzyme to free enzyme agrees with the hypothesis that enzyme release is one of the main effects of ionizing radiation.[238]

The activity of DNAase in the homogenates of radiosensitive tissues such as spleen, thymus and bone-marrow, is increased after irradiation. The activity of the DNAases increases in the serum 18 hours after irradiation and urinary excretion of these enzymes is three times the normal level.[191]

The origin of the activation of nucleases is possibly the breakdown of granules to which the enzymes had been bound, allowing contact between the nucleases and their substrates. A marked synthesis could also occur. The nucleases are extremely important as the fate of the nucleic acids, after irradiation, depends to a certain extent on these enzymes.

1-5.2(i) Metabolism of Other Compounds

Beta-carotene is more stable in water than fat solvents. The presence of sugars can also increase its stability to radiation. A dose of 1 Mrad destroys 38% of the carotenoids in orange juice and 24% of those in mango pulp.[242]

Chloroplasts and their associated enzymes have been the subject of several investigations utilizing ionizing radiation. Gailey and

Tolbert[243] reported that 50 and 150 Krad delayed chlorophyll synthesis in etiolated plants subsequently exposed to light. The inhibition of chlorophyll biosynthesis has also been reported in irradiated potato tubers. The effect was proportional to doses of 10 Krad and higher.[196]

1-5.3 The Stimulation of Growth

The stimulation of the growth of various organisms as a result of small doses of radiation have been reported.

Accelerated germination of X-radiated seeds was first reported by Maldiney and Thouvenin in 1898. In 1919 the stimulation of the germination of rice seed was reported but this claim has not been substantiated.[246] Germination of the peach, Prunus persica, is improved following irradiation. Doses of 2.5 to 20 Krad cause acceleration of germination in Oenothera bertieriana.[244] Low doses of gamma-radiation resulted in an apparent radiostimulatory effect on the germination of conifer species.[245] This occurred after exposures up to 250 rads for white pine seeds, up to 1,500 rads for Jack pine and up to 3,400 rads for Black spruce. Exposures below 500 rads stimulated the germination of Scotch pine, White spruce and Red pine seeds.[245]

Seeds of peas, vetch and lupine exposed to a dose of 50 and 150 rads gave a 10-15% increase in the plant yield, compared to the non-irradiated controls.[247] The plants from these irradiated seeds had an elevated nitrogen and a reduced carbohydrate content. Doses of 750-1000 rads for rye, 350-500 rads for pea, 500-1000 rads for radish, 1000-2000 rads for cabbage and 100-300 rads for cucumber seeds led to increased yields. A dose of 1000 rads gave the maximum root length and a dose of 750 rads gave the maximum root diameter. This increase in root diameter was due to an increased number of cells, not to an increase in cell size.[248] Heavily irradiated plants of Kalanchoe gave plants

containing larger, broader and heavier leaves than those of the controls. Much of this increase also resulted from the production of more cells rather than larger cells.[249]

Chronic irradiation of rye seeds, giving a total dose of 1000 rads over a period of 27 days resulted in an increased yield of 20%. Seed irradiation of legumes not only increased the yields by as much as 20-30% but also promoted the time of flowering.[246] Long and Kersten[250] treated seeds of soya bean, Glycine max, with a range of doses of X-rays. Unlike most authors they report the full details of a planned statistical analysis of the growth of these plants. They found a stimulation of over three times the probable error of control but the order of magnitude was only 3%. Bilquez[251] treated seeds of radish with 750, 1020 and 1275 rads of X-rays. A stimulation of 20% of the fresh weight of the roots was found significant at the 5% level of probability.

The stimulatory effects of ionizing radiations were investigated for seeds (Helianthus and Fagopyrum), plants (Fagopyrum) and leaf discs Nicotiana and Phaseolus). The authors[252] used over 1600 plants and made 28,000 individual measurements. Small but significant growth increases were produced in certain instances but these results were not always reproducible from one experiment to another. Also, when increases did occur, they were not always produced by the same exposure levels. Jefferson[177] failed to show any statistically significant increase of dry weight of radish, lettuce (Lactuca sativa), flax (Linum usitatissimum), duckweek (Lemna minor), clover (Trifolium fragiferum), or cabbage (Brassica oleracea) when these plants were treated with 25 to 1,500 rads in their early stages.

The stimulation of the growth of hair in mammals has been reported as a result of small doses of radiation.[253] Small doses to the immature stages may cause increased emergence of insects and mites or produce

larger and longer-lived adults.[177] Cell division in bacteria and fungi is stimulated in a large number of species, often for a short period of time. Cell proliferation leading to cancerous growths is a form of radiation stimulation well known in mammals but which can also occur in insects, higher plants and ferns.

1-5.4 Mutations and Plant Breeding

Plant breeding is essentially concerned with exploiting the natural variation existing in varieties of crop plants or closely related species, and the variation released after hybridisation. The various forms of ionizing radiation can produce heritable changes in cells by transferring their/energy through ionizations or excitations to sites within or near the genes which are located on the chromosomes, thereby increasing the chemical reactivity of these sites. After exposure to radiation the gene may be changed or lost and the chromosome damaged or broken. The term mutation is often loosely defined to cover all such changes.

Mutations may arise spontaneously, due to a number of factors but at a very low rate of 1 in 10^5 - 1 in 10^8 genes. After exposure to radiation this rate may be increased up to a thousandfold. Most mutations, however, whether spontaneous or artificially induced, are deleterious and of no value to a plant breeder. It has been estimated that possibly one out of every 800 mutations induced could be potentially of value. One of the major problems of producing radiation induced mutants is that the amount of chromosome damage induced may be so great that cell division is abnormal and the organism may be sterile. The rarity of the required event necessitates growing large populations of plants and making many thousands of measurements.

In Sweden barley, wheat, oat, pea, soybean, flax and mustard mutants have been produced which vary in yield, straw-strength,

earliness and other characters. From Germany breeders have reported cereal variants having higher grain-yield, stronger straw and improved disease resistance. A marked change in terms of size of bunches, taste, seed number and earliness have also been reported in blackcurrants. A self-fertile form of cherry has been produced from the normally self-sterile type. As these are independent of insects for pollination they should give a better yield in colder regions and unfavourable seasons.

Other than gene mutation itself, chromosome mutation may also occur. This occurs when a chromosome is broken into two or more pieces and this happens only rarely under normal conditions but much more frequently under the effects of radiation. When a chromosome is broken one of the pieces, that which lacks the centromere, lacks the ability to migrate to its appointed place when the cell divides. As a result one of the newly formed cells does not get its full quota of genes and generally dies. However, when a chromosome breaks both ends of the break are chemically active and if the two ends come close together they will rejoin without deleterious effects. Consequently with a single break there are two possible outcomes, either one piece is lost at the next cell division or prior to the division the broken ends rejoin. Other possibilities arise if two or more breaks occur in a cell simultaneously. One or more of the chromosome fragments may be lost on division leading to the death of the newly formed cell or the breaks may rejoin to form the original chromosomes. Two chromosome fragments which lack centromeres may join, or two fragments including centromeres may join; cells in which this happens are not viable. The last possibility is that a piece of one chromosome lacking a centromere may join the piece of another chromosome with a centromere and the other pieces may also join, thus forming 2 new chromosomes containing one and only one centromere each. Such new chromosomes which contain genes previously carried on different chromosomes may function normally during cell

division. Genes are sufficiently independent in their action to produce a normally viable and healthy organism even if reshuffled between different chromosomes.

Normally such chromosome breakages are undesirable features accompanying the desired gene mutations in the breeding on new plants by irradiation but occasionally the breakage phenomena may have great value. It offers the possibility of separating 2 genes, one of which is useful and the other deleterious, and which are closely linked together on the chromosome.

The outstanding use of the breakage phenomena in plant breeding has been demonstrated by the American geneticist Dr Sears. One of the problems in America was the rust diseases which attacked cultivated wheats. He used X-rays to transfer rust resistance from a grass-like wild wheat species to a cultivated variety of wheat. Hybridization of the two species had been tried but the wild wheat had many deleterious features which could not be separated from the rust resistance. By irradiating the hybrids the linkage with the undesirable features was broken and the segment of chromosome bearing the gene for rust resistance was separated and attached to one of the wheat chromosomes. Eventually a variety was produced, virtually indistinguishable from wheat but having the very potent disease resistance of the wild wheat.[177]

1-5.5 Sterilization by Irradiation

Wyckoff[254] in 1930 found that the fraction of organisms (E. scherichia coli or Salmonella aertrycke) surviving irradiation decreased exponentially as the dose increased. Much work has been directed towards the preservation of food, mainly because of the great potential advantages if food could be stored indefinitely at room temperature. The radiation preservation of food is beset by many

difficulties, not the least of which are the chemical changes undergone in the food itself. Radiation sterilization of medical or scientific equipment has fewer problems and is already well developed on a commercial scale, e.g. TVL in Wellington, N.Z.

The determination of a sterilizing dose depends very largely on the degree of contamination which can be tolerated in a given application. Take as an example a batch of 1,000 articles exposed to irradiation. If one article is not sterile and 999 are the degree of contamination would be 0.001. Such a degree of contamination would be unlikely to give a positive result in a routine laboratory examination of a small number of these articles. The inactivation of cells follows a probability law and the implications of this were first realised by Crowther in 1924 when he stated that "it is not possible to attach any precise significance to the idea of a "lethal dose". After exposure to a dose, however large, there would always be a probability of some cell surviving, although the probability might be extremely minute..."

The calculation of a sterilizing dose for a given set of environmental conditions is dependent upon (i) the final degree of contamination which can be tolerated and (ii) the initial viable count. The ratio of initial viable count to final viable count is the "inactivation factor". Thus to reduce 10^{3} organisms per gram to 10^{-3} organisms per gram requires a dose capable of producing an inactivation factor of 10^{6}. Factors of 10^{8} to 10^{12} have been found necessary for Clostridium botulinum in foodstuffs.[177] To calculate the dose of radiation necessary to achieve the required factor a survival curve must be plotted for the most resistant organism likely to be encountered. Table 1-5.5.1 shows the inactivation factors for various microorganisms for a dose of 2.5 Mrads.

Katahdin potatoes treated at 20 Krad remained free of sprouts and of excellent appearance for periods up to 18 months. Irradiation is also effective in inhibiting sprouting in stored onions although

Table 1-5.5.1

Approximate Inactivation Factors for Various Organisms for a Dose of 2.5 Mrad of γ-rays.[177]

Organism	Conditions During Irradiation	Inactivation Factor
B. coagulans	phosphate buffer pH 7	10^{21}
"	Saline	10^{39}
B. subtilis	Distilled water	10^{12}
"	Phosphate buffer pH 7	10^{13}
B. pumilus	Dried on paper discs	10^{13}
B. globigii	" " " "	10^{20}
Cl. tetani	" " " "	10^{19}
Cl. welchii	" " " "	10^{9}
Micrococcus radiodurans	Nutrient broth	10^{3}
	Phosphate buffer pH 7	10^{6}
Staphylococcus aureus	Phosphate buffer, aerobic	10^{167}
	" " anaerobic	10^{63}
Escherichia coli	" " aerobic	10^{143}
	" " anaerobic	10^{50}
Salmonella gallinarum	" " aerobic	10^{176}
	Liquid egg	10^{68}

there is some evidence that irradiation can in fact stimulate sprouting. If the treatment is carried out immediately after harvest a dose of 2 Krad is sufficient, but if delayed 9 or 15 weeks a dose of even 250 Krad is not effective.[177]

Insects can also be killed by irradiation and possible sterilization of grain before use in breadmaking has been investigated. Smaller doses of irradiation can render male insects sterile. The rearing and release

of sufficient numbers of these is an effective method of biological control.[195]

Seeds of gymnosperms and angiosperms exposed to between 5 and 100 Krad have been shown to give rise to plants which do not form viable seeds.[177] Autopolyploids often bear larger flowers, fruits and leaves than their diploid counterparts but they are characterized by reduced fertility.[256]

1-5.6 Growth Inhibition and Death

Radiation doses of 10 to 20 Krad prevent sprouting in potatoes,[255] onions,[257] Jerusalem artichokes, carrots,[258] sugar beet and mangolds.[259]

Medium doses of irradiation can severely inhibit plant growth and this is frequently correlated with chromosome damage. In Vicia faba roots 1 Krad causes little or no reduction in root growth despite the fact that an appreciable proportion of cells contain exchange aberrations.[236] By within-seed comparisons of chromosome abnormalities (from roots excised at 24-36 hrs) with height (attained by 7-9 days) in irradiated barley seeds Conger and Stevenson[260] showed that the damage to the height and to the chromosomes were closely correlated. The seedling height was not decreased until 20-30% of the cells had chromosomal abnormalities.

Most authors have used growth inhibition at a specified time e.g. 7 days as a measure of the radiosensitivity of seeds, and have shown a wide range of sensitivities between species, mustard normally being considered to be one of the most radioresistant seeds. Dormant seeds of Triticum vulgare exposed to 30 Krad formed retarded seedlings but at 50 Krad growth was completely inhibited and the plants died within a short period.[261] The LD_{50} for the survival of seedlings at 20 days for Zea mays was 54 Krad at 10.6% H_2O content and 10 Krad at 1.9%.[262] On the basis of survival of plants at maturity weed seeds have been shown

to have LD_{50} values between 1 and 5 Krads.[263] Lily seeds show practically no growth after 2 Krads but seeds of cabbage and radish are practically unaffected by 64 Krad.[264] Doses of 10-12 Krad caused a delay in the germination of Phaseolus vulgaris seeds. Doses of 15-20 Krad caused delayed germination and the roots formed began to enlarge but the hypocotyls became chlorotic and the seedlings died.[265] Table 1-5.6.1 shows the LD_{50} for 30 plants based on the death of seedlings.

Dry wheat seeds exposed to 0.8 Mrads of Co^{60} gamma-rays could form small seedlings without cell division.[217] Lettuce seeds after receiving 0.6 Mrads germinated in the absence of cell division and mitotic activity was not evident until 2 days after germination.[223] A dose of 0.5 Mrad given to wheat seeds gave an embryo which grew by cell enlargement only.[237]

At a dose rate of 130 rads/day, 16 Krad had no effect on the germination of Pinus rigida but at a dose rate of 295 rad/day only 8 Krads resulted in a reduction in germination. At the latter rate the LD_{50} for germination was 13 Krads. When a dose rate of 840 rads/day a dose of 16.8 Krads gave 84% germination 22.7 Krad gave 45% and the LD_{50} (i.e. 50% germination) was calculated to be 22 Krad. The moisture content of these seeds was between 10 and 14% of the dry weight.[270]

Peanut seeds exposed to 250 Krads gave 50% germination, to 500 Krads gave 30% germination and to 700 Krad, or above, no germination at all.[241] Wheat seeds irradiated with a dose of 1 Megarad showed reduced germination and after a dose of 5 Megarad no germination was observed within 1 month.[230] Saris[268] irradiated maize seeds with a dose of 15 Krad and only obtained 20% germination. Haber and Luippold[267] reported no germination within 7 days of wheat seed exposed to a dose of 1 Megarad.

Extensive studies on the effect of irradiation on the germination of conifer seeds have been reported. The LD_{50} values for the germination of Interior Douglas fir, Coastal Douglas fir, Western hemlock and Sitka

Table 1-5.6.1

LD_{50} (Krad) Values for 30 Plants after Co^{60} Gamma-irradiation of the Dry Seed[266]

Common Name	Scientific Name	Dose Rate Rad/min	LD_{50} Krad.
Alfalfa	Medicago sativa	844	38-62
Barley	Hordeum vulgare	844-850	13-20
Clover, button	Medicago orbiculatus	844	21
Clover, crimson	Trifolium incarnatum	844-1240	25-64
Clover, red	Trifolium pratense	795-1270	35-108
Clover, sweet	Melilotus sp.	844	59
Cowpea	Vigna sinensis	1260	11
Dallisgrass	Paspalum dilatatum	710	32
Fescue	Festuca elatior	844	19
Grape	Vitis spp.	790-1240	4-5
Guava	Psidium guajava	1240	17
Lespedeza, Korean	Lespedeza stipulacea	795	40
Lupine, blue	Lupinus augustifolius	750	40
Maize	Zea mays	840	15
Millet, German	Setaria italica	760	14
Oats	Avena sativa	840	17-27
Orchardgrass	Dactylis glomerata	844	11
Papaya	Carica papaya	650	12
Peanut	Arachis hypogaea	1260	10
Pepper	Capsicum frutescens	1260	24
Pigeon pea	Cajanus cajan	1260	15
Rice	Oryza sativa	650-1260	15-42
Rye	Secale cereale	714-840	8-16
Sericea	Lespedeza cuneata	795-840	37-46
Sorghum, grain	Sorghum vulgare	1260	40
Soybean	Glycine max	1260	11
Tomato	Lycopersicon esculentum	609-1240	13-37
Vetch, hairy	Vicia villosa	840	17
Watermelon	Citrullus vulgaris	1280	60
Wheat	Triticum vulgare	670-840	14-25

spruce were 7.5, 5.5, 2.5 and 1.9 Krads respectively.[269] Between 10.9 and 16.1% moisture content the LD_{50} values for the germination of Pinus rigida seeds after gamma-radiation were between 20 and 21 Krad. There was no loss of germination capacity up to 12 Krad although seedling development was retarded. After exposure to 48 Krad the germination was only 2% that of the control (96%).[290]

Clark et al[245] irradiated several species with various doses. Their results are shown in Table 1-5.6.2. The doses which totally prevented germination were found to range from 1.5 Krad to 11.2 Krad for the species examined. Heaslip[271] exposed various seeds of deciduous trees to 10 Krad γ-rays. She then examined the germination of these seeds, both in the field and in the laboratory. Most of the species showed decreased germination and for several species germination was severely inhibited. For seeds of Quercus alba a dose of 10 Krad was sufficient to prevent germination completely. The results of these experiments are shown in Table 1-5.6.3.

For radiation exposure of animals it is normal to specify that death must occur within 30 days. On this basis the LD_{50} for Man, rats and snails are 300-500 rads, 600-700 rads and 20,000 rads respectively.[272] By examining the soil from atomic bomb test sites it has been shown that fungi belonging to most species of the Moniliaceae are killed by doses of 0.25 to 1 Megarad but members of the Dematiaeae are not affected.[273] The giant unicellular algae Acetabularia mediterranea can recover from a dose of 0.5 Megarads.[274] The spores of Clostridium tetani and of Bacillus pumilus require a dose of 1.4-2.1 Mrads and 2.1 Mrads respectively to prevent growth.[275] The inhibition of germination of pollen (i.e. pollen tube growth) occurs only after massive doses of radiation. The LD_{50} values range up to 0.55 Mrads.[276] The pollen of Oenothera berteriana was shown to have an LD_{50} for germination of 20 Krad and an LD_{100} of 80 Krad.[224] The tobacco mosaic virus requires a dose of 1.8 Mrad before it is inactivated.[277]

Table 1-5.6.2

The Effect of Gamma-Radiation on the Germination over 30 Days of Conifers

Species	LD_0 (Rads)	LD_{50} (Rads)	LD_{100} (Rads)
White pine	400	685	1500
Scotch pine	600	935	3400
White spruce	650	1110	4300
Red pine	650	4900	9500
Black spruce	3700	5495	11200
Jack pine	1900	6310	11200

KEY LD_0 = Highest dose not giving inhibition of germination.
LD_{50} = Dose giving 50% of control germination.
LD_{100} = Lowest dose at which no germination occurred.

Table 1-5.6.3

The Effect of Irradiation of Dormant Seeds with 10 Krads[271]

Species	% Germination			
	Laboratory		Field	
	Control	Irrad.	Control	Irrad.
Acer saccharinum	85.2	48.0	72.5	29.2
A. saccharum	16.5	16.0	12.2	11.5
A. rubrum	73.2	69.0	*	*
Aesculus octandra	94.3	82.0	88.0	69.0
Carya ovata	72.0	14.0	79.3	4.6
Cercis canadensis	86.2	85.0	46.2	6.1
Fraxinus americana	48.5	60.0	54.2	63.4
Juglans nigra	28.0	20.0	32.0	62.0
Liquidambar styraciflua	47.6	39.0	*	*
Nyssa sylvatica	17.0	11.0	*	*
Pinus rigida	41.3	6.0	*	*
Platanus occidentalis	23.3	13.2	*	*
Quercus alba	87.4	0.0	*	*
Q. prinus	73.7	1.2	43.2	0.0
Q. velutina	45.5	5.7	48.8	16.8
Robinia pseudoacacia	64.5	67.0	34.4	12.8
Ulmus fulva	50.0	1.7	*	*
U. americana	75.5	72.0	20.5	16.5

* no field tests conducted.

1-5.7 Factors Modifying the Effects of Irradiation

(i) The Oxygen Effect

In vegetative systems, the oxygen effect is generally characterized by the following features. (a) In the absence of oxygen or at reduced oxygen pressure the effects of gamma- or X-rays are diminished but not abolished. (b) For maximum effect the oxygen has to be present during irradiation. (c) The effects of densely ionizing radiations such as neutrons or alpha-particles are much less sensitive to the presence of oxygen than are those for sparsely ionizing gamma- or X-rays.

Radiation induced seedling injury and chromosomal aberration frequencies following irradiation of germinating barley seeds were less in a partial vacuum than in air.[278] For dry barley seeds (7-11% moisture) an oxygen atmosphere enhances, while a nitrogen atmosphere retards the development of radiation after-effects occurring on storage.[279,280] Similar results have been obtained with dry seeds of Crepis capillaris,[281] onion[282] and tomatoes.[283] Neary[284] showed that the oxygen effect in bean roots was independent of temperature and was not directly related to metabolic processes.

(ii) Sulfhydryl Compounds

Hydrogen sulphide reduces the lethal effect of X-rays on dry spores by 50% when given after irradiation and by 75% when present during irradiation.[285] Both glutathione and cysteine protect plants from the effects of irradiation.

It was originally thought that such compounds protected biological systems by keeping the -SH groups in a reduced state. However, sulfhydryl compounds can also protect synthetic polymers, not containing -SH groups, from the effects of irradiation.[175]

(iii) Amines, Amino Acids and Peptides

Amino acids are weak protectors. The amines, especially aromatic amines, are much more active than their corresponding amino acids. Even the simple amines such as methylamine or aniline have some protective action.

(iv) Water Content and Storage

Desiccated seeds show lower radiosensitivity than do seeds with a high water content. The minimum sensitivity for barley seeds occurs when the water content is 4-8% above dry weight.[207] Wolf and Sicard[286] showed that normally-dry barley seeds (10% water content) stored after irradiation under normal conditions or desiccated seeds stored in a desiccator underwent no post-irradiation storage effect; but if desiccated seeds were stored under normal conditions the damage decreased. When normally-dry seeds were irradiated and then stored in a desiccator before planting the seedling height decreased with increased storage time and by 18 days showed as much damage as if the seeds had been desiccated at the time of irradiation.[286,287]

Conger and Randolp[288] found that the electron spin resonance, from radiation-induced free radicals, decreased in a similar manner to the decrease in damage to the Himalaya barley seeds on storage in moist conditions. Wheat germ (8.5% moisture content) had fewer active radicals induced in them by a given dose of radiation than did desiccated wheat germ. If desiccated wheat germ was brought to 8.5% moisture content after irradiation the electron spin resonance signals decreased very rapidly. When the wheat germ irradiated at 8.5% moisture content was stored in a desiccator the pattern decayed very slowly. The authors deduced that any moisture present could react with the radicals and dissipate them, without causing biological damage.

Osborne[289] has shown that flax seeds with normal moisture content at the time of irradiation, stored in normal moisture contents for 3 weeks following irradiation, showed a consistent significant recovery effect. Damage resulting from storage was observed only in seeds with a low moisture content at the time of irradiation and with doses up to 40 Krad. With higher doses recovery occurred. Normal moisture during storage produced the best recovery effect.[289] Increased moisture content up to 8.9% decreased the radiosensitivity of Pinus rigida seeds. Recovery increased with increasing stratification period and moisture content.[290]

(iv) Nuclear Volume

Several authors have found correlations between the average nuclear volumes of the cells and the radiosensitivity of the plant concerned. Sparrow et al[291] studied the radiobiological responses of a large number of species of higher plants. In diploid species a clear relationship was shown between the average nuclear volume of apical meristerm cells and tolerance to chromic gamma radiation. The larger the nuclear volume the greater the sensitivity of the nucleus and ultimately of the whole plant. Their data also indicated a relationship between the average amount of DNA per nucleus and radiosensitivity, i.e. the greater the amount of DNA the greater the sensitivity.

In Sedum plants the influence of polyploidy was shown. As the chromosome number was doubled from 20 to 40 the LD_{50} increased from 850 rads to 1300 rads. In polyploids a chromosome depletion would be expected to be less serious for the nucleus in the case where 4 or more genomes exist as compared to the diploid with only 2 genomes. This protective effect must, however, be balanced against the enhancing effect associated with increased nuclear volume.[291]

Lunden has carried this analysis further. His findings are consistent with those reported on the correlation between radiosensitivity and nuclear volume but he showed that the overall dose-response was a complex function depending on the cellular volume and structure as well. The seedling dose-response is determined by the structural features of the seed being treated, is largely dependent upon nuclear volume and overall epicotyl development, and can be predicted from multiple regression of nine embryonic factors on dose.

1-5.8 Recovery

(i) Mechanisms

Several processes of recovery are conceivable:

(a) Neutralisation of the primary effects produced at the molecular level by free radicals or by direct action, e.g. in a model system

$$\underset{\text{polymer}}{PH} + OH\cdot \longrightarrow \underset{\substack{\text{polymer}\\\text{radical}}}{P\cdot} + H_2O$$

$$P\cdot + \underset{\substack{\text{protective}\\\text{agent}}}{XH} \longrightarrow \underset{\substack{\text{repaired}\\\text{polymer}}}{PH} + X\cdot$$

This has been shown to occur *in vitro* but is highly improbable *in vivo* as the damaged molecules rapidly undergo further irreversible changes. Such a protective agent would need to be present during irradiation.

(b) Neutralisation of the secondary effects. The cells resynthesize or repair molecules which irradiation has inactivated or destroyed.

(c) In a given tissue, many cells may be killed but those which are left divide actively after a latent period and replace the dead cells.

(ii) Evidence for Recovery

Seeds stored at higher water content than that at which they were irradiated show significant recovery in terms of the height of 7 day seedlings.[286,290,293,294] The number of plant deaths which occurred over 8 years of chronic gamma-radiation of 11 woody species of plants indicated a decline in the rate of death with increasing exposure time.[295] This implies that a highly effective repair mechanism may develop at least in the range of exposure which reduces survival by 50% for these plants.

Reversals of radiation effects by various plant growth promoters have been noted in several instances. Among these are: (i) the reversal of radiation-stunted growth of corn seedlings by gibberellic acid,[296] (ii) the reversal of the radiation-inhibited geotrophic response in corn seedlings by auxin[297] (iii) the reversal of the radiation-induced potato dormancy by gibberellic acid,[298] and (iv) reversal of the radiation-induced delay in germination of lettuce seeds by gibberellic acid.[267]

UV-induced mutations in maize pollen can be repaired by post-irradiation treatment with light.[299] Chlorophyll synthesis in etiolated barley leaves was inhibited by ionizing radiation but it could be partially reversed by exposing the leaves for short periods to red light immediately after irradiation.[196] Carbon dioxide fixation of Chlorella was markedly inhibited by radiation but completely recovered following a 5 hour exposure to light.[300]

Repair is discerned most readily when split-doses are administered and the residual capacity for reproduction or growth is used as an index of radiation damage. A quantitative estimate of recovery can be obtained either by the reduction of damage per unit dose, or by the increased dose required to achieve equivalent damage with split doses as opposed to a single dose. The presence of repair phenomena has been shown using this

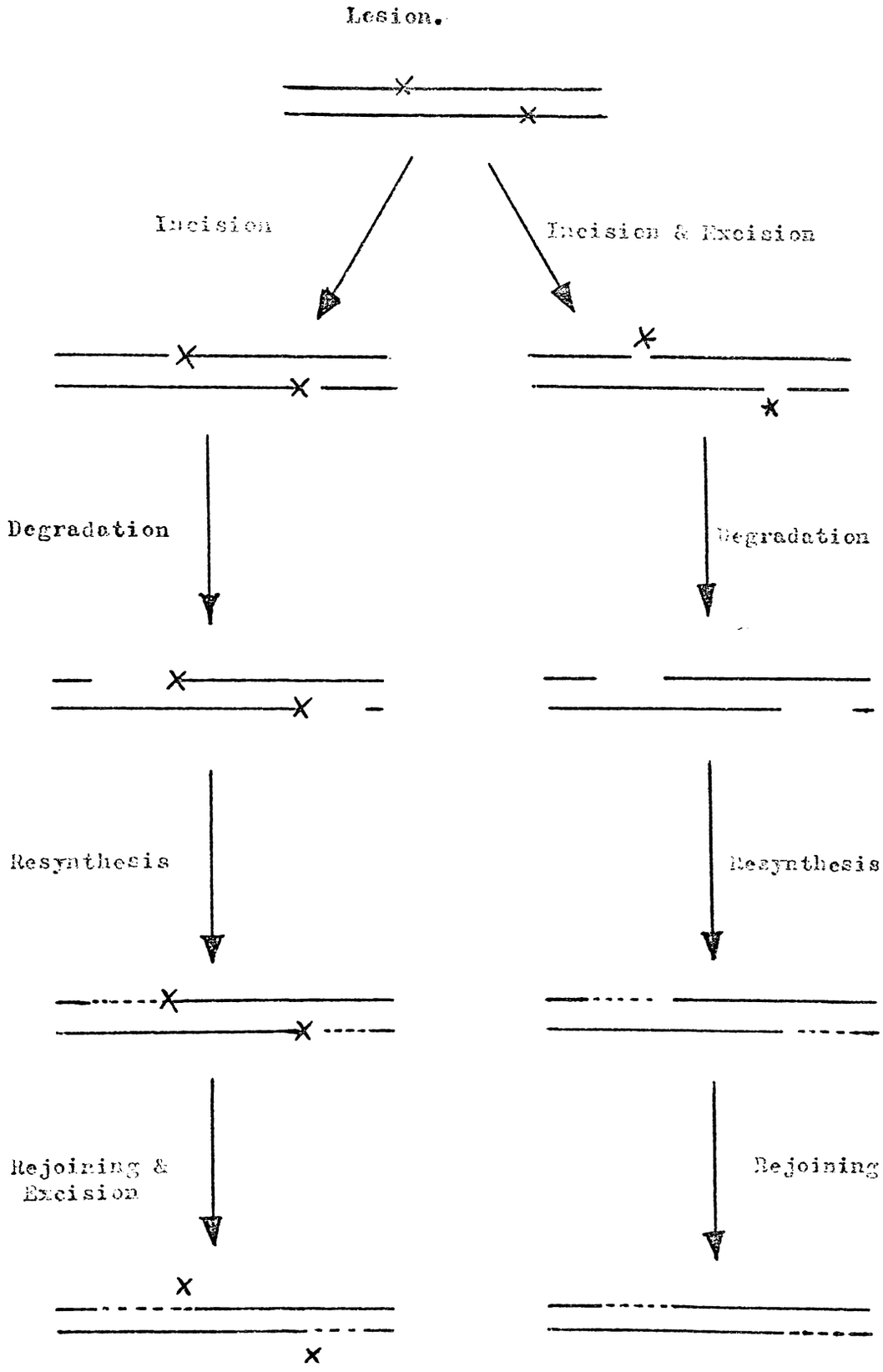
split-dose technique in rice and grape seeds. Clear evidence for recovery processes in the meristem cells of Vicia faba have been shown by Hall and Lajtha.[301] Split-dose effects were noted, with additional evidence that the rate of repair was dependent on the temperature.

If repair processes are active during the actual irradiation treatment, different dose rates giving the same total dose would be expected to affect the final damage observed. In Vicia faba seedlings the amount of damage caused decreased with lower dose rates from 8.4 to 1.52 rads/hr.[302] At a dose rate of 130 rads/day 16 Krad had no effect on the germination of Pinus rigida but with a dose rate of 295 rad/day 8 Krads was sufficient to cause a reduction in the germination percentages.[270]

Shoots of several apple varieties have been shown to recover from a dose of 3 Krad of X-rays.[303] The mechanism involved the replacement of the dead cells by the rapidly growing cells surrounding them, the latter having escaped from severe radiation damage.

In simple systems, probably including dry seeds, DNA is the most important target of the ionizing radiations. Dimers between adjacent pyrimidine residues of DNA are produced in large numbers during the irradiation process. In radiosensitive bacteria and viruses these dimers are thought to be the main cause of cell death.[304] Photo-reactivating enzymes, which have been found present in these cells, have been shown to repair such dimers and to form DNA identical to the DNA present before irradiation. Two schematic pathways for the mechanism of this enzyme are illustrated in Fig. 1-5.8.1. The repair process in bacteria involves 4 enzymes and is effected by the removal of an oligonucleotide of which the dimer is part. The average size of the

FIG. 1-S.S.1.



TWO SCHEMATIC PATHWAYS ILLUSTRATING THE POSSIBLE SEQUENCES IN THE REPAIR OF DNA.

oligonucleotide is 500 nucleotides and using this process up to 4000 dimers per chromosome can be replaced.[305] In a normal cell such a combination of enzymes could be used to scan newly synthesized DNA for the presence of any mispaired bases which if found can be effectively removed and replaced by the correct bases. In E. coli single strand breaks in DNA increase linearly with dose from 0 to 80 Krads. At lower doses, up to 16.2 Krad the enzyme systems in E. coli are capable of repairing the breaks that have occurred within 45 minutes.[306] At higher doses the repair processes take much longer and, in some radiosensitive strains are ineffective. Repair of single strand breaks and, possibly, double strand breaks has been demonstrated in cells of the Chinese hamster.[307]

Ligases can also join together breaks produced in a single strand of DNA as a result of exposure to radiation. Their role in repair is, however, secondary and perhaps "accidental" to their primary function in DNA synthesis. The ligases can only join those radiation induced breaks which have as their ends 3'-OH and 5'-P groups. Breaks with a different chemical composition such as 5'-OH and 3'-P cannot be directly acted upon. The rejoining of radiation induced 3'-OH and 5'-P breaks is probably so effective in all dividing cells that such breaks do not contribute to radiation injury.[308] In the repair of breaks of different chemical composition excision to form 3'-OH and 5'-P groups could occur first and be followed by repair due to the action of the ligase. The action of the ligase does, however, require ATP and/or NAD and hence these must be in ample supply for the repair process to be rapid.

Approximately one double break is produced for every 10 single breaks when cells are irradiated with X-rays. In Micrococcus radiodurans double breaks are effectively rejoined but the mechanism has not been resolved. In single strand breaks the other strand keeps the two broken ends close together and acts as a template for repair processes. In

double strand breaks it is thought that the histone which surrounds the DNA molecules acts as a "splint", keeping the DNA portions in place. This could also, however, be achieved by the secondary valence forces between the parallel bases as long as the electrostatic repulsion between the phosphate groups produced at the break are reduced. Heavy metal ions or cell membranes could be important for this.

If cells are irradiated in the stationary phase, held in salt solution in the dark and not permitted to divide for a few hours and then placed on nutrient agar they will show a much higher rate of survival than if they were placed on agar immediately.[309] Similarly if bacteria are kept for 2 hours at temperatures where no cell division occurs, but enzyme activity can take place (e.g. 15°C for E. coli) the survival is once again enhanced. These results are thought to be due to the activity of repair enzymes which need to be effective before mitosis takes place. If E. coli are kept at a temperature of 45-47°C after irradiation recovery will be evident when later placed on nutrient agar. This could be due to either retardation of mitosis at the high temperature or to the decomposition of heat-labile toxins, e.g. peroxides produced by the irradiation.[310]

2. PRINCIPLES OF ENZYMATIC ANALYSIS

2-0 Introduction

Many diverse techniques have been developed in order to determine what chemical reactions are occurring in living systems, to measure the effects of assorted variables upon these reactions and to determine the rates at which these reactions occur. In all experimental science, in contrast to purely observational science it is necessary to apply some disturbing condition to the system under observation and to measure the effect of that disturbance. In biochemistry this means that one must experimentally modify or alter the organism or tissue being studied. The applied disturbance can range from the addition of a minute amount of isotopically labelled nutrient to the isolation of an enzyme in a pure state from the organism.

2-1 Tracer Techniques

2-1.0 The Advantages of Radioisotope Methods

Radioactive elements can be detected with extreme sensitivity, permitting the use of extremely small quantities of radioactive material. Once the required apparatus has been set up the materials needed are relatively cheap as so little is required for each experiment and the techniques involved are simple.

The metabolism of the biological system can be studied "in vivo". In animals the isotope can be included with the food and with seeds the tracer, tritiated water, can be used as this readily penetrates the cell walls. The incorporation of tritium into various metabolites gives an in vivo indication of enzyme activity. The standard biochemical

procedure of obtaining cell-free systems of enzymes while yielding much useful information requires extrapolation of *in vitro* results to *in vivo* conditions. Unless some care is taken the validity of the extrapolation can be questionable.

The use of tracers, in particular $C^{14}O_2$ and THO (tritiated water), is applicable to certain studies which cannot be conveniently undertaken by other means. An example of this is the study of the resting metabolism of seeds for which either $C^{14}O_2$ or THO vapour can be used.

2-1.1 Isotope Effects

In chemical reactions isotopic fractionation may arise from two causes:

(a) A small, but significant difference between the equilibrium constants of reactions in which the two species are involved.

(b) There may be a large difference in the rates at which the two species react.

Since the mass of tritium is three times that of the normal form of hydrogen, isotope effects will be large, especially when tritium is substituted at the reaction centre.[311] These effects would not, however, be so great for carbon-14 which is closer in mass to that of the nonradioactive isotope.

Lewis[312] showed that two varieties of *Pisum sativum* germinated in water containing up to 40% D_2O but at values greater than this no germination occurred. Wheat seeds have also been examined[313,314] and found to germinate in 94% D_2O. No macroscopic differences were detected, nor was there any difference in respiration between the treated seeds and the control. Spedding[315] has shown that seeds treated with THO for a period of time before the addition of water will germinate normally.

The isotope effect for tritium can theoretically be as large as 60 as compared to the value of 1.5 for $C^{14}:C^{12}$. However, in the tritiated water used, with a specific activity of 5 curies per gram, only 0.15% of the hydrogen atoms have been replaced by tritium atoms. This would not be expected to have an appreciable effect on the operation of metabolic sequences or on metabolic pool sizes. The C^{14} tracers would have an even smaller effect than that of tritium. Most of the work reported here is qualitative rather than quantitative and any changes in the rates of enzymic reactions due to the use of tracers are unlikely to alter the interpretation of results, although the intensity of labelling may not be strictly proportional to the metabolic pool sizes.

2-1.2 Radiation Damage

Spedding and Wilson[173] estimated the amount of radiation to which a single Sinapis alba seed is exposed during a one hour tritiated water experiment to be approximately $3x10^4$ reps. The work reported in this thesis shows that seeds can endure doses of over $1x10^6$ rep without a decrease in percentage germination. The C^{14} compounds used had a much lower activity and for these experiments the dose of radiation received by the seed would also be proportionately lower.

One of the types of "radiation damage" which does occur in the use of tracers in biological systems is due to the decay of these compounds. Carbon-14 decays by beta-radiation to form nitrogen. If some of the carbon-14 added to the biological system becomes incorporated into the DNA and then decays the carbon will in effect be replaced by a nitrogen atom. Such a substitution may or may not have serious consequences on the activity of the DNA.

2-1.3 Self-decomposition of Labelled Organic Compounds

Carbon-14-labelled methyl iodide turns red during storage.[316] Assuming that an average of 32.5 eV of energy is expended for every ion pair formed, the authors calculated that the carbon-14 beta particle of average energy destroys 1570 molecules of methyl iodide in addition to the molecule that contains the disintegrating carbon-14 atom.

The G-values obtained for amino acids by Tolbert vary from 2 to 30 and on decomposition ammonia, amines and carbon dioxide are formed. This radiation induced decomposition is a serious problem for the storage of radioactive tracers. The rate at which this self-decomposition occurs depends upon the following factors (i) the specific activity of the sample, (ii) the average energy, $\bar{E}$, of the radiation, (iii) the fraction of $\bar{E}$ absorbed by the organic compound, (iv) the G-values of the system and (v) the half life of the nucleotide. The fraction of $\bar{E}$ absorbed by the sample will approach unity for the weak beta emitters such as tritium and carbon-14.

If high-level labelling is necessary it is not possible to dilute the radioactive sample with inactive carrier. The compound may be mixed with a finely divided solid such as powdered glass or if suitable dissolved in benzene.

The presence of even small quantities of strongly radioactive impurities can lead to errors in the interpretation of results. If the desired radioactive material is contaminated in this manner it must be purified before used in experiments. If self-decomposition is forming radioactive impurities then the radioactive compound must be purified immediately before each experiment. Each batch of material purchased should be checked for radiochemical purity before use, not withstanding

the manufacturers comments, as serious contamination of newly purchased compounds, of supposedly 99% purity, is not uncommon.[318,319]

2-1.4 Interpretation of Results, Using Tritiated Water as a Tracer

When a living system is allowed to metabolize in the presence of tritiated water the tritium atoms are incorporated and eliminated at many points of the metabolic sequences, leading to the formation of labelled compounds. The stability of the label in a particular metabolite falls into one of the following categories.

(i) Labile Hydrogen. The hydrogen atoms in the groups -OH, -COOH, $-NH_2$, =NH, and -SH are readily ionizable and, in solvents such as water, will exchange rapidly with the surrounding protons. In tritiated water these groups are labelled very rapidly but when the specific activity of the surrounding solution is reduced, as during extraction and chromatography, this label will be readily lost.

(ii) Semi-labile hydrogen. Certain groups such as those found in the keto-enol system are characterized by a slow rate of exchange.

$$R - CH_2 - \underset{\underset{O}{\|}}{C} - R \rightleftharpoons R - CH = \underset{\underset{OH}{|}}{C} - R$$

The hydrogen atoms adjacent to the carbonyl group exchange slowly with the solvent. Such labels will be lost during experimental procedures.

(iii) Non-labile hydrogen. Hydrogen atoms bound directly to a carbon atom and not involved in a keto-enol system can be removed in a biological system only by a biochemical reaction. An example of such a reaction is

$$\begin{array}{l} HO - CH - COOH \\ \quad\quad | \\ {}^{3}H - CH - COOH \end{array} \xrightleftharpoons{THO} \begin{array}{l} CH - COOH \\ \| \\ CH - COOH \end{array}$$

the addition of water to fumaric acid yielding malic acid, as occurs in the TCA cycle. This compound could theoretically undergo keto-enol

tautomerism but the rate of this is so slow that the label is not lost during experimental procedures.

The essential feature of this method is that non-exchangeable incorporation of tritium from tritiated water can occur only as a result of metabolic activity. Thus, incorporation of tritium may be used as an index of metabolism. For example as quoted above, malic acid will become labelled if the TCA cycle is operating. If labelled malic acid is found it must have been involved in some metabolism which may or may not have been the TCA cycle. If other labelled acids, such as citric acid, which occur in the same metabolic sequence are found, then the evidence for the operation of the cycle is stronger. If malic acid was present but not labelled then it is improbable that the TCA cycle is in operation.

This argument has been shown to hold true for all the common biochemical pathways and is the basis on which the results using tritiated water obtained in this thesis have been interpreted.

3. EXPERIMENTAL MATERIALS

3-1 Seeds

Sinapis alba (white mustard) seeds were purchased in bulk from a local retailer.

Lactuca sativa (lettuce) - varieties "Webbs Wonderful", "White Cos" and "Great Lakes" were purchased in bulk from F. Cooper Ltd, Wellington.

Allium cepa (onion) - variety "White Silverskin" - were purchased in bulk from F. Cooper Ltd, Wellington.

Pisum sativa (pea) seeds were purchased from F. Cooper Ltd, Wellington.

Vicia faba (broadbean) seeds were purchased from F. Cooper Ltd, Wellington.

Triticum sativum (wheat) was purchased from a local retailer.

Avena sativa (oat) of the variety "Algerian Oat" was purchased from a local retailer.

Solanum lycopersicum (tomato). Packets of Cooper's "Frost-Resistant" and Cooper's "Beafsteak" varieties were purchased.

Cucumis sativus (cucumber). Packets of Cooper's "Heinz Gherkin" were purchased.

Cucurbita pepo (squash). Packets of Cooper's "Butternut" variety were purchased.

Cucumis melo (watermelon). Packets of Cooper's variety "Black Diamond Florida Giant" were purchased.

Solanum melonga (egg plant) - Packets of Cooper's "New York Purple" were purchased.

Pastinaca sativa (parsnip). Packets of Cooper's "Hollow Crown Select" variety were purchased.

Spinacia oleracea (spinach). Packets of Cooper's variety - "Long Round Standing Viking" were purchased.

Trifolium repens (white clover) seeds - variety "Grasslands Huia" - were purchased in bulk from Dalgety and N.Z. Loan Ltd, Wellington. The analysis of these Government certified seeds is shown in Fig. 3-1.1.

_____ (Ryegrass) seeds were purchased in bulk from Dalgety and N.Z. Loan Ltd, Wellington.

3-2 Soils

Potting mixture: The potting mixture used was purchased in quantity from a local retailer. It was called "Utility Potting Mixture" made to the University of California's formula and was manufactured by Asquith Nurseries, Mount Albert, Auckland.

Soil: Steam sterilized top soil was used for the large scale statistical analysis carried out in the glasshouse. The pots used were plastic 8 in. Win Pots.

3-3 Nutrient Solution

The following solutions were prepared.[325]

(a) 1M $MgSO_4 \cdot 7H_2O$

(b) 1M $Ca(NO_3)_2 \cdot 4H_2O$.

(c) 1M KH_2PO_4

(d) Iron solution:- 20.8 gms of sequestrene (supplied by Ivan-Watkins Dow, N.Z.) in one litre distilled water.

NEW ZEALAND DEPARTMENT OF AGRICULTURE
Farm Advisory Division
Seed Testing Station

S.T.S.No.69/15513

CERTIFICATE OF ANALYSIS - GOVT. CERTIFIED SEED

Seed: "Grasslands Huia"
White Clover

Pure Seed: 98.1%

Class: Certified Seed
2nd Generation

Germination: 93+1%

Reg. No. R/881-C

P. & G. No. 1907/R Sacks: 29

PURITY ANALYSIS

Pure Seed	Other Crop Seed	Weed Seed	Inert Matter
98.1%	1.6%	0.1%	0.2%

Found in Working Sample

Crop Seed:	Weed Seed:
Trifolium dibium 1.6%	Stellaria graminea

Pure seed includes a trace of insect damaged seed.

Inert Matter: Broken and decorticated seed.

GERMINATION TEST OF PURE SEED

Interim Count	Final Count	Hard Seed	Abnormal Growths	Remainder
90% in 3 days	93% in 7 days	1%	4%	2%

PALMERSTON NORTH
6.5.69

The examination reported above was made on a sample drawn from sealed sacks by an officer of the Dept of Agriculture

A. V. Lithgow
Superintendent

Figure 3-1.1

(e) Supplementary solution:

2.86 gms H_3BO_3

1.81 gms $MnCl_2.4H_2O$

0.11 gms $ZnCl_2$

0.05 gms $CuCl_2.2H_2O$

5.046 gms $NaMoO_4.2H_2O$

Distilled water to one litre.

The Nutrient solution was prepared by adding together:

2.3 mls of (a)

4.5 mls of (b)

2.3 mls of (c)

1.0 mls of (d)

1.0 mls of (e)

Distilled water to one litre.

3-4 Tracers

Tritiated water. A 5 ml (5 curies/ml) supply of tritiated water was obtained from the Radiochemical Centre, Amersham, England, Working supplies of 1 ml were transferred to a Quickfit stoppered glass vessel as required.

3-5 Chromatography

Paper. The paper used for most experiments was Whatman No.4 paper, cut into 10½" x 6" rectangles. Whatman No.3 MM and Whatman No.1 cut into 16" x 8½" rectangles and whole sheets of Whatman No.4 were used for the analysis of amino acids and for cleaning the contaminated tracers.

Solvents

(i) 100 gms redistilled phenol:39 mls distilled water, stored in the dark.

(ii) a 937 ml n-butanol:63 ml distilled water.

b 440 ml propionic acid:560 ml distilled water.

Immediately before use b was added to a until a single phase system was just formed.[321]

(iii) 3 vols 2:4/2:5 butidene:1 vol 2:4:6-collidine saturated with distilled water.

(iv) 9 vol n-butanol:1 vol glacial acetic acid saturated with water.[46]

(v) 1 vol n-butanol:1 vol. pyridine:1 vol. distilled water.

(vi) 18 vols ethanol:1 vol. conc. ammonia:1 vol. distilled water.

(vii) 4 vols iso-propanol:1 vol distilled water (IPrAq).

(viii) 7 vols n-propanol:1 vol ethylacetate:2 vols distilled water (PrEtAc)

The two solvents 1PrAq and PrEtAc were used only for the chromatography of carbohydrates.

Sprays

(i) For amino acids a 0.2% solution of ninhydrin in absolute ethanol was used.

(ii) For organic acids three sprays were used.

(a) Sulphanilamide Reagent[322]. Chromatograms are first sprayed with a solution of 2% sulphanilamide and 0.5% 2-napthol in 95% ethanol. After airdrying at room temperature for half an hour they were sprayed with 1% aqueous sodium nitrite. Acids appear as orange spots on an almost colourless background.

(b) A solution of 0.1% mercurchrome in 95% ethanol[323] was occasionally used for chromatograms which had not been immersed in scintillating liquid or which had been washed with toluene after autography. Under ultraviolet light acids fluoresce as purple spots on a green background.

(c) Aniline Xylose Reagent.[324] Dissolve 1 gram of xylose in 3 mls of water, add 1 ml of aniline and make up to 100 mls with methanol. Spray the chromatograms, air dry for 5-10 minutes to remove the methanol and then heat the damp paper at 105-110°C for 5-10 mins. The acids appear as brown spots on a pale yellow background. Pyruvic, glutaric and glyceric acids react weakly.

(iii) For sugars, a solution of 0.9 gm aniline and 1.66 gm phthalic acid in 100 mls of water saturated n-butanol. On heating to 100°C for 10 mins, reducing sugars give brownish colours.

Amino Acids. Shandon 0.01M solutions in 10% iso-propanol were used.

Sugars and Organic Acids. 1% solutions in 10% iso-propanol were used.

3-6. Scintillation Autography

Scintillating Liquid: 6-7 gms p-diphenylbenzene (terphenyl) were added to one winchester (2.5 litres) of purified toluene. This voluene was obtained by passing technical grade toluene slowly through a 3x75 cm column containing chromatographic alumina covered by a layer of finely powdered silica gel. Sulphur compounds, which inhibit the action of the scintillator were removed by this procedure.

Photographic Film. Kodak "Royal Blue" Duplitized X-ray Films in 14 x 17 inch sheets were used.

4. INITIAL EXPERIMENTS INVOLVING IRRADIATED MUSTARD SEEDS

4.0 Introduction

It was desired to produce a non-viable seed by supplying a sufficient dose of gamma-radiation so that germination was prevented. It was hoped that by comparing the metabolism of a viable seed (i.e. one which could germinate) to that of a nonviable seed, produced by irradiation, a clue to the germination mechanism could be obtained.

With this aim Sinapis alba (white mustard) seeds were irradiated with various doses and their ability to germinate compared to that of a control group which had undergone exactly the same procedures with regard to environmental conditions excepting the actual irradiation. The effect of storage on these seeds was also examined. The results obtained were extremely interesting and led on to the accummulation of further data on the physiological aspects of gamma-irradiated seeds. The initial experiments are described in this section.

4-1 Experimental Methods

4-1.1 Irradiation Procedure

Seeds in paper envelopes were equilibrated overnight at 75% relative humidity, above a slurry of sodium chloride and water in a desiccator. The seeds were then irradiated with various doses of gamma-radiation from a cobalt-60 source at the Institute of Nuclear Science, Gracefield, Wellington. The dose, dose-rate and exposure times of the two sets of irradiated seeds are shown in the tables.

4-1.2 Germination Studies

For the germination tests a random selection of one hundred seeds of each dosage group were placed on two 11 cm No.5 Whatman Filter papers in Petri dishes. Distilled water was then added, ensuring that

each seed was moistened. The seeds were separated from one another to ensure adequate gas exchange. The germinated seeds were counted at regular intervals of twentyfour hours, taking the protrusion of the radicle as the criterion for germination.

4-1.3 Growth of Plants

Ten seeds chosen at random from each of the 0.0, 1.19, 2.98 and 10.22 Mrep dose groups were placed on filter paper in labelled Petri dishes and allowed to germinate. In addition 10 seeds from a newly purchased batch of seeds (called the Control 1969) were used to check that the 0.0 Mrad group had not undergone damage during storage. Three days later ten seeds from each of the 0.52 and 5.08 Mrad groups were similarly placed in Petri dishes. Three days later the seedlings were transferred to a pot, each seed being placed at the same depth in the soil. This pot which was purchased from "Woolworths" measured 15" x 5" and was made of polystyrene foam. Drainage holes were formed and then a layer of stones added to help provide adequate drainage. The pot was filled with "Utility" potting mixture and the young seedlings were carefully transferred. The pot was divided into seven equal areas, one for each dose group and these were marked using pins and cotton thread. The seedlings for each dose group were planted in rows running from the front to the back of the pot. Twenty days later all but one of the Control 1969 plants appeared to be infected by a fungus or virus. The leaves were discoloured and the plants themselves were stunted and appeared to be dying. To minimize the risk of infection spreading to the other plants in the pot these seedlings were removed.

Three months after germination the pot which had previously been on a household windowsill in Kelburn, Wellington, was transferred to a Victoria University glasshouse with controlled temperature and

lighting. The lower leaves of the plants were turning yellow, showing nitrogen deficiency. From this time onwards they were fed the nutrient solution.

4-2 Germination Studies

The percent of the seeds which germinated within a given time interval (i.e. the rate percentage germination) and the total percentage germination have been plotted as functions of time for the various storage conditions examined. The values tabulated in column (a) in the Tables were plotted versus time to give the former graph and those in column (b) to give the latter graph.

Table 4-2.1 shows the germination values obtained for seeds irradiated in February 1968 and not stored before the tests were begun. Graphs 4-2.1(a) and 4-2.1(b) were plotted using these results. The "Percentage Germination" versus "Time" curve differs for the various doses of gamma-rays received. The seeds which had received 2.98, 5.08 or 10.22 Mrep showed no germination at all within three weeks. The maximum time for such an experiment was determined by the onset of fungus attack although the seeds did not appear to have rotted before this occurred. Graph 4-2.1(a) is, in effect, the first derivative of the "Percentage Germination" versus "Time" curve. It can be seen that the maximum rate of germination decreases and becomes progressively later with increasing dose.

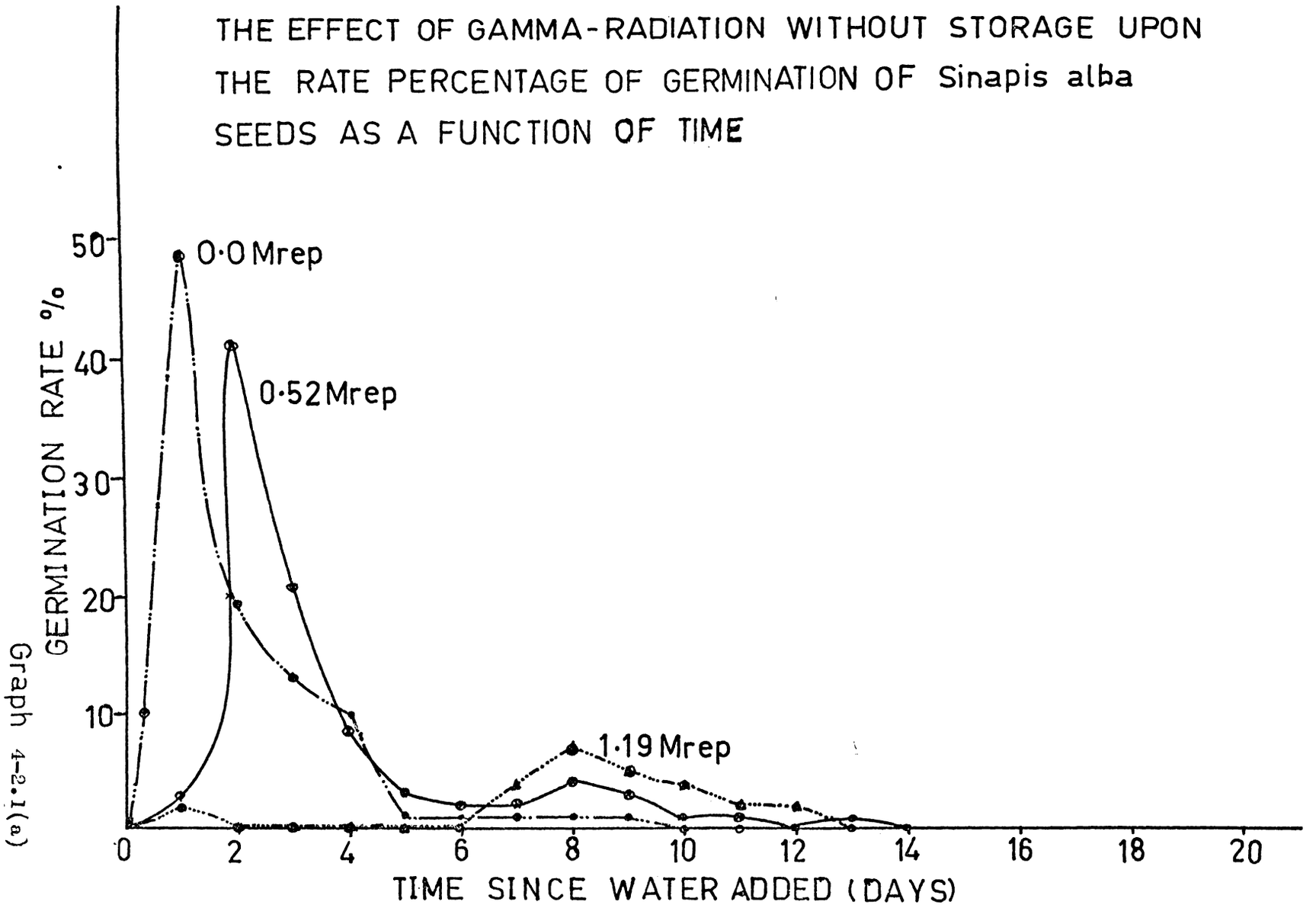
The seeds used in this test were then allowed to stand for five months enclosed in their envelopes, in a drawer in the laboratory - designated "in air" in the results. After this period of time the germination tests were repeated. The results obtained are tabulated in Table 4-2.2, from which graphs 4-2.2(a) and 4-2.2(b) were drawn. The

Table 4-2.1

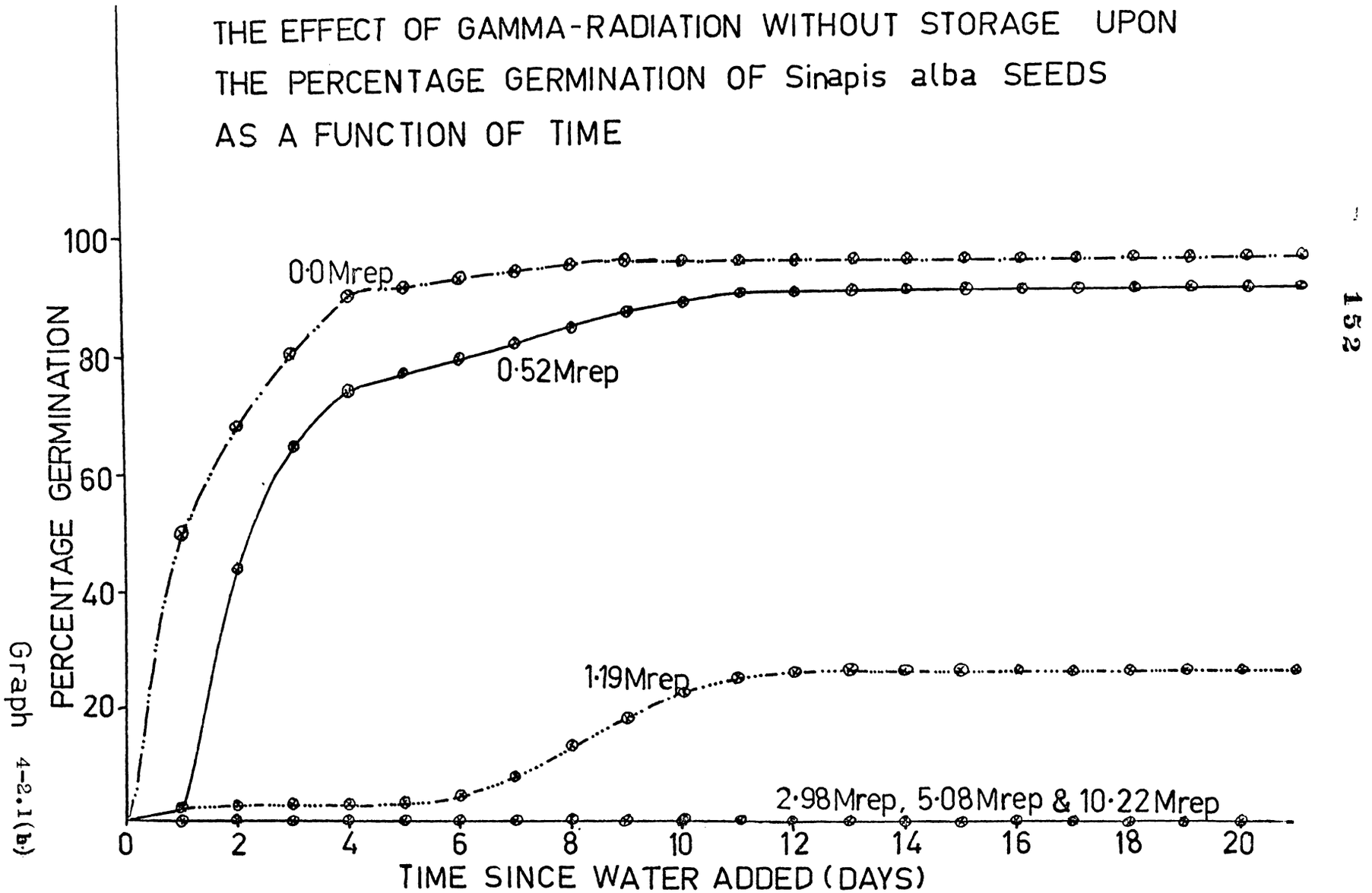
The Effect of Gamma-Radiation Without Storage upon the Germination of Sinapis alba Seeds [338]

Dose (Mrad)	0.00		0.52		1.19		2.98		5.08		10.22	
Dose Rate Mrad/hr	0		0.214		0.214		0.214		0.214		0.214	
Time Exposed (hrs)	0		2.4		5.5		13.8		23.6		47.5	
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	49	49	2	2	2	2	0	0	0	0	0	0
2	19	68	41	43	0	2	0	0	0	0	0	0
3	13	81	22	65	0	2	0	0	0	0	0	0
4	10	91	9	74	0	2	0	0	0	0	0	0
5	1	92	3	77	0	2	0	0	0	0	0	0
6	1	93	2	79	0	2	0	0	0	0	0	0
7	1	94	2	81	4	6	0	0	0	0	0	0
8	1	95	4	85	7	13	0	0	0	0	0	0
9	1	96	3	88	5	18	0	0	0	0	0	0
10	0	96	1	89	4	22	0	0	0	0	0	0
11	0	96	1	90	2	24	0	0	0	0	0	0
12	0	96	0	90	2	26	0	0	0	0	0	0
13	0	96	1	91	0	26	0	0	0	0	0	0
14	0	96	1	91	0	26	0	0	0	0	0	0
15	0	96	0	91	0	26	0	0	0	0	0	0
16	0	96	0	91	0	26	0	0	0	0	0	0
17	0	96	0	91	0	26	0	0	0	0	0	0
18	0	96	0	91	0	26	0	0	0	0	0	0
19	0	96	0	91	0	26	0	0	0	0	0	0
20	0	96	0	91	0	26	0	0	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.


THE EFFECT OF GAMMA-RADIATION WITHOUT STORAGE UPON
THE RATE PERCENTAGE OF GERMINATION OF Sinapis alba
SEEDS AS A FUNCTION OF TIME
GERMINATION RATE %
50
40
30
20
10
0·0 Mrep
0·52 Mrep
1·19 Mrep
0
2
4
6
8
10
12
14
16
18
20
TIME SINCE WATER ADDED (DAYS)

Graph 4-2·1(a)


THE EFFECT OF GAMMA-RADIATION WITHOUT STORAGE UPON
THE PERCENTAGE GERMINATION OF Sinapis alba SEEDS
AS A FUNCTION OF TIME
PERCENTAGE GERMINATION
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
TIME SINCE WATER ADDED (DAYS)
0·0Mrep
0·52Mrep
1·19Mrep
2·98Mrep, 5·08Mrep & 10·22Mrep

Graph 4-2.1(a)

seeds showed complete recovery of their germinating ability as even those having previously received a dose of 10.22 Mrep (approximately $3x10^4$ times the dose required to kill 50% of exposed humans) displayed 90% germination. Graph 4-2.2(a) of the "Germination Rate" versus "Time" shows that recovery had also occurred in the rate at which the seeds germinated. The period of maximum rate was the same for all of the dose groups.

Graphs 4-2.3(a) and 4-2.3(b) were drawn from the results obtained by W.J.H. Baillie[320] for his seeds irradiated in February, 1964. These results show similar trends to those for the seeds irradiated in 1968 as illustrated in Graphs 4-2.1(a) and 4-2.1(b). However the difference between seeds having received 1.0 Mrep and 1.19 Mrep is worthy of note. These results imply that the LD_{50} (the dose at which 50% of the species are killed) would lie between 1.0 and 1.19 Mrep but the differences in experimental procedure would not permit an exact extrapolation. The recovery shown by the seeds stored "in air" does pose a problem in terminology as although these seeds were not able to germinate without storage they had not been "killed". Instead a secondary dormancy seems to have been produced by the gamma-radiation.

The seeds irradiated by Baillie[320] were stored over silica-gel (at approximately 10% water content) in a desiccator for 4 years. A germination test was carried out to examine for any recovery from the effects of irradiation. The results obtained are tabulated in Table 4-2.4 and shown in graphs 4-2.4(a) and 4-2.4(b). An increase in damage is apparent. The percentage germination for the seeds which had received 0.5 and 1.0 Mrep had fallen from 100% to 95% and 98% to 94% respectively. Although this decrease in total percent germination could not be considered [redacted] significant, a noticeable retardation does occur

in the periods of maximum germination. The seeds in the 1.0 Mrep group no longer showed a period of maximum germination between the 2nd and 3rd days but instead show a period extending from the 3rd to the 9th day, over which 60% of the germination occurred. A smaller change causing a shoulder to appear on the graph is also apparent for the period of maximum germination of seeds belonging to the 0.5 Mrep dose group.

These seeds were then allowed to stand in air (at normal room relative humidities) for five months as were those which had been irradiated in 1968. After this period further germination tests were carried out. The results obtained are tabulated in Table 4-2.5 and shown in Graphs 4-2.5(a) and 4-2.5(b). The latter graph of the "Germination Percentage" versus "Time" shows a slight recovery in the 0.5 Mrep dose group. This curve approaches more closely the curve obtained for the Control than was the case in graphs 4-2.4(b) or 4-2.3(b). Those seeds having received 1.0 Mrep of gamma-rays however, do not show an increased percentage germination or a more marked period of maximum rate.

These seeds which had been irradiated in 1968 were further examined during prolonged storage in air. Table 4-2.6 shows the results of a germination study after 1.5 years' storage in air and Table 4-2.7 that after 2.0 years' storage in air. The graphs 4-2.6(a) and (b) represent the results after 1.5 years. One interesting feature of these graphs is the enhanced damage undergone by the 0.5 Mrep seeds on prolonged storage. The period of maximum germination has occurred at the same time as that of the control seeds but a pronounced shoulder has appeared on the right hand side of this curve showing that a delay was occurring in the germination of a considerable number of the seeds. In contrast, however, the 1.0 Mrep seeds are displaying

Table 4-2.2

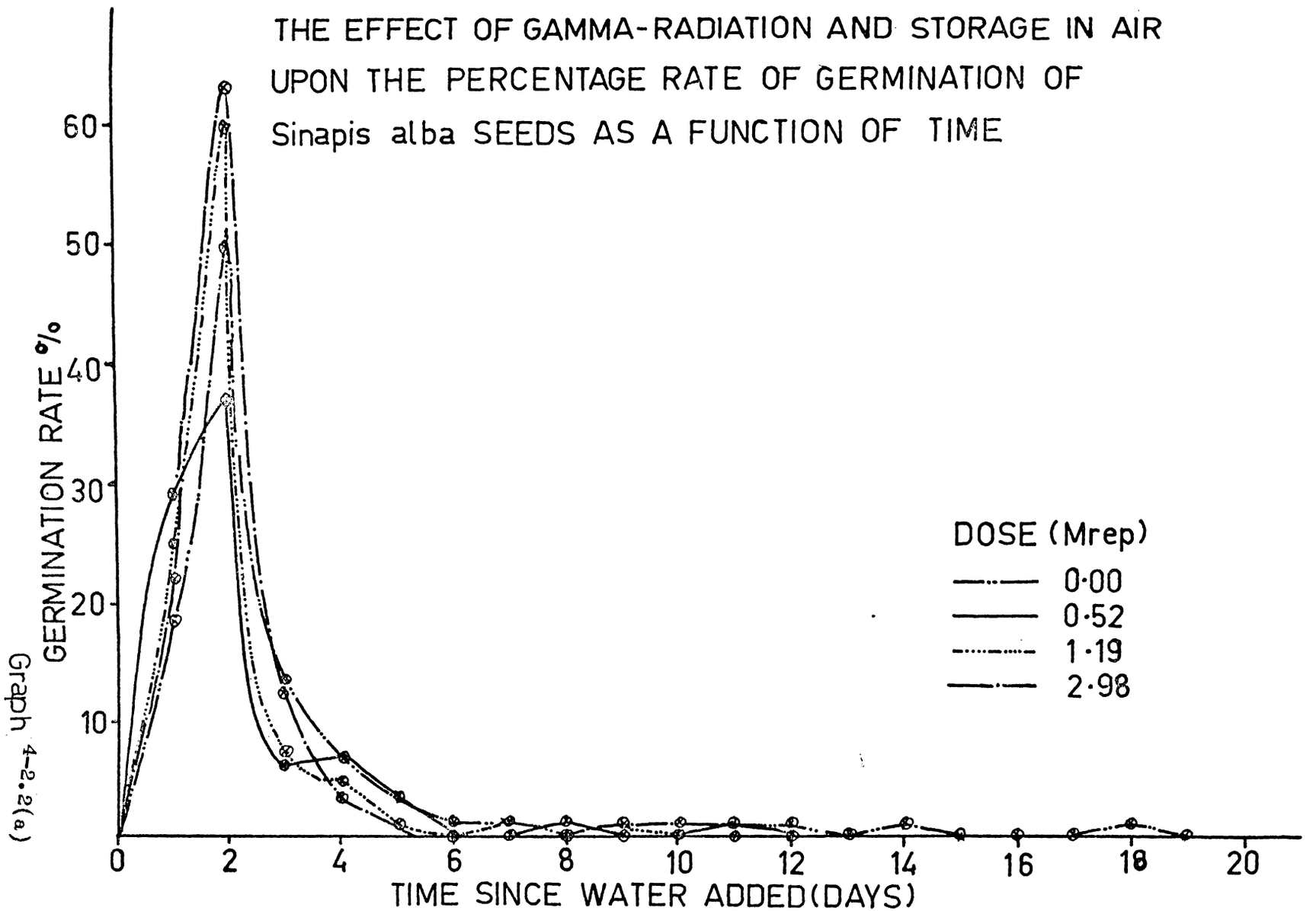
The Germination of Sinapis alba Seeds after 5 months storage "in air". [338.]

Dose (Mrad)	0.00		0.52		1.19		2.98		5.08		10.22	
Dose Rate Mrad/hr	0		0.214		0.214		0.214		0.214		0.214	
Time Exposed (hrs)	0		2.4		5.5		13.8		23.6		47.5	
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	18	18	29	29	23	23	20	20	21	21	25	25
2	51	69	37	66	60	83	63	83	57	78	50	75
3	10	79	6	72	7	90	12	95	8	86	8	83
4	4	83	7	79	5	95	3	98	8	94	3	86
5	3	86	3	82	1	96	1	99	0	94	0	86
6	1	87	0	82	0	96	0	99	0	94	0	86
7	1	88	0	82	1	97	0	99	2	96	1	87
8	0	88	1	83	0	97	0	99	1	97	2	89
9	1	89	0	83	1	98	0	99	0	97	1	90
10	1	90	0	83	0	98	0	99	0	97	0	90
11	1	91	1	84	0	98	0	99	0	97	0	90
12	1	92	0	84	0	98	0	99	0	97	0	90
13	0	92	0	84	0	98	0	99	0	97	0	90
14	1	93	0	84	0	98	0	99	0	97	0	90
15	0	93	0	84	0	98	0	99	0	97	0	90
16	0	93	0	84	0	98	0	99	0	97	0	90
17	0	93	0	84	0	98	0	99	0	97	0	90
18	1	94	0	84	0	98	0	99	0	97	0	90
19	0	94	0	84	0	98	0	99	0	97	0	90
20	0	94	0	84	0	98	0	99	0	97	0	90

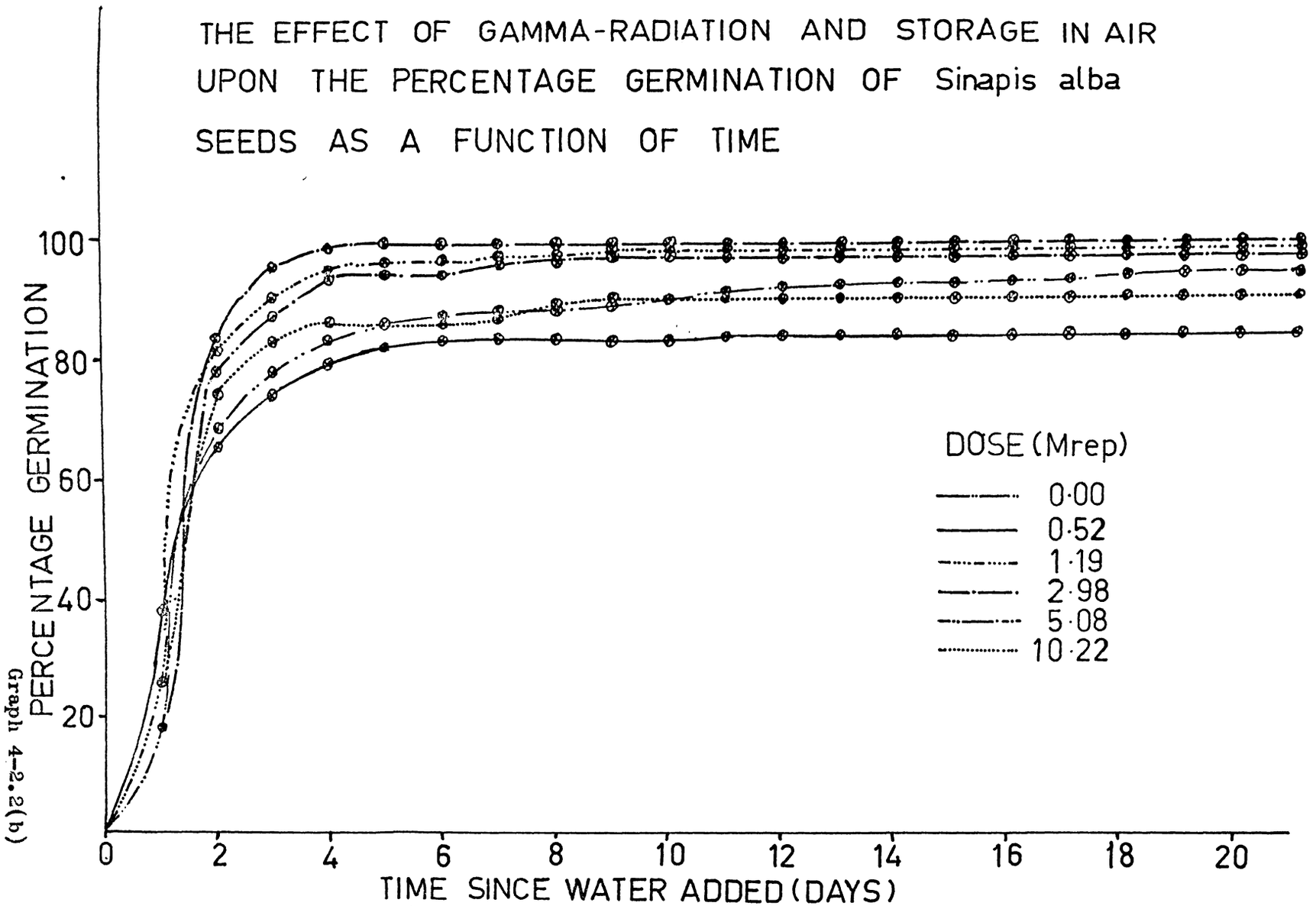
* (a) % Germinated previous 24 hours

* (b) Total % germination.

Note: The values for seeds having received 5.08 and 10.22 Mrep have not been shown on graph 4-2.2(a) to avoid overcrowding the graph. The time interval in which the maximum rate of germination occurred was, as can be seen clearly above, the same as that for seeds having recovered from smaller doses of radiation.


THE EFFECT OF GAMMA-RADIATION AND STORAGE IN AIR
UPON THE PERCENTAGE RATE OF GERMINATION OF
Sinapis alba SEEDS AS A FUNCTION OF TIME
GERMINATION RATE %
60
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
TIME SINCE WATER ADDED(DAYS)
DOSE (Mrep)
0·00
0·52
1·19
2·98

Graph 4-2.2(a)


THE EFFECT OF GAMMA-RADIATION AND STORAGE IN AIR
UPON THE PERCENTAGE GERMINATION OF Sinapis alba
SEEDS AS A FUNCTION OF TIME
PERCENTAGE GERMINATION
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
TIME SINCE WATER ADDED (DAYS)
DOSE (Mrep)
0·00
0·52
1·19
2·98
5·08
10·22

Graph 4-2.2(b)

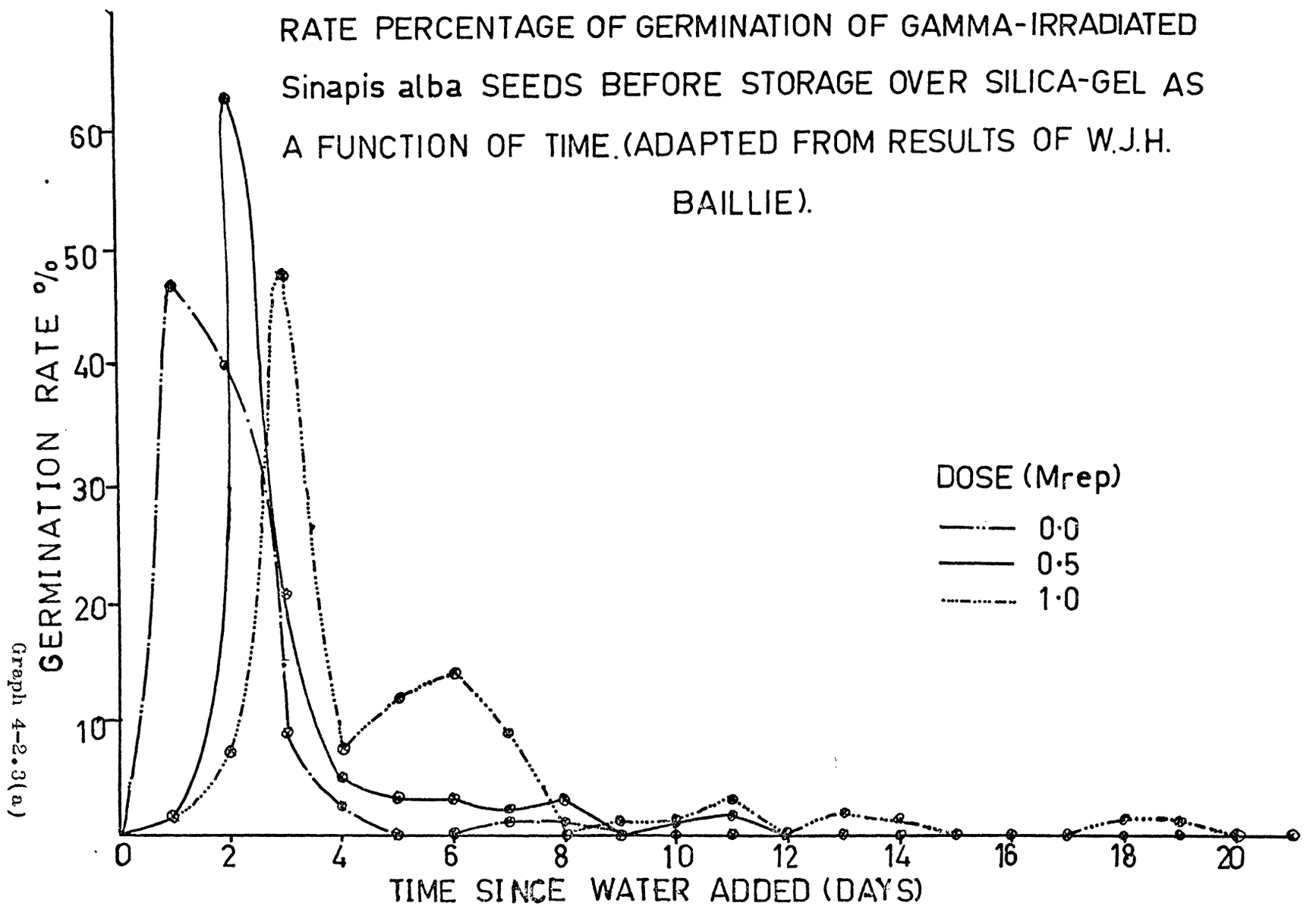
Table 4-2.3

The Effect of Gamma Radiation before Storage over Silica Gel on Sinapis alba Seeds (adapted from the results of W.J.H. Baillie[320]).

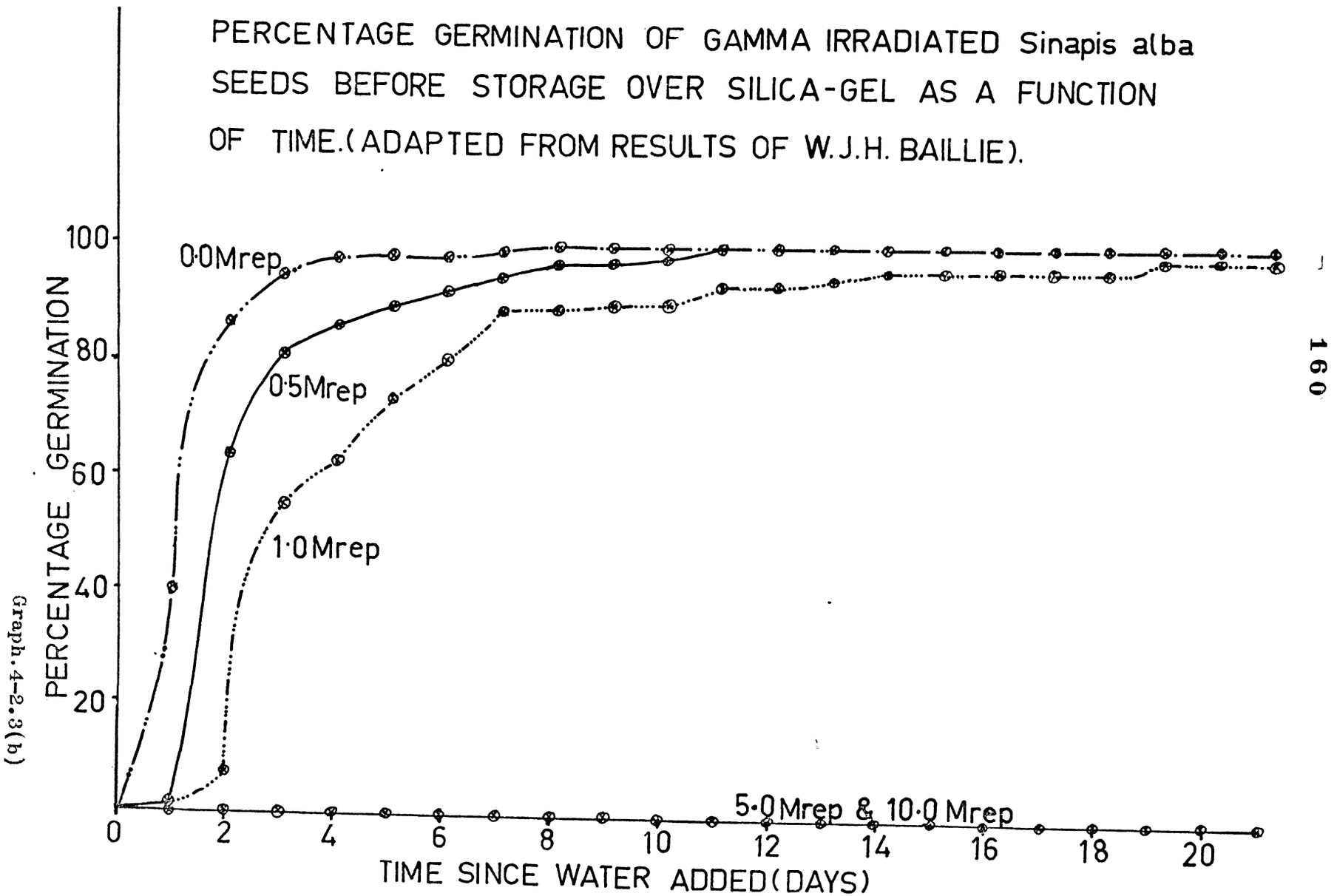
Dose (Mrad)	0.0		0.5		1.0		5.0		10.00			
Dose Rate Mrad/hr	0.00		7.14		14.3		64.0		64.0			
Time Exposed (hrs)			70		70		71		140			
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	47	47	1	1	1	1	0	0	0	0		
2	40	87	63	64	6	7						
3	3	96	17	81	48	55						
4	2	98	5	86	7	62						
5	0	98	3	89	12	74						
6	0	98	3	92	6	80						
7	1	99	2	94	9	89						
8	1	100	3	97	0	89						
9	–	100	0	97	1	90						
10	–	100	1	98	0	90						
11	–	100	2	100	3	93						
12	–	100	–	100	0	93						
13	–	100	–	100	2	95						
14	–	100	–	100	1	96						
15	–	100	–	100	0	96						
16	–	100	–	100	0	96						
17	–	100	–	100	0	96						
18	–	100	–	100	1	97						
19	–	100	–	100	1	98						
20	–	100	–	100	0	98						

* (a) % Germinated previous 24 hours

* (b) Total % germination.


RATE PERCENTAGE OF GERMINATION OF GAMMA-IRRADIATED Sinapis alba SEEDS BEFORE STORAGE OVER SILICA-GEL AS A FUNCTION OF TIME. (ADAPTED FROM RESULTS OF W.J.H. BAILLIE).
GERMINATION RATE %
TIME SINCE WATER ADDED (DAYS)
DOSE (Mrep)
0·0
0·5
1·0
0
2
4
6
8
10
12
14
16
18
20
10
20
30
40
50
60

Graph 4-2.3(a)


PERCENTAGE GERMINATION OF GAMMA IRRADIATED Sinapis alba SEEDS BEFORE STORAGE OVER SILICA-GEL AS A FUNCTION OF TIME.(ADAPTED FROM RESULTS OF W.J.H. BAILLIE).
PERCENTAGE GERMINATION
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
TIME SINCE WATER ADDED(DAYS)
0·0Mrep
0·5Mrep
1·0Mrep
5·0Mrep & 10·0 Mrep

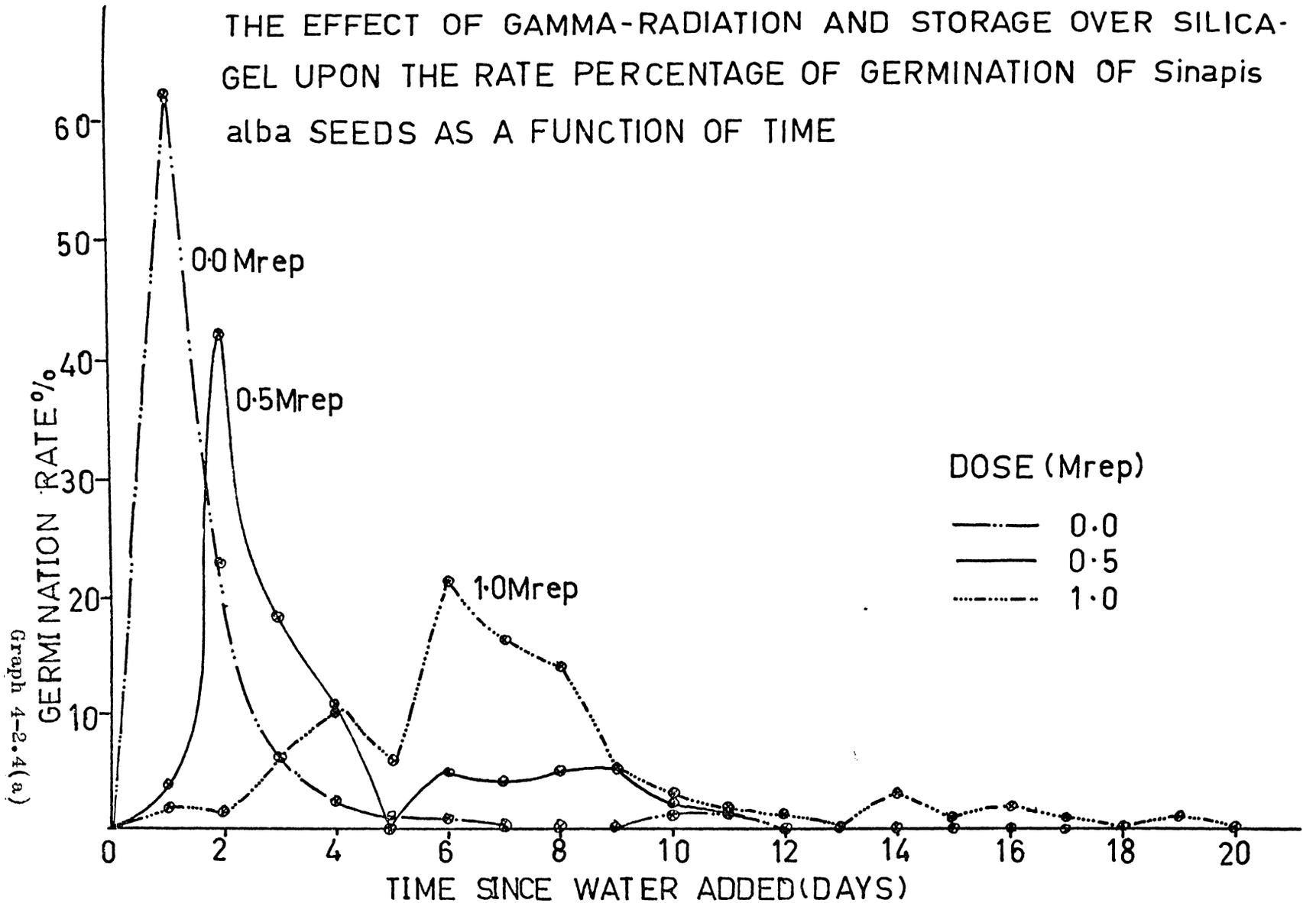
Graph. 4-2.3(b)

Table 4-2.4

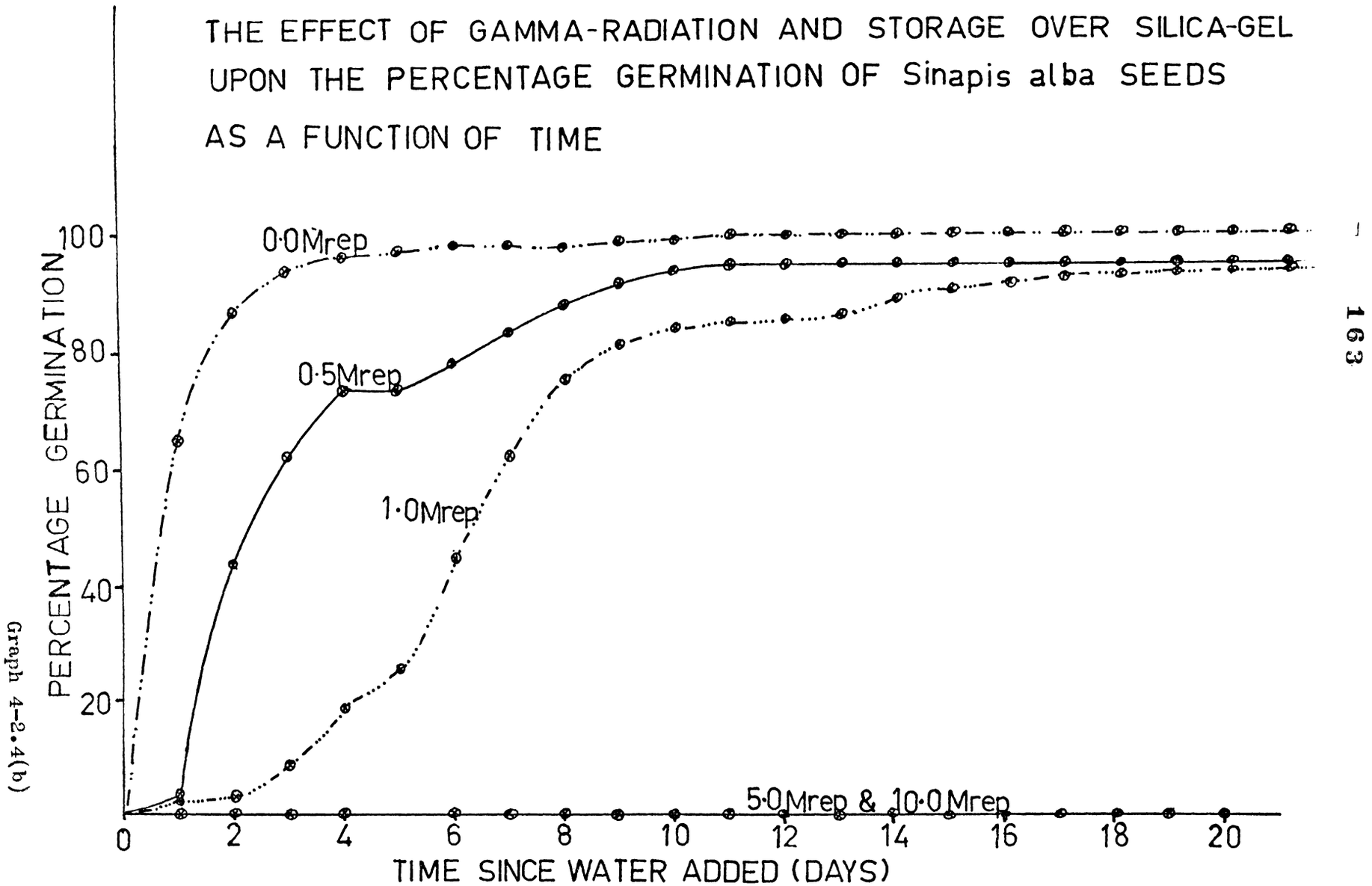
The Effect of Gamma Radiation and Storage over Silica-Gel upon the Germination of Sinapis alba Seeds.[338]

Dose (Mrad)	0.0		0.5		1.0		5.0		10.0			
Dose Rate Mrad/hr	0.00		7.14		14.3		64.0		64.0			
Time Exposed (hrs)			70		70		71		140			
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	65	65	2	2	2	2	0	0	0	0		
2	23	88	42	44	1	3						
3	6	94	18	62	6	9						
4	2	96	11	73	10	19						
5	1	97	0	73	6	25						
6	1	98	5	78	21	46						
7	0	98	4	82	16	62						
8	0	98	5	87	14	76						
9	0	98	5	92	5	81						
10	1	99	2	94	3	84						
11	1	100	1	95	1	85						
12	-	100	0	95	1	86						
13	-	100	0	95	0	86						
14	-	100	0	95	3	89						
15	-	100	0	95	1	90						
16	-	100	0	95	2	92						
17	-	100	0	95	1	93						
18	-	100	0	95	0	93						
19	-	100	0	95	1	94						
20	-	100	0	95	0	94						

* (a) % Germinated previous 24 hours
* (b) Total % germination.


THE EFFECT OF GAMMA-RADIATION AND STORAGE OVER SILICA-GEL UPON THE RATE PERCENTAGE OF GERMINATION OF Sinapis alba SEEDS AS A FUNCTION OF TIME
GERMINATION RATE %
TIME SINCE WATER ADDED (DAYS)
0·0 Mrep
0·5 Mrep
1·0 Mrep
DOSE (Mrep)
0·0
0·5
1·0
0
2
4
6
8
10
12
14
16
18
20
10
20
30
40
50
60

Graph 4-2.4(a)


THE EFFECT OF GAMMA-RADIATION AND STORAGE OVER SILICA-GEL
UPON THE PERCENTAGE GERMINATION OF Sinapis alba SEEDS
AS A FUNCTION OF TIME
PERCENTAGE GERMINATION
100
80
60
40
20
0·0Mrep
0·5Mrep
1·0Mrep
5·0Mrep & 10·0Mrep
0
2
4
6
8
10
12
14
16
18
20
TIME SINCE WATER ADDED (DAYS)

Graph 4-2.4(b)

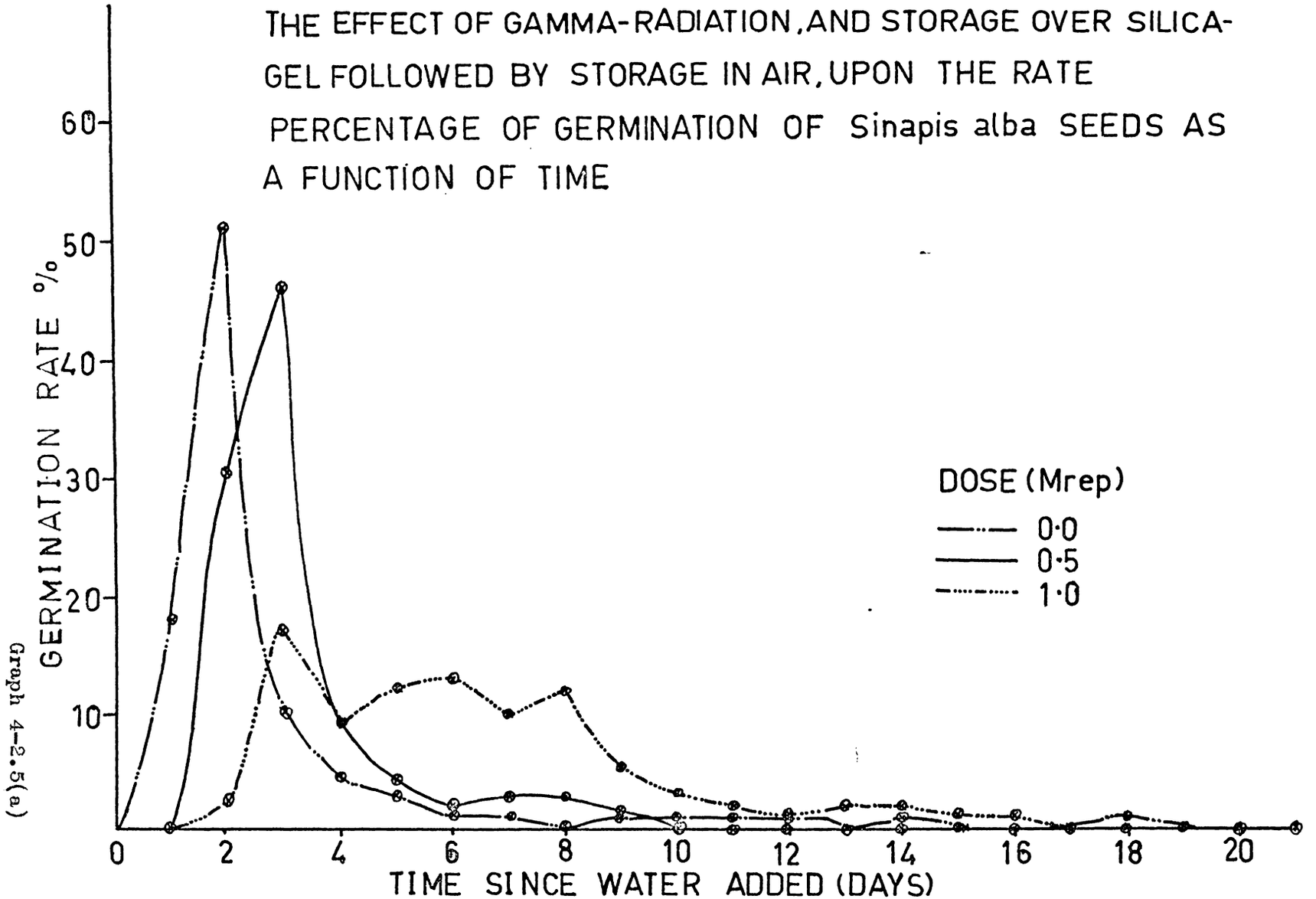
Table 4-2.5

The Effect of Gamma-Radiation and Storage over Silica-Gel followed by Storage in Air, upon the Germination of Sinapis alba Seeds.[338]

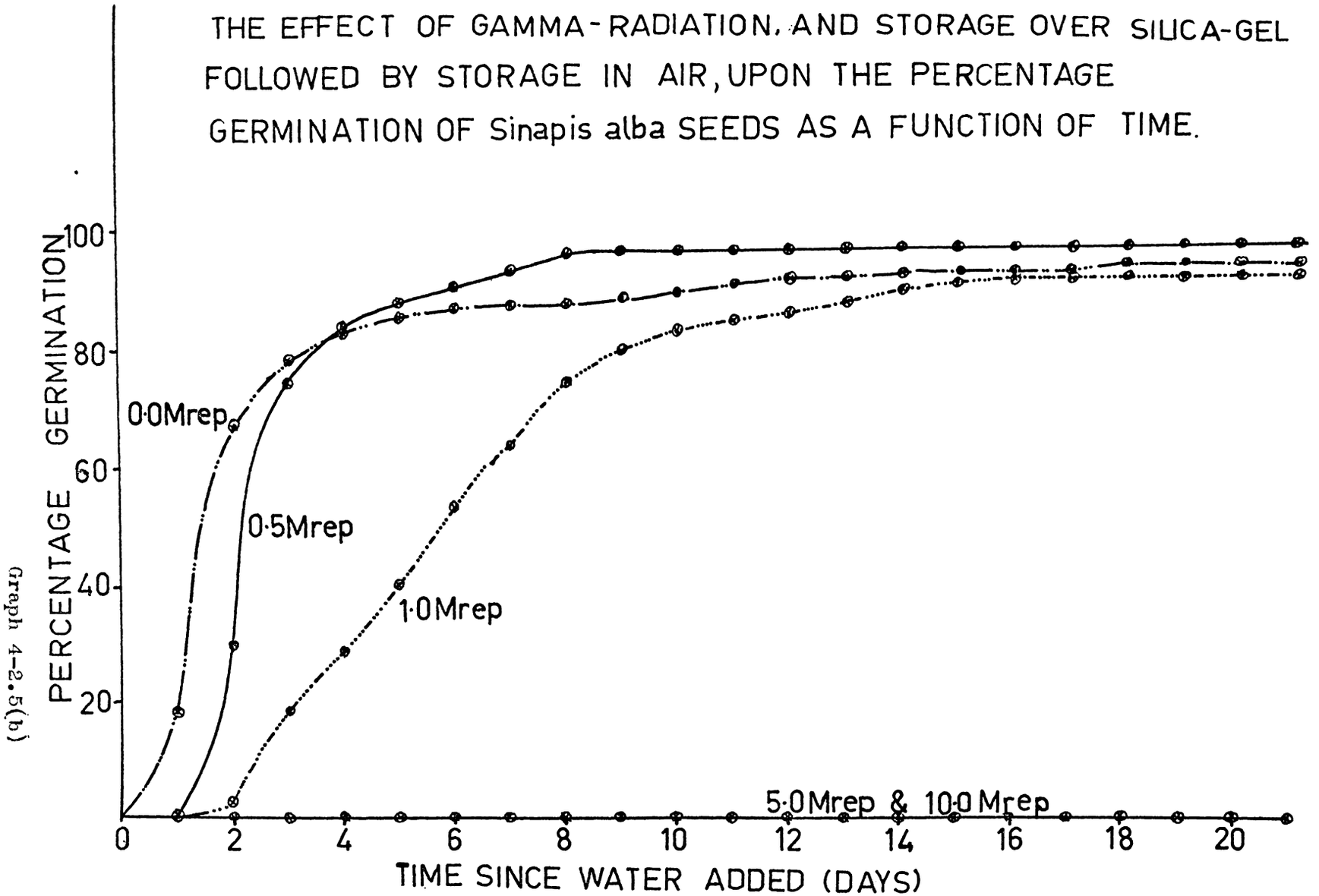
Dose (Mrad)	0.0		0.5		1.0		5.0		10.0			
Dose Rate Mrad/hr	0.00		7.14		14.3		64.0		64.0			
Time Exposed (hrs)	0		70		70		71		140			
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	18	18	0	0	0	0	0	0	0	0		
2	51	69	29	29	2	2						
3	10	79	46	75	17	19						
4	4	83	9	84	9	28						
5	3	86	4	88	12	40						
6	1	87	2	90	13	53						
7	1	88	3	93	10	63						
8	0	88	3	96	12	75						
9	1	89	1	97	5	80						
10	1	90	0	97	3	83						
11	1	91	0	97	2	85						
12	1	92	0	97	1	86						
13	0	92	0	97	2	88						
14	1	93	0	97	2	90						
15	0	93	0	97	1	91						
16	0	93	0	97	1	92						
17	0	93	0	97	0	92						
18	1	94	0	97	0	92						
19	0	94	0	97	0	92						
20	0	94	0	97	0	92						

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Note: These seeds had been stored in air for 5 months after removal from the silica-gel. (c.f. the seeds which recovered fully).


THE EFFECT OF GAMMA-RADIATION, AND STORAGE OVER SILICA-GEL FOLLOWED BY STORAGE IN AIR, UPON THE RATE PERCENTAGE OF GERMINATION OF Sinapis alba SEEDS AS A FUNCTION OF TIME
GERMINATION RATE %
60
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
TIME SINCE WATER ADDED (DAYS)
DOSE (Mrep)
0·0
0·5
1·0

Graph 4-2.5(a)


THE EFFECT OF GAMMA-RADIATION, AND STORAGE OVER SILICA-GEL FOLLOWED BY STORAGE IN AIR, UPON THE PERCENTAGE GERMINATION OF Sinapis alba SEEDS AS A FUNCTION OF TIME.
PERCENTAGE GERMINATION
100
80
60
40
20
0·0Mrep
0·5Mrep
1·0Mrep
5·0Mrep & 10·0Mrep
0
2
4
6
8
10
12
14
16
18
20
TIME SINCE WATER ADDED (DAYS)

Graph 4-2.5(b)

complete recovery, and in fact this group germinated more rapidly than the control group. After only 6 months' storage in air a slight recovery was evident but the period of maximum germination for these seeds extended from the 2nd to the 9th day. The third important feature of these graphs is the 4% germination which was obtained for the seeds which had received a dose of 2.0 Mrep. These seeds did not germinate in previous trials involving a shorter period of storage and hence they too are showing significant recovery.

After 2.0 years' storage in air the 0.5 Mrep group had undergone further damage. This can be readily seen in Graph 4-2.7(a) in which the "shoulder" of graph 4-2.6(a), corresponding to the 5th day of germination, has now become the highest peak. The maximum rate of germination of the 0.5 Mrep group has therefore decreased and occurred progressively later on prolonged storage in air. The reverse is true for the 1.0 Mrep group. After 2 years' storage at room humidities these seeds are showing complete recovery. Their ability to germinate in terms of both rate and total percent, surpasses that of the control, non-irradiated seeds. In the first 24 hours of imbibition 70% of the 1.0 Mrep germinated as compared to the value of only 32% for the control seeds. The total % germination of these groups was 89% and 85% respectively. Although a difference of only 5% is not [redacted] significant it is interesting that a similar result was obtained after 1.5 years' storage in air. A small number of the 2.0 Mrep group retained the ability to germinate first displayed after 1.5 years' storage at room humidities.

These results show that mustard seeds which have been exposed to various doses of gamma-radiation can recover in their germinating ability by storage at normal room humidities but not by storage over silica-gel.

Table 4-2.6

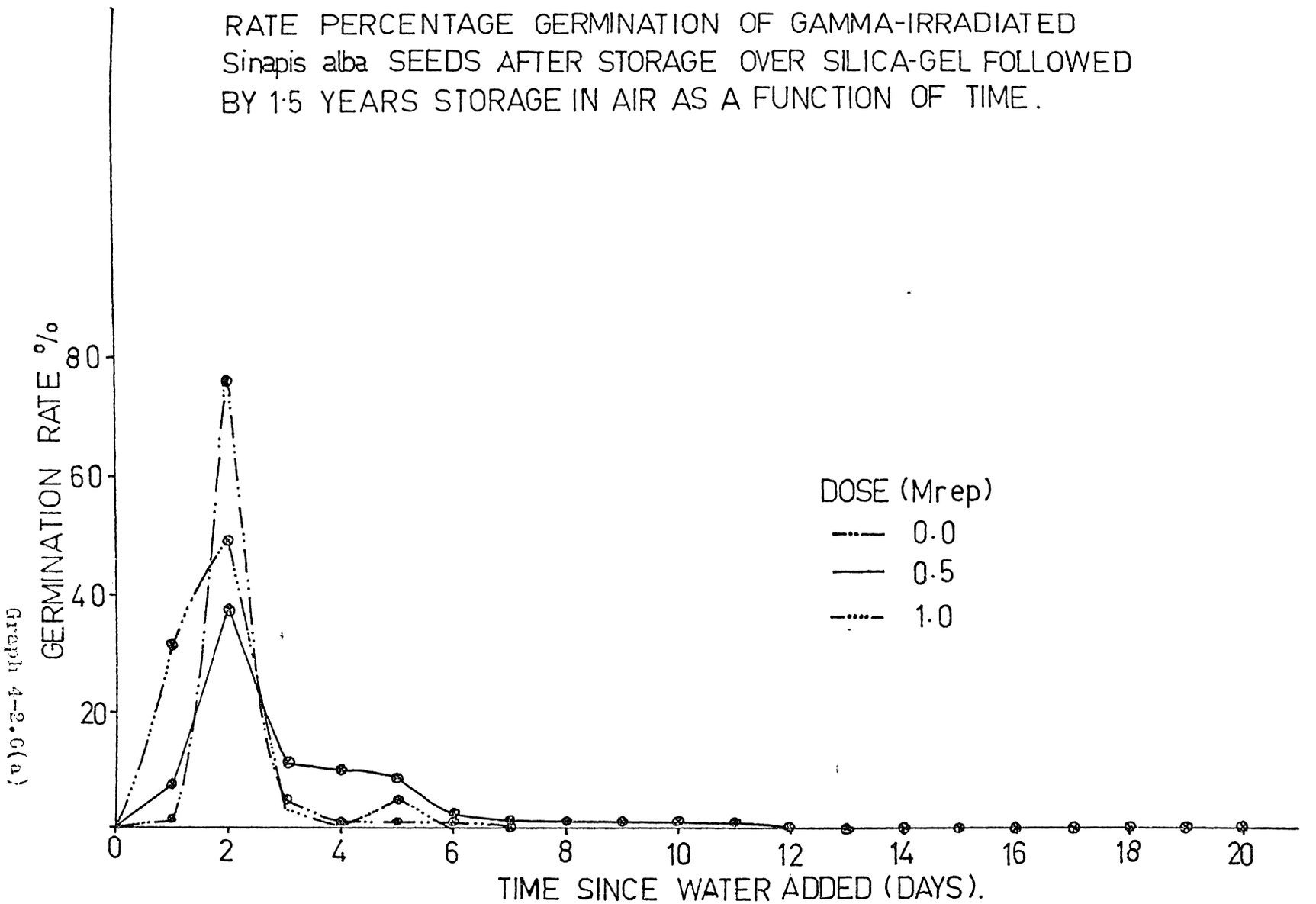
The Effects of Gamma-Radiation, Storage over Silica-Gel and 1½ Years' Storage in Air Upon the Germination of Sinapis alba Seeds.

Dose (Mrad)	0.0		0.5		1.0		2.0+		5.0		10.0	
Dose Rate Mrad/hr	0.00		7.14		14.3		28.6		64.0		64.0	
Time Exposed (hrs)	0		70		70		70		71		140	
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	1	1	0	0	31	31	1	1	0	0	0	0
2	76	77	7	7	49	80	0	1				
3	5	82	38	45	4	84	2	3				
4	1	83	11	56	1	85	0	3				
5	1	84	10	66	5	90	1	4				
6	1	85	9	75	0	90	0	4				
7	0	85	2	77	0	90	0	4				
8	0	85	1	78	0	90	0	4				
9	0	85	1	79	0	90	0	4				
10	0	85	1	80	0	90	0	4				
11	0	85	1	81	0	90	0	4				
12	0	85	1	82	0	90	0	4				
13	0	85	0	82	0	90	0	4				
14	0	85	0	82	0	90	0	4				
15	0	85	0	82	0	90	0	4				
16	0	85	0	82	0	90	0	4				
17	0	85	0	82	0	90	0	4				
18	0	85	0	82	0	90	0	4				
19	0	85	0	82	0	90	0	4				
20	0	85	0	82	0	90	0	4				

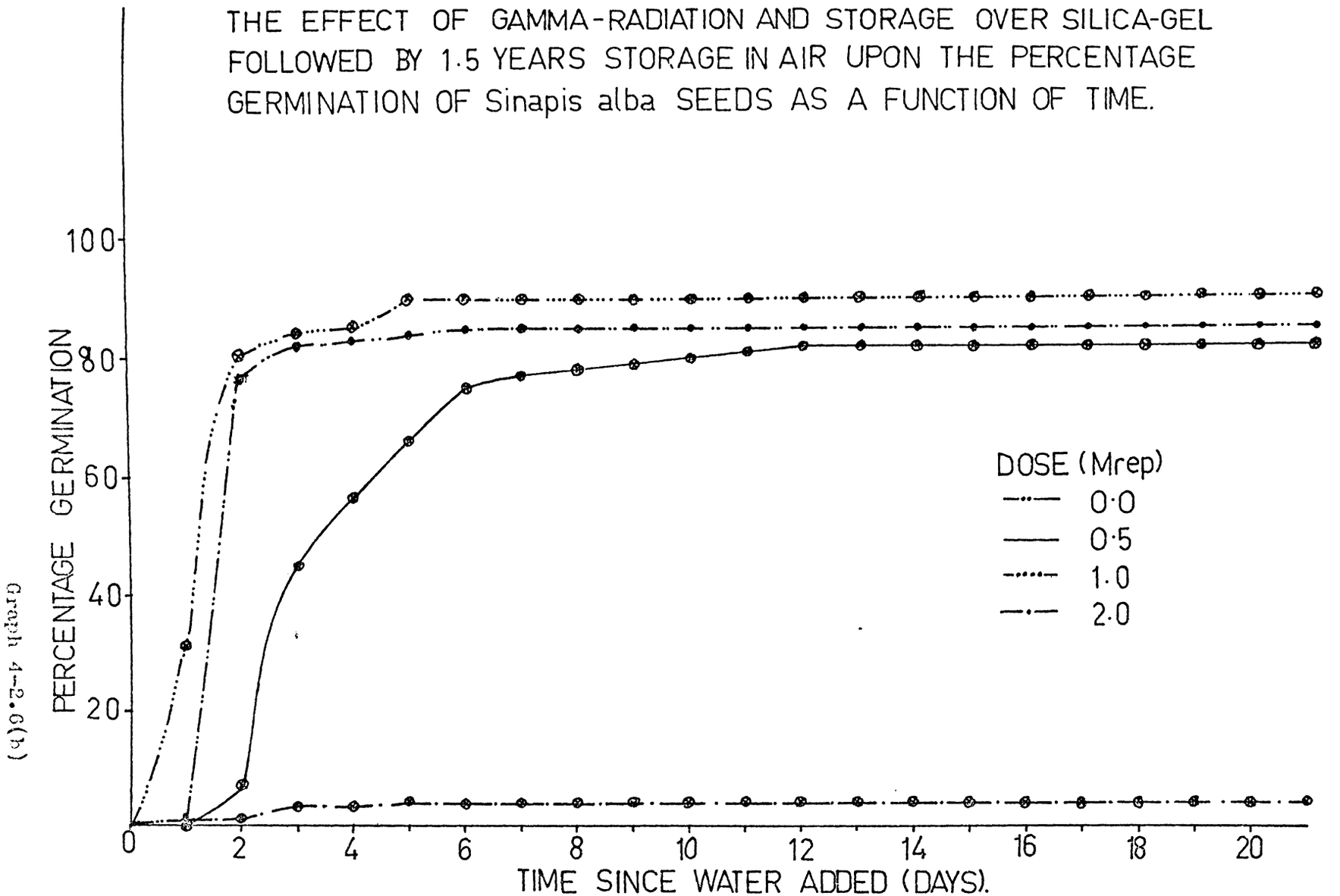
* (a) % Germinated previous 24 hours

* (b) Total % germination.

+Note: The 2.0 Mrep group have not been included in the previous tables but no germination of these seeds had been obtained previous to this germination run. Hence, the values shown on the earlier tables for the 5.0 and 10.0 Mrep groups also apply to these seeds.


RATE PERCENTAGE GERMINATION OF GAMMA-IRRADIATED
Sinapis alba SEEDS AFTER STORAGE OVER SILICA-GEL FOLLOWED
BY 1·5 YEARS STORAGE IN AIR AS A FUNCTION OF TIME.
GERMINATION RATE %
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
TIME SINCE WATER ADDED (DAYS).
DOSE (Mrep)
0·0
0·5
1·0

Graph 4-2.6(a)


THE EFFECT OF GAMMA-RADIATION AND STORAGE OVER SILICA-GEL FOLLOWED BY 1.5 YEARS STORAGE IN AIR UPON THE PERCENTAGE GERMINATION OF Sinapis alba SEEDS AS A FUNCTION OF TIME.
PERCENTAGE GERMINATION
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
TIME SINCE WATER ADDED (DAYS).
DOSE (Mrep)
0·0
0·5
1·0
2·0

Graph 4-2.6(b)

Table 4-2.7

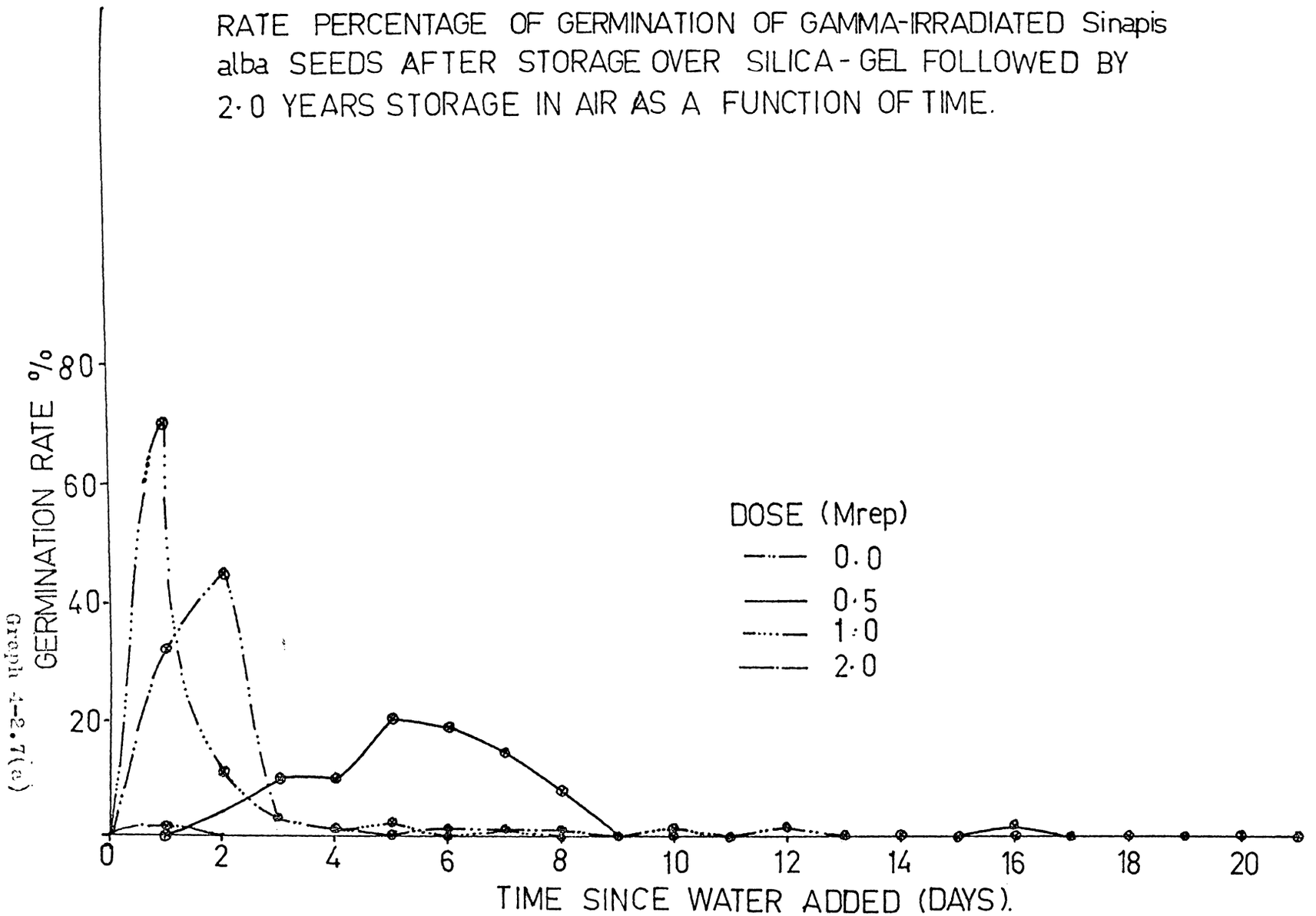
The Effect of Gamma-Radiation, Storage over Silica-Gel (4 years) and 2 years Storage in Air Upon the Germination of Sinapis alba Seeds.

Dose (Mrad)	0.0		0.5		1.0		2.0		5.0		10.0	
Dose Rate Mrad/hr	0.0		7.14		14.3		28.6		64.0		64.0	
Time Exposed (hrs)	0		70		70		70		71		140	
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	32	32	0	0	70	70	1	1	0	0	0	0
2	45	77	4	4	11	81	0	1				
3	3	80	10	14	3	84	0	1				
4	1	81	10	24	1	85	0	1				
5	0	81	20	44	2	87	0	1				
6	1	82	19	63	0	87	0	1				
7	1	83	5	68	1	88	0	1				
8	1	84	8	76	0	88	0	1				
9	0	84	0	76	0	88	0	1				
10	0	84	0	76	1	89	0	1				
11	0	84	0	76	0	89	0	1				
12	1	85	1	77	0	89	0	1				
13	0	85	0	77	0	89	0	1				
14	0	85	0	77	0	89	0	1				
15	0	85	0	77	0	89	0	1				
16	0	85	1	78	0	89	0	1				
17	0	85	0	78	0	89	0	1				
18	0	85	0	78	0	89	0	1				
19	0	85	0	78	0	89	0	1				
20	0	85	0	78	0	89	0	1				

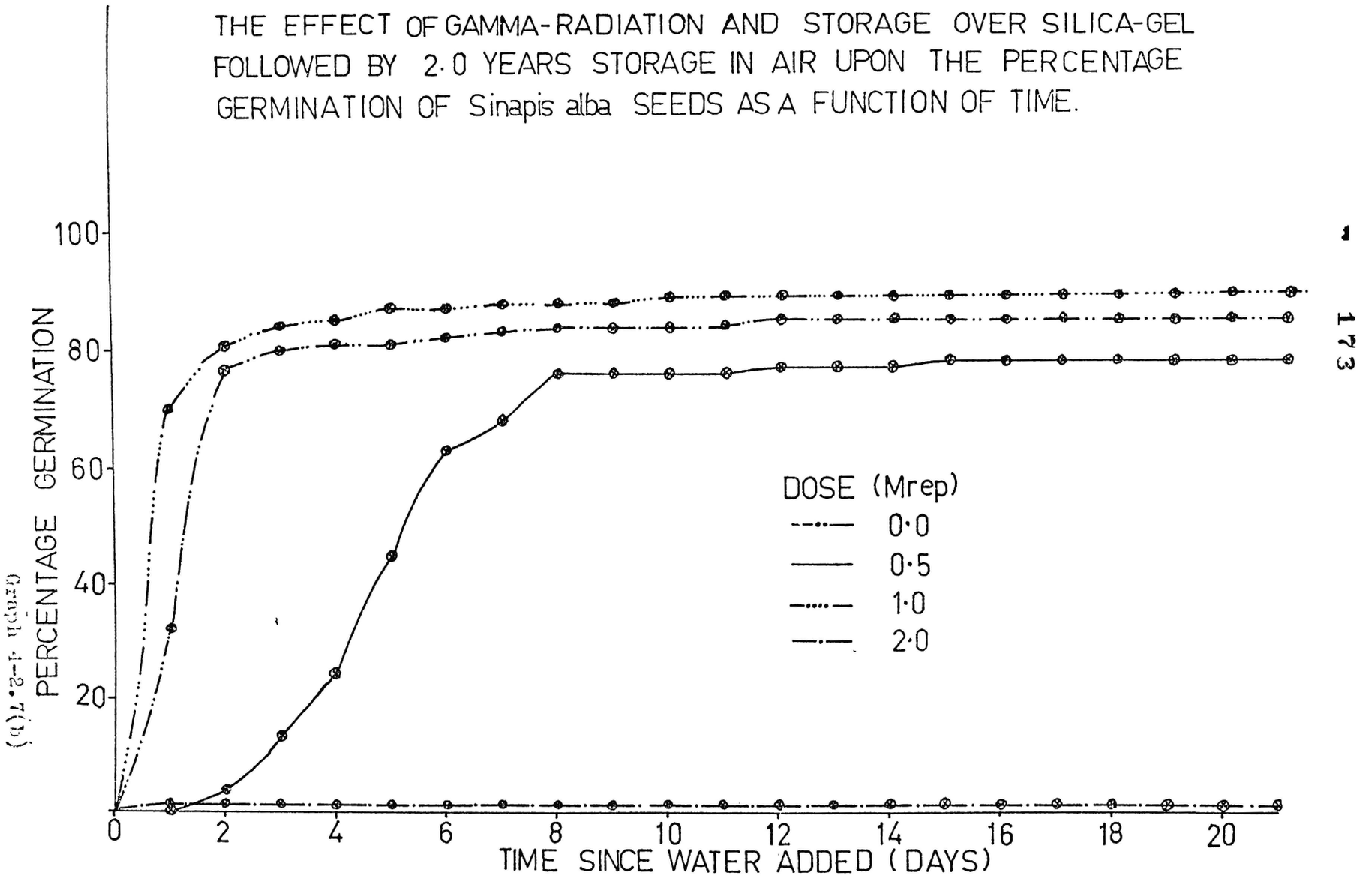
* (a) % Germinated previous 24 hours

* (b) Total % germination.

RATE PERCENTAGE OF GERMINATION OF GAMMA-IRRADIATED Sinapis alba SEEDS AFTER STORAGE OVER SILICA-GEL FOLLOWED BY 2·0 YEARS STORAGE IN AIR AS A FUNCTION OF TIME.



Graph 4-2.7(a)


THE EFFECT OF GAMMA-RADIATION AND STORAGE OVER SILICA-GEL FOLLOWED BY 2·0 YEARS STORAGE IN AIR UPON THE PERCENTAGE GERMINATION OF Sinapis alba SEEDS AS A FUNCTION OF TIME.
PERCENTAGE GERMINATION
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
TIME SINCE WATER ADDED (DAYS)
DOSE (Mrep)
0·0
0·5
1·0
2·0

The problem encountered in this section was to obtain an adequate definition of a dead seed. Baillie[320] in his discussion concluded that a seed which cannot germinate may be said to have been killed by irradiation. From the results presented here it is obvious that we cannot say death was caused by irradiation of Sinapis alba seeds. A secondary dormancy was induced in the seeds by the gamma-rays and the subsequent storage conditions control the effect of irradiation more completely than has been supposed by previous workers.

Death is usually defined as the irreversible stoppage of "vital activity". It may occur as the ultimate conclusion of the process of senescene, or it may occur earlier due to an environmental or hereditary agent. Induced-death is regarded either as a response to any one type of insult exceeding its tolerance limit, or the result of a sum of varied kinds of injury exceeding a critical threshhold. At the single cell level, death is usually specified in terms of reproductive ability. Bacteria which fail to divide are said to be dead. In higher forms, the individual is considered dead when activities have ceased in the cells which are essential for co-ordination of the organism. Certain tissues or even separate cells may remain functional for hours or days after the organism is considered dead. This process is not, however, normally considered reversible, i.e. once an organism is classified as dead, the level of injury in each cell will increase and eventually no cells will remain functional.

Combining the data obtained on the germination of gamma-irradiated mustard seeds before storage of those irradiated in 1968 with those irradiated in 1964 the total germinations expressed as % of control shown in Table 4-2.8 are obtained.

Table 4-2.8

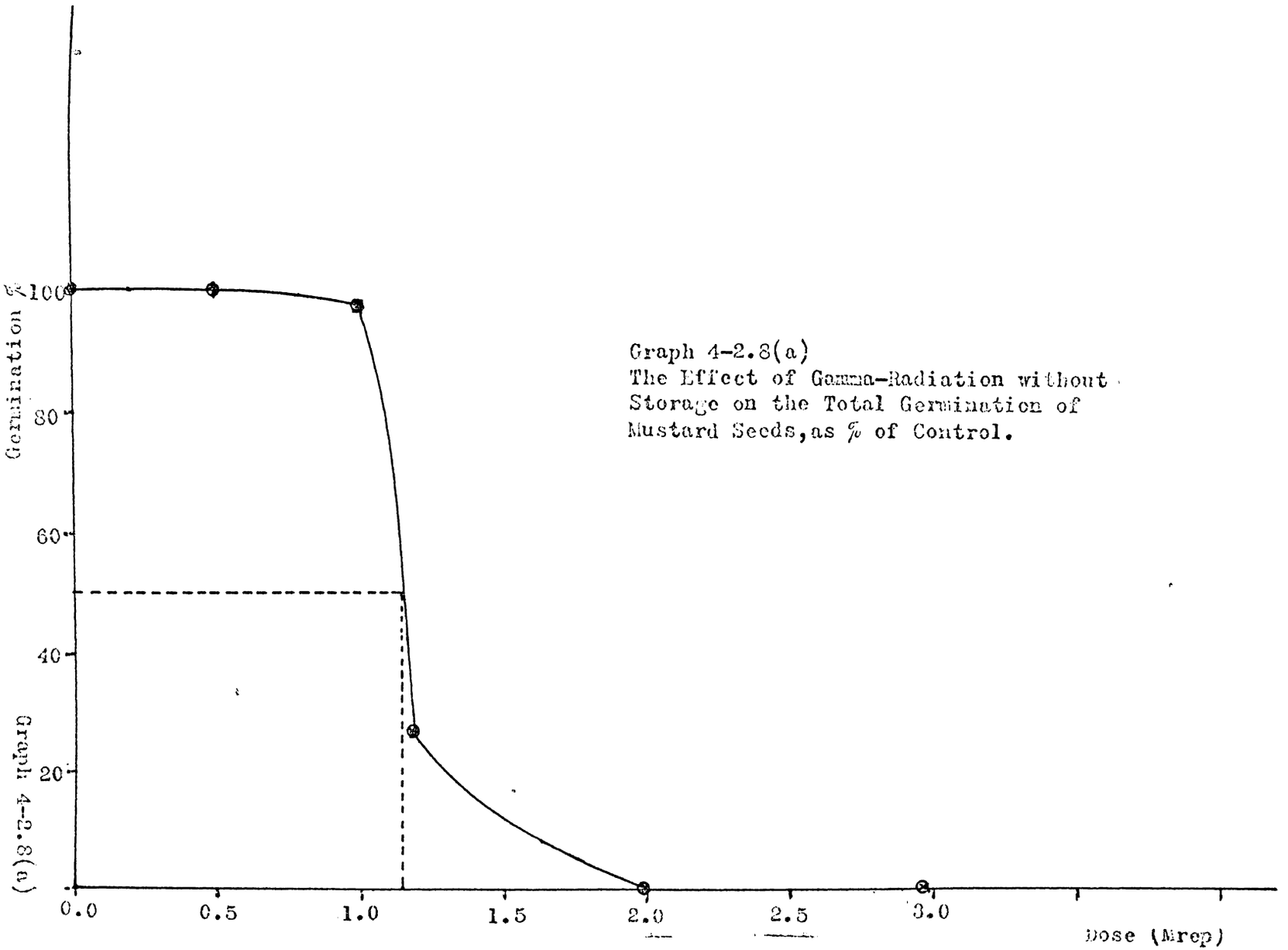
The Effect of Gamma-Radiation without Storage upon the Total Germination of Mustard Seeds, Expressed as Percentage of the Control

Dose (Mrep)	0.0	0.5	1.0	1.19	2.0	2.98
% Control	100%	100%	98%	27%	0%	0%

The effects of gamma-radiation on seedling growth has been shown to depend on dose rate. As shown in the earlier tables the dose rates differ among the dose groups used in this table. Assuming a similar relationship exists between germination and dose-rate, the data shown above will only give an approximate value for the LD_{50} for the germination of mustard seeds.

The germination expressed as a percentage of the control was plotted against the dose received and this is shown in graph 4-2.8(a). From this graph it can be seen that the approximate LD_{50} for the germination of Sinapis alba is 1.15 Mrep. The curve of the dependence of a character, X, on the dose received is generally S-shaped, as was obtained using this data. Because of the shape of the curve the LD_{50} dose often differs little from the LD_{90} dose in practice. Theoretically the LD_{100} dose is never attained so that LD_{100} values obtained in experiments are normally quoted as the "practical LD_{100}".[177]

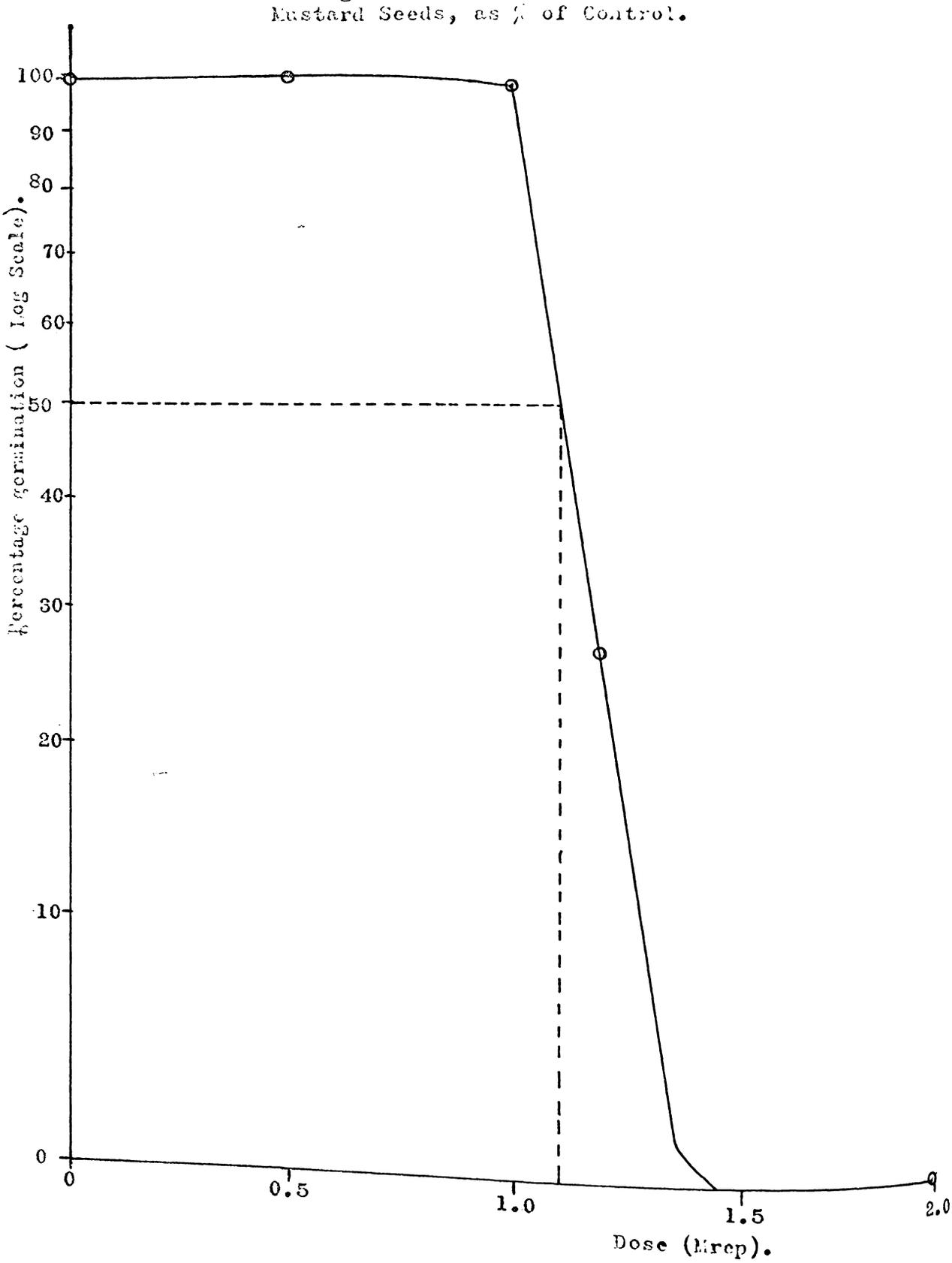
The target theory predicts that a graph of % survival in log units versus dose received will be a straight line. Assuming this to be true graph 4-2.8(b) was drawn using logarithmic paper. The value obtained by this method corresponding to the LD_{50} for the germination of Sinapis alba seeds was 1.1 Mrep.



Graph 4-2.8(a)
The Effect of Gamma-Radiation without Storage on the Total Germination of Mustard Seeds, as % of Control.

(See Over)

Graph 4-2.8(b)
The Effect of Gamma-Radiation without Storage on the Total Germination of Mustard Seeds, as % of Control.




CONTROL
(1969 seeds)
3/4/69
BLANK
1968
3/4/69
0.52
6/4/69
1.19
.3/4
2.98
3/4
5.08.
6/4
10.22
3/4

Plate 1.
The Growth of the Plants.

Control
Blank
1.19 Mrep
2.98 Mrep
10.22 Mrep

15 Days

0.52 Mrep
5.08 Mrep

12 Days

4-3 The Growth of Plants from Irradiated Seeds

Before storage only the 0.0, 0.52 and 1.19 Mrep groups showed the ability to germinate. Plants grown from the irradiated seeds showed the stunted growth, considered to be typical for plants grown from heavily irradiated seeds. Typical results from the growth of such seeds are shown in Fig. 4-3.1 which is of the growth of plants from the seeds irradiated in 1964 by Baillie.[320] Similar results were obtained for the seeds irradiated in 1968 before storage.

After recovery had occurred it was decided to investigate the growth of the plants from the recovered seeds. In particular it was desired to determine whether or not the plants formed were viable, and if so whether they could be grown to maturity to give sterile or fertile seeds. The method used is described in Section 4-1.3.

The growth of these plants was watched carefully and photos taken at various time intervals. It soon became obvious that the plants from the irradiated but recovered seeds were larger than those from either the blank or control seeds. Plates one, two and three show these plants at various stages of their early growth. The difference between the 0.52 Mrep group and the control group can be seen even in the 15 or 12 day old seedlings. As the plants grew, those from the remaining groups of irradiated seeds also became obviously larger than those of the blank or control groups.

Although the growth of the plants had not been undertaken in a controlled environment and the layout used was not randomised it was decided to measure various characteristics and compare these between the dose groups. When the control, blank, 1.19 Mrep, 2.98 Mrep and 10.22 Mrep groups were 33 days old and the 0.52 and 5.08 Mrep groups were 30 days old, the height of the plant, the length of the cotyledons, of the primary leaves and of the secondary leaves were measured. As it was

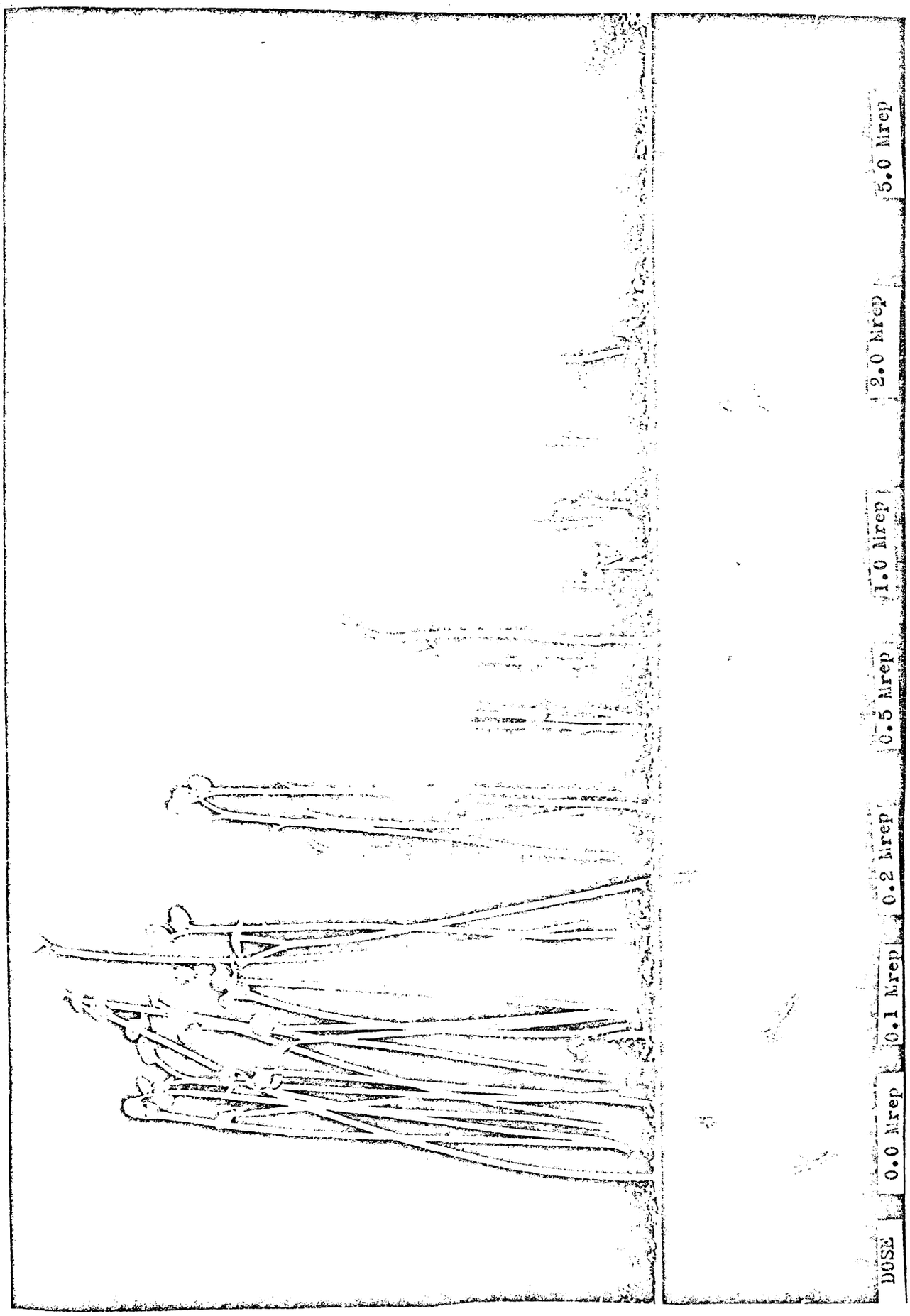


Fig. 4-3.1
The Growth of Plants from freshly irradiated Sinapis alba Seeds.

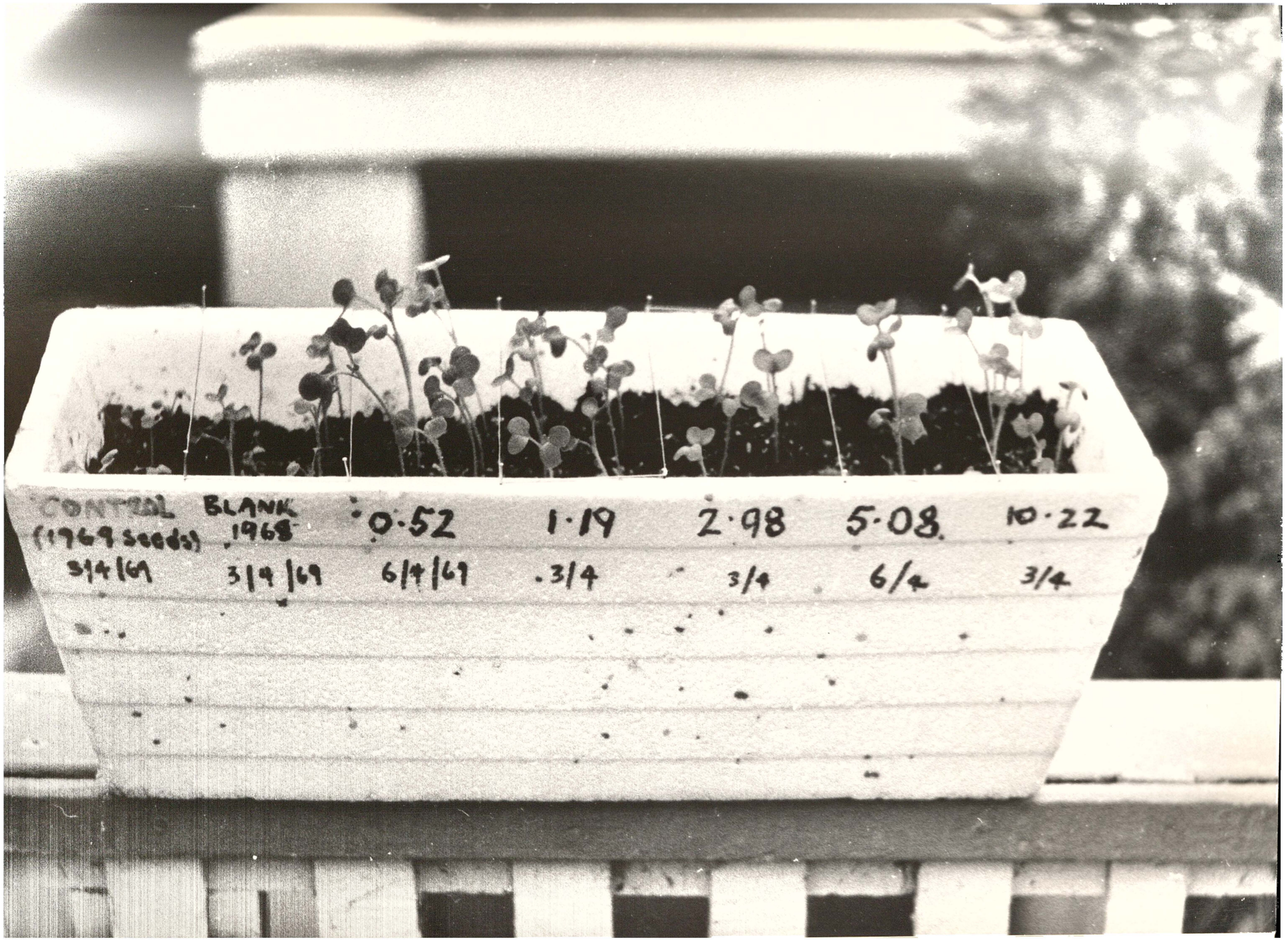

CONTROL
(1969 seeds)
3/4/69
BLANK
1968
3/9/69
0·52
6/4/69
1·19
3/4
2·98
3/4
5·08
6/4
10·22
3/4

Plate 2.
The Growth of the Plants.

Control
Blank
1.19 Mrep — 16 Days
2.98 Mrep
10.22 Mrep

0.52 Mrep — 13 Days
5.08 Mrep

not desired to destroy the plants the measurements made had to be limited to these few. The results obtained are shown in Table 4-3.1. In the following Table (4-3.2) a summary of the average values obtained for each dose group is shown.

Although the numbers involved were small and a random layout had not been used the figures obtained were analysed using statistics to determine whether or not this apparent enhanced growth obtained from the recovered seeds was significant.

Students "t" test was used and, assuming similar environmental factors during plant growth, the results were indeed statistically significant. The probability of selecting a sample from the blank group with the same heights as the 0.52 Mrep group was P=0.005, for the 1.19 Mrep group P = 0.01, for the 2.98 Mrep group P = 0.025, for the 5.08 group P = 0.05 and for the 10.22 Mrep group P = 0.01. It was therefore decided to plan a complete statistical analysis experiment involving random layout to compare the growth of the plants from the recovered seeds to that of the non-irradiated group. These experiments are described in Section 5 of this thesis.

4-4 Analysis of Cell Size in the Plants

The plants from the seeds which had recovered from irradiation remained larger than those from the blank group throughout this experiment. As they grew towards maturity the 10.22 Mrep group competed strongly with the 0.52 Mrep group for the group containing the largest plants. At maturity these groups were obviously taller, their stalks thicker and their leaves larger. Two side stems were removed from a plant in the blank group and a plant in the 10.22 Mrep group.

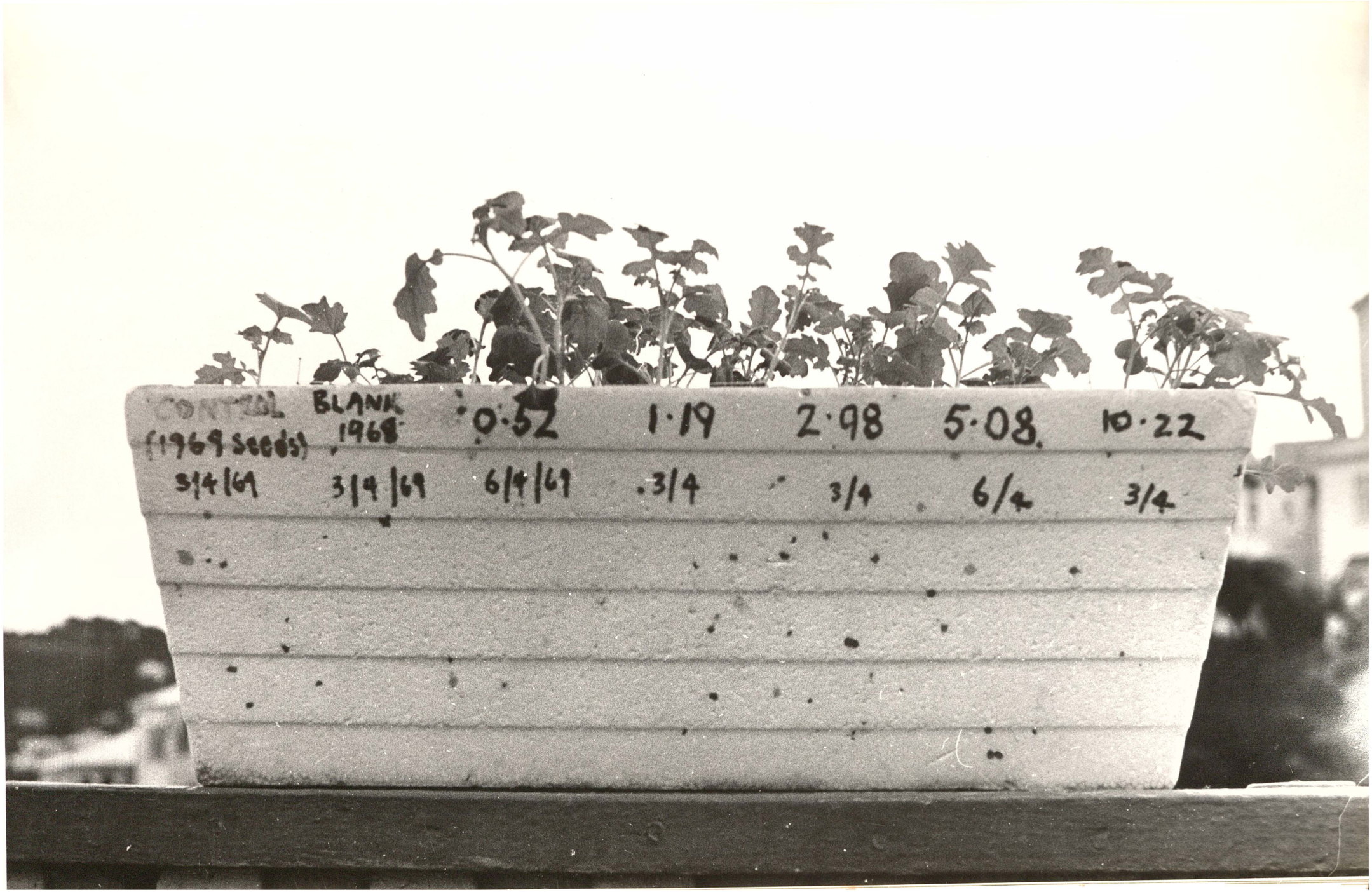

CONTROL
(1969 seeds)
3/4/69
BLANK
1968
3/4/69
0·52
6/4/69
1·19
3/4
2·98
3/4
5·08
6/4
10·22
3/4

Plate 3.

The Growth of the Plants.

Control Blank 1.19 Mrep 2.98 Mrep 10.22 Mrep	37 Days
0.52 Mrep 5.08 Mrep	34 Days

Table 4-3.1

Comparison of the Characteristics of the Plants Grown from Seeds which had Recovered from the Effects of Gamma-Radiation

Dose (Mrep)	No. of Plants	Characteristic	Plant Numbers 1	2	3	4	5	6	7	8	Av.
			cms	cms	cms	cms	cms	cms	cms	cms	cms
0.0	8	Height Plant	3.7	4.2	5.7	7.0	4.7	5.2	8.3	6.2	5.6
		cotyledon	0.8	0.8	0.9	0.9	1.0	0.6	0.9	0.9	0.85
		1° leaf	1.1	2.1	2.1	1.9	2.1	1.5	1.9	1.9	1.8
		2° leaf	0.2	0.3	0.8	none	0.6	0.8	0.7	0.7	0.5
0.52	8	Height plant	13.1	8.7	8.2	7.7	9.0	9.2	11.7	8.7	9.6
		cotyledon	1.2	1.2	1.4	1.2	1.1	1.3	1.4	1.1	1.24
		1° leaf	2.6	2.3	2.4	2.0	3.0	1.5	2.6	2.3	2.34
		2° leaf	1.9	0.9	0.7	none	0.8	0.3	1.0	0.2	0.7
1.19	8	Height plant	7.7	8.7	6.7	6.5	8.1	6.5	7.2	8.0	7.4
		cotyledon	1.1	1.3	1.1	1.3	1.0	1.0	1.3	1.2	1.2
		1° leaf	2.5	2.3	1.2	1.2	2.0	1.7	1.7	3.0	2.0
		2° leaf	1.7	0.6	0.4	0.2	0.3	0.4	none	1.7	0.7
2.98	6	Height plant	5.7	1.7*	6.5	8.5	9.7	9.2			7.9**
		cotyledon	0.8	1.1	0.9	1.4	0.9	0.9			1.0
		1° leaf	2.1	1.4	2.4	4.1	2.2	2.5			2.7
		2° leaf	0.8	0.6	1.1	0.7	0.9	1.1			0.9
5.08	3	Height plant	6.3	10.8	7.8						8.3
		cotyledon	1.1	1.0	1.1						1.1
		1° leaf	2.5	2.6	3.0						2.7
		2° leaf	1.8	1.1	1.1						1.3
10.22	6	Height plant	6.1	6.7	7.3	8.3	8.5	8.7			7.6
		cotyledon	1.1	0.8	1.0	1.2	1.6	1.2			1.2
		1° leaf	2.6	2.6	2.1	3.1	2.9	2.5			2.6
		2° leaf	0.8	0.7	1.8	1.0	1.3	1.0			1.1

Notes: * The plant appeared to be a mutation. The primary and secondary leaves were long and smooth and lacked the characteristic feather-type pattern of the normal mustard plants. The plant did not possess a centre stalk.

** The average values were calculated excluding those from the mutated plant.

Table 4-3.2

Summary of the Average Values Obtained for the Characteristics of the Plants Grown from Seeds which had Recovered from the Effects of Gamma-Radiation

Dose (Mrep)	0.0	0.52	1.19	2.98	5.08	10.22
Number of Plants	8	8	8	5	3	6
Age of Plants (Days)	33	30	33	33	30	33
Average of Characteristic (cms)						
Plant height	5.6	9.6	7.4	7.9	8.3	7.6
Cotyledon	0.85	1.24	1.2	1.0	1.1	1.2
Primary leaf	1.8	2.34	2.0	2.7	2.7	2.6
Secondary leaf	0.5	0.7	0.7	0.9	1.3	1.1

Table 4-3.3

Statistical Analysis of the Variation in Heights Between these Plants

Students t Test

Dose (Mrep)	0.0	0.52	1.19	2.98	5.08	10.22
Mean (cms)	5.63	9.54	7.43	7.92	8.30	7.60
D^2	16.66	24.40	5.89	12.10	10.50	4.66
σ^2	2.38	3.63	0.84	3.03	5.25	0.93
σ_d	-	0.87	0.63	0.95	1.43	0.67
t	-	4.5	2.86	2.41	1.87	2.94
No. degrees freedom	-	14	14	11	9	12
Significant	-	Yes	Yes	Yes	Yes	Yes
Level (p)	-	0.005	0.01	0.025	0.05	0.01

Note: D = Sum of Squares of Deviation of Samples

σ = Standard deviation

$$\sigma_d^2 = \frac{\sigma^2_{\text{blank}}}{8} + \frac{\sigma^2_{\text{sample}}}{\text{No. in sample}}$$

$$t = \frac{\text{"Sample" mean} - \text{"Blank" mean}}{\sigma_d}$$

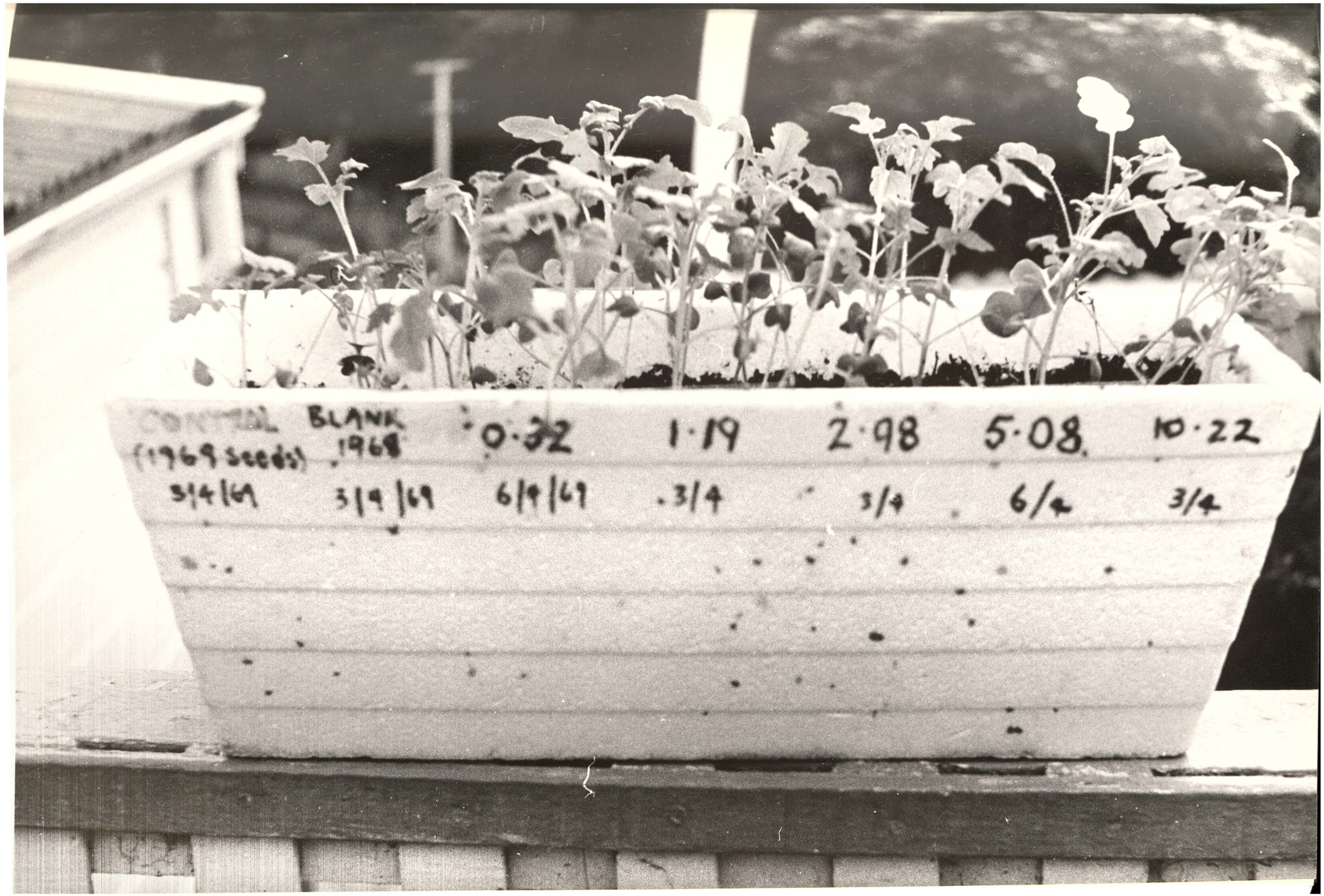

CONTROL
(1969 seeds)
3/4/69
BLANK
1968
3/19/69
0·22
6/9/69
1·19
3/4
2·98
3/4
5·08
6/4
10·22
3/4

Plate 4.

The Growth of the Plants.

Control
Blank
1.19 Mrep — 37 Days
2.98 Mrep
10.22 Mrep

0.52 Mrep
5.08 Mrep — 34 Days

The diameters of the stems used for the analysis of cell size were 0.4 cms from the 10.22 Mrep group and 0.25 cms from the blank group. Cross-sections of each stem were prepared. The cell size was measured using a microscope containing an eyepiece with divisions. Fifty eyepiece divisions standardised against a measuring scale were found to equal 120 microns (1 micron = 10^{-6} metre = 10^{-3} mm). Therefore one eyepiece division (ED) was equal to 2.4 u.

The number of cell layers outside the vascular tissue was nine in both samples. The larger stem (10.22 Mrep) contained a larger hole in the centre of the pith (0.1 cms diameter) as compared to the smaller stem from the blank group for which the diameter of this hole was extremely small. The average trans-section of the cells from the layer under the epiderm was found to be equal to 11 ED for the stem from the 10.22 Mrep plant and 7 ED for the stem from the blank plant. The difference in stem size largely arose from the holes in the centre of the pith. It was thought that the pith cells in the 10.22 Mrep plants could possibly have reached a maximum size, after which the cell walls burst, forming the hole whereas in the "blank" plant very few cells had reached this size maximum. There was a small difference in the size of the pith cells which remained intact in the plants. In the 10.22 Mrep plant there were many cells of approximately 17 ED whereas in the blank plant the largest cells measured 13.5 ED. A rough scan of the two cross-sections could not detect any obvious difference in cell numbers outside the pith. Within the pith the 10.22 Mrep section seemed to contain fewer cells but those present were larger than those in the blank sample. These results are summarized and converted to millimetre units in Table 4-4.1.

Table 4-4.1

The Analysis of the Difference in Stem Size between Plants Grown from the Seeds which had Recovered from a Dose of 10.22 Mrep and from those which had not been Exposed to Radiation.

Dose received by seed (Mrep)	0.0	10.22
Diameter of stem (cms)	0.25	0.40
No. of cell layers outside vascular tissue	9	9
Size of hole in the pith (cms)	negligible	0.1
Size of pith cells (ED) (μ) (mm)	13.5 (largest) 32.4 3.24×10^{-2}	17.0 (many) 40.8 4.08×10^{-2}
Trans-section of cells in layer under epiderm (ED) (μ) (mm)	7 16.8 1.68×10^{-2}	11 26.4 2.64×10^{-2}

This analysis shows that the increased growth of the plants formed from the irradiated but recovered seeds was due to an increase in cell size and not to an increase in cell number. Such an increase in size could arise from increased metabolic activity which was not accompanied by increased cell division.

4-5 Germination of the Seeds from the Plants Grown

After transferring the plants grown from the seeds which had recovered from the effects of radiation, these plants were grown to maturity. Flowering began at approximately the third month. After 6 months growth, seeds were well-developed and had started to turn brown. From the beginning of the seventh month the seeds, from pods which had dried and were almost ready to break open, were collected and


CONTROL BLANK 0·52 1·19 2·98 5·08 10·22
(1969 seeds) 1968
3/4/69 3/4/69 6/4/69 3/4 3/4 6/4 3/4

Plate 5.

The Growth of the Plants.

Control Blank 1.19 Mrep 2.98 Mrep 10.22 Mrep	48 Days
0.52 Mrep 5.08 Mrep	45 Days

stored in paper envelopes in groups corresponding to the dose of gamma-radiation which the seed of the parent plant had received.

After sufficient seeds had been collected the germination of these seeds was examined. The results obtained are shown in Table 4-5.1. The results obtained for the 0.0, 0.52 and 1.19 Mrep groups are plotted on graph 4-5.1. As can clearly be seen from both the table and the graph the seeds from these plants are not adversely affected in their ability to germinate, and nor were the parent plants sterile. The seeds produced from the first generation plants were perfectly normal in every aspect.

The seeds which had germinated were allowed to continue to grow within their petri dishes and the growth of the plants produced compared by eye. The plants from the recovered seeds did not show retarded growth as compared to the control and possibly were in fact, larger and healthier. They quickly developed a larger root system than did the control plants.

These results show that the original seeds which had been irradiated and recovered had recovered in all aspects of plant growth. The plants produced were viable and contained abundant seeds all of which germinated normally.

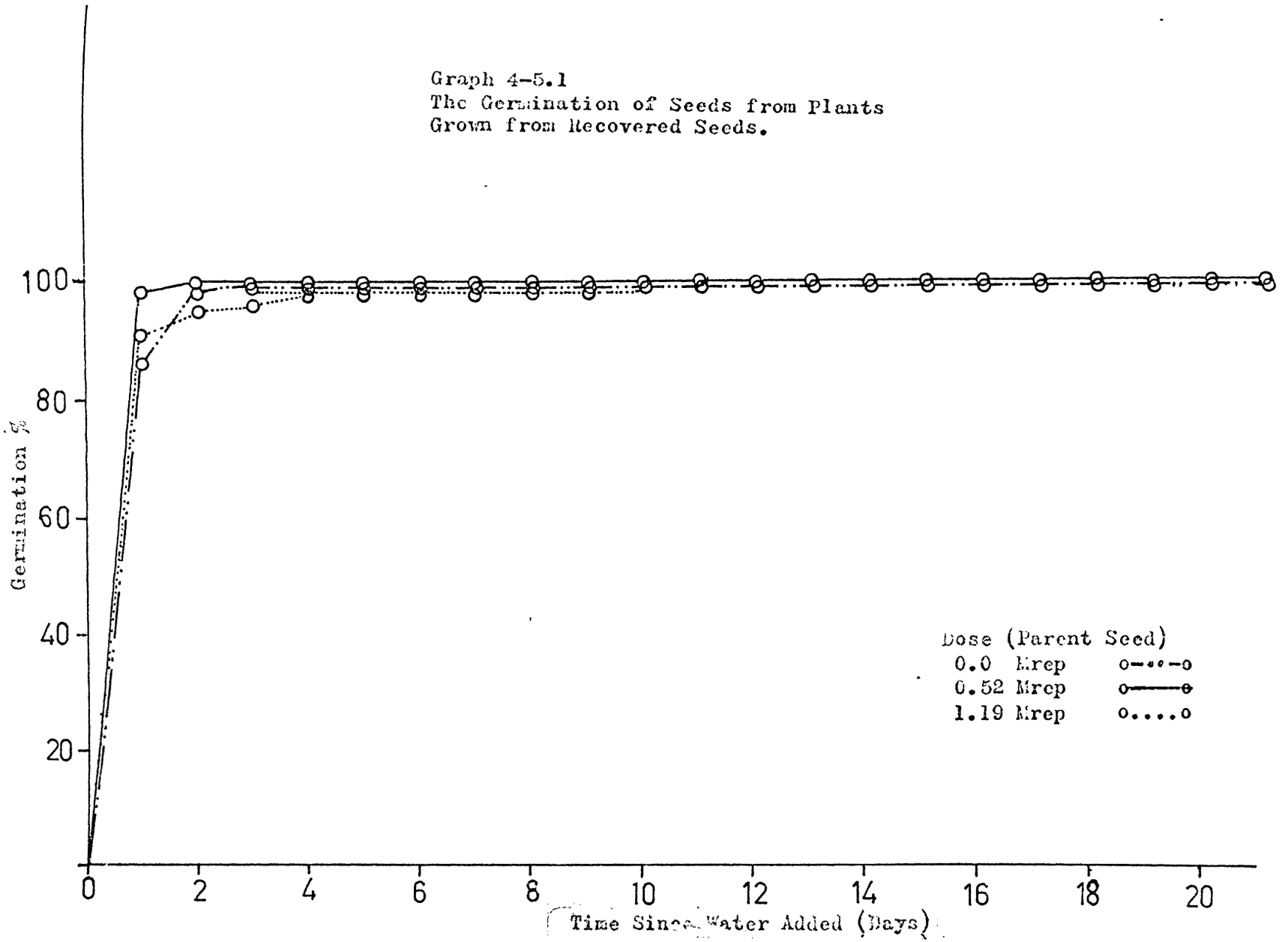
Table 4-5.1

The Germination of Seeds from the First Generation Plants, Grown from Seeds which had received Irradiation Treatment.

Dose (Mrad)	0.0		0.52		1.19		2.98		5.08		10.22	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	86	86	98	98	91	91	95	95	96	96	87	87
2	12	98	2	100	4	95	5	100	3	99	12	99
3	1	99	-	100	1	96	-	100	0	99	0	99
4	0	99	-	100	2	98	-	100	0	99	0	99
5	0	99	-	100	0	98	-	100	0	99	1	100
6	0	99	-	100	0	98	-	100	0	99	-	100
7	0	99	-	100	0	98	-	100	0	99	-	100
8	0	99	-	100	0	98	-	100	0	99	-	100
9	0	99	-	100	0	98	-	100	0	99	-	100
10	0	99	-	100	1	99	-	100	0	99	-	100
11	0	99	-	100	0	99	-	100	0	99	-	100
12	0	99	-	100	0	99	-	100	0	99	-	100
13	0	99	-	100	0	99	-	100	0	99	-	100
14	0	99	-	100	0	99	-	100	0	99	-	100
15	0	99	-	100	0	99	-	100	0	99	-	100
16	0	99	-	100	0	99	-	100	0	99	-	100
17	0	99	-	100	0	99	-	100	0	99	-	100
18	0	99	-	100	0	99	-	100	0	99	-	100
19	0	99	-	100	0	99	-	100	0	99	-	100
20	0	99	-	100	0	99	-	100	0	99	-	100

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Graph 4-5.1
The Germination of Seeds from Plants
Grown from Recovered Seeds.



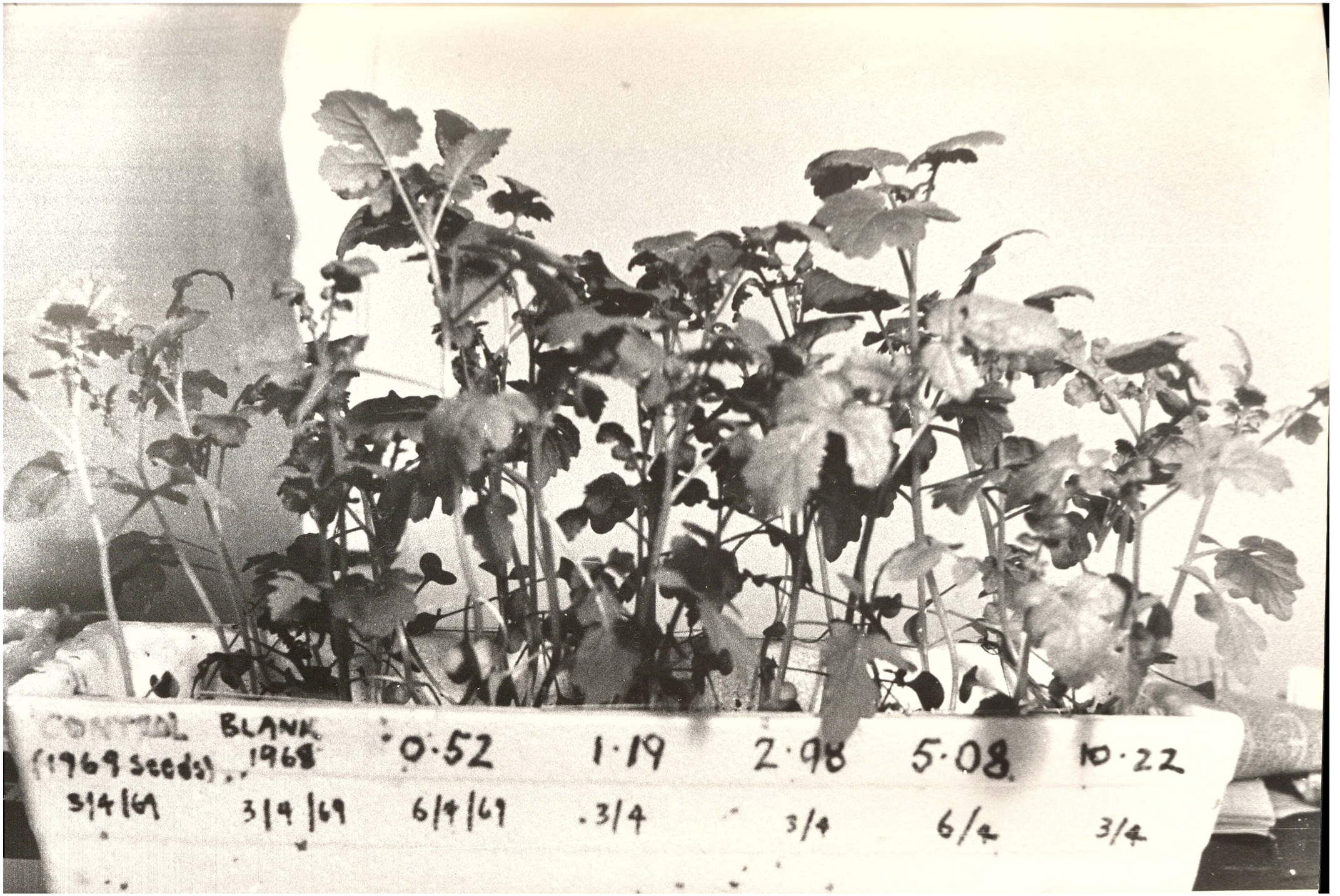

CONTROL BLANK 0·52 1·19 2·98 5·08 10·22
(1969 seeds) 1968
3/4/69 3/4/69 6/4/69 3/4 3/4 6/4 3/4

Plate 6.

The Growth of the Plants.

Control Blank 1.19 Mrep 2.98 Mrep 10.22 Mrep	48 Days
0.52 Mrep 5.08 Mrep	45 Days

5. LARGE SCALE STATISTICAL ANALYSES OF GROWTH OF PLANTS FROM IRRADIATED BUT RECOVERED SEEDS

5-1 Analysis Number One

Pots were prepared from four large blocks of polystyrene foam using heated metal to melt the foam. The pots prepared were 3 inches diameter, 2 inches deep and had one centre drainage hole. Stones were placed in the bottom and covered by "Utility" potting mixture. Three Petri dishes, each containing 100 seeds, were used for each dose group. The seeds were allowed to germinate in the dishes and transferred to the pots immediately after germination. The first 140 seeds to germinate were used for each dose group. Four plants, one from each of the four dose groups to be compared were placed in each pot. The pots were in four blocks of 35 and each block is referred to as a "Light Group" i.e. L_1, L_2, L_3 and L_4. The position of each plant within a pot was fixed by taking 35 sets of random number groupings from Random Number Tables.[328] This layout was repeated for each Light Group.

Each pot was divided into quadrants and the positions designated a, b, c and d in clockwise order beginning with "a" in the North-West quadrant. The dose groups used were 0.0, 0.52, 2.98 and 10.22 Mrep groups. These were given a code number each from one to four and the random groupings shown in Table 5-1.1 determined using the random number tables. The code and the plan for pot numbers are also shown here.

Three time intervals were used for this experiment namely 10, 20 and 30 days. At the end of the required time period the pots from which all four plants were to be removed were selected by using random number tables. Eleven pots were selected from each light group for

Table 5-1.1

The Layout used for the First Statistical Analysis of the Difference in Growth between Plants Grown from Irradiated but Recovered Seeds.

Pot No.	a	b	c	d	Pot No.	a	b	c	d
1	2	1	3	4	19	3	4	1	2
2	1	3	4	2	20	3	4	2	1
3	4	2	1	3	21	1	2	4	3
4	3	1	2	4	22	1	3	4	2
5	3	4	1	2	23	1	4	3	2
6	3	1	2	4	24	4	3	2	1
7	2	1	3	4	25	4	2	1	3
8	2	3	1	4	26	3	1	2	4
9	1	2	3	4	27	4	1	2	3
10	3	1	4	2	28	4	2	3	1
11	3	1	4	2	29	2	1	4	3
12	4	3	2	1	30	1	2	3	4
13	3	1	4	2	31	1	2	3	4
14	1	3	2	4	32	4	3	1	2
15	2	1	4	3	33	1	3	4	2
16	2	4	1	3	34	3	1	2	4
17	3	1	4	2	35	2	4	1	3
18	1	2	4	3					

Code:	1	2	3	4
Dose (Mrep)	0.0	0.52	2.98	10.22

North

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31	32	33	34	35

South

The Pot Numbers shown in a Typical Light Block.

each time interval. The constant dry weight was determined for each plant and the results are shown in Table 5-1.2. The height of each plant was also determined immediately after removal from the soil and the values obtained are shown in Table 5-1.3.

The results for the Dry Weight were first analysed by calculating machine using the analysis shown in Table 5-1.4, and later by computer as a check for the programme devised.

The computer used was an IBM 1130 model. The Fortran programme devised to carry out the analysis is contained in a pocket at the back of this thesis. The results obtained for the analyses are shown in Tables 5-1.5 and 5-1.6. The difference in dry weights between treatments was found to be significant at the 5% level and between heights to be significant at the 1% level.

This data was then further analysed on the difference between the mean for each treatment and that of the 0.0 Mrep or Control group. The analyses are shown in Table 5-1.10. The value "Z" as calculated is used to determine whether or not the results of the treatments are significantly different to those of the control.

The Growth 10.22 Mrep group is significantly better than the control at the 0.10 level based on both a dry weight and a height basis. The 2.98 Mrep group which was showing slightly inferior growth to the control group did not, however, differ significantly. The growth of the plants in the 0.52 Mrep group gave the most highly significant result. On a dry weight basis the difference between these plants and the controls is significant at the 0.005 level and on a height basis, at greater than the 0.002 level.

Table 5-1.2

The Dry Weights Obtained for the Plants Involved in the First Statistical Analysis

Dose (Mrad)	Block No.	10 days		20 days		30 days	
		Pot No.	Dry Weight (grams)	Pot No.	Dry Weight (grams)	Pot No.	Dry Weight (grams)
0.00	A	3	0.00830	2	0.01109	1	0.00920
		10	0.00530	6	0.01080	4	0.00790
		11	0.00616	7	0.00733	8	0.01068
		14	0.00690	9	0.00660	13	0.01182
		16	0.00857	12	0.00666	15	0.00979
		20	0.00654	19	0.00865	17	0.01055
		24	0.00735	21	0.01095	18	0.00955
		26	0.00720	23	0.00695	22	0.00204
		27	0.00868	28	0.00735	25	0.01096
		32	0.00844	30	0.00850	29	0.01074
		33	0.00593	35	0.00938	31	0.00960
0.00	B	7	0.00869	1	0.00732	8	0.00766
		10	0.00774	2	0.00954	9	0.00857
		12	0.01166	3	0.00785	18	0.00670
		13	0.00841	4	0.00851	19	0.00924
		14	0.00713	11	0.00946	20	0.00633
		16	0.00576	15	0.00957	25	0.00620
		24	0.00566	17	0.00749	29	0.00960
		26	0.00694	21	0.00834	30	0.00760
		27	0.00721	22	0.00781	33	0.00708
		31	0.00672	23	0.00743	34	0.00869
		32	0.00950	28	0.00860	35	0.00966
0.00	C	3	0.00873	1	0.01176	2	0.01578
		5	0.00625	4	0.01036	6	0.01134
		10	0.01057	7	0.01106	9	0.00936
		12	0.00574	8	0.00712	11	0.01134
		14	0.00630	16	0.01035	13	0.01323
		15	0.00696	18	0.00881	17	0.00780
		21	0.01184	23	0.00950	19	0.01010
		22	0.00604	27	0.00976	25	0.00852
		26	0.00814	28	0.01158	29	0.01145
		31	0.00841	30	0.00856	32	0.00915
		35	0.00645	33	0.00905	34	0.01140
0.00	D	1	0.00721	11	0.01156	2	0.01355
		4	0.00900	12	0.01121	3	0.01363
		6	0.00850	13	0.01033	5	0.01120
		9	0.00816	14	0.00946	7	0.01469
		16	0.00828	19	0.00999	8	0.02378
		17	0.00770	20	0.01109	15	0.01491
		18	0.00901	24	0.00921	21	0.01909
		22	0.00816	25	0.00691	27	0.01123
		30	0.00830	26	0.00854	29	0.02308
		32	0.00971	28	0.01271	33	0.01320
		35	0.00720	31	0.01189	34	0.01882

/continued...

Table 5-1.2 continued

Dose (Mrad)	Block No.	10 days		20 days		30 days	
		Pot No.	Dry Weight (grams)	Pot No.	Dry Weight (grams)	Pot No.	Dry Weight (grams)
0.52	A	3	0.00593	2	0.00937	1	0.00934
		10	0.00609	6	0.00810	4	0.00920
		11	0.01175	7	0.00859	8	0.00803
		14	0.00639	9	0.00815	13	0.00750
		16	0.00771	12	0.00780	15	0.00755
		20	0.01414	19	0.00989	17	0.00808
		24	0.00580	21	0.00818	18	0.00881
		26	0.00694	23	0.00815	22	0.00848
		27	0.00589	28	0.00880	25	0.01075
		32	0.00588	30	0.01010	29	0.00730
		33	0.00669	35	0.01000	31	0.00911
0.52	B	7	0.00623	1	0.01000	8	0.00834
		10	0.00889	2	0.01313	9	0.00840
		12	0.00736	3	0.01138	18	0.01155
		13	0.00638	4	0.00842	19	0.00680
		14	0.00636	11	0.00665	20	0.01004
		16	0.00668	15	0.00824	25	0.00831
		24	0.01169	17	0.00681	29	0.01191
		26	0.01083	21	0.00900	30	0.00850
		27	0.01023	22	0.00868	33	0.01200
		31	0.00824	23	0.00792	34	0.00695
		32	0.00620	28	0.00915	35	0.00988
0.52	C	3	0.00679	1	0.01048	2	0.00800
		5	0.00806	4	0.00915	6	0.01066
		10	0.00710	7	0.00910	9	0.00921
		12	0.00840	8	0.01110	11	0.01357
		14	0.00740	16	0.00935	13	0.00700
		15	0.00798	18	0.00990	17	0.01663
		21	0.00765	23	0.01439	19	0.00955
		22	0.01037	27	0.00962	25	0.01290
		26	0.00710	28	0.01115	29	0.01476
		31	0.01046	30	0.01308	32	0.01257
		35	0.01045	33	0.01085	34	0.00991
0.52	D	1	0.01084	11	0.01324	2	0.01881
		4	0.01130	12	0.01100	3	0.02232
		6	0.00420	13	0.00811	5	0.02255
		9	0.00969	14	0.01642	7	0.01992
		16	0.00925	19	0.01335	8	0.01410
		17	0.00930	20	0.00990	15	0.00766
		18	0.00940	24	0.01279	21	0.01510
		22	0.00931	25	0.01045	27	0.02041
		30	0.00836	26	0.00781	29	0.01243
		32	0.00820	28	0.00999	33	0.00872
		35	0.00642	31	0.01126	34	0.01805

/continued...

Table 5-1.2 continued

Dose (Mrad)	Block No.	10 days		20 days		30 days	
		Pot	Dry Weight (grams)	Pot	Dry Weight (grams)	Pot	Dry Weight (grams)
2.98	A	3	0.01064	2	0.00875	1	0.00914
		10	0.00686	6	0.00815	4	0.01079
		11	0.00854	7	0.01022	8	0.00935
		14	0.00874	9	0.00978	13	0.00753
		16	0.00812	12	0.00864	15	0.00665
		20	0.00762	19	0.00797	17	0.00911
		24	0.00680	21	0.00831	18	0.01047
		26	0.00851	23	0.00741	22	0.00855
		27	0.00668	28	0.01099	25	0.00889
		32	0.00496	30	0.00896	29	0.00909
		33	0.00584	35	0.01025	31	0.00869
2.98	B	7	0.0755	1	0.00861	8	0.00399
		10	0.00631	2	0.00696	9	0.00901
		12	0.00729	3	0.00600	18	0.00851
		13	0.00989	4	0.00815	19	0.00521
		14	0.00991	11	0.01081	20	0.00931
		16	0.00686	15	0.00909	25	0.01583
		24	0.00575	17	0.01160	29	0.01289
		26	0.00760	21	0.00781	30	0.00893
		27	0.00899	22	0.00956	33	0.00740
		31	0.00746	23	0.01144	34	0.00635
		32	0.00877	28	0.00815	35	0.01084
2.98	C	3	0.01001	1	0.00807	2	0.01141
		5	0.00625	4	0.00984	6	0.00990
		10	0.00885	7	0.00868	9	0.00929
		12	0.00978	8	0.00828	11	0.00953
		14	0.00840	16	0.01010	13	0.01280
		15	0.00662	18	0.00851	17	0.00866
		21	0.00568	23	0.00976	19	0.00747
		22	0.00580	27	0.01199	25	0.01321
		26	0.00681	28	0.00983	29	0.01003
		31	0.00644	30	0.00939	32	0.01286
		35	0.00632	33	0.00830	34	0.01050
2.98	D	1	0.00591	11	0.00829	2	0.01144
		4	0.00920	12	0.01101	3	0.01102
		6	0.00831	13	0.01030	5	0.01283
		9	0.00676	14	0.01036	7	0.01496
		16	0.00935	19	0.00881	8	0.01540
		17	0.00611	20	0.00929	15	0.01160
		18	0.00781	24	0.01040	21	0.01411
		22	0.00680	25	0.01063	27	0.00950
		30	0.00907	26	0.00796	29	0.01765
		32	0.00526	28	0.01026	33	0.01630
		35	0.00727	31	0.01521	34	0.01283

/continued...

Table 5-1.2 continued

Dose (Mrad)	Block No.	10 days Pot No.	10 days Dry Weight (grams)	20 days Pot No.	20 days Dry Weight (grams)	30 days Pot No.	30 days Dry Weight (grams)
10.22	A	3	0.00369	2	0.00760	1	0.00890
		10	0.00801	6	0.00891	4	0.00946
		11	0.00556	7	0.00613	8	0.01076
		14	0.01105	9	0.00711	13	0.00796
		16	0.00893	12	0.00519	15	0.00912
		20	0.00619	19	0.01011	17	0.00828
		24	0.00806	21	0.00732	18	0.00627
		26	0.00941	23	0.00869	22	0.01055
		27	0.00875	28	0.00984	25	0.00939
		32	0.00966	30	0.00835	29	0.01150
		33	0.00799	35	0.00951	31	0.01060
10.22	B	7	0.00944	1	0.00743	8	0.00918
		10	0.00853	2	0.01101	9	0.00681
		12	0.00883	3	0.00941	18	0.00873
		13	0.00914	4	0.00732	19	0.00966
		14	0.00628	11	0.00865	20	0.01143
		16	0.00832	15	0.01119	25	0.00850
		24	0.00709	17	0.01104	29	0.01059
		26	0.00980	21	0.00837	30	0.00673
		27	0.00759	22	0.00901	33	0.00594
		31	0.00821	23	0.01176	34	0.01063
		32	0.00553	28	0.00853	35	0.01101
10.22	C	3	0.00870	1	0.00992	2	0.01168
		5	0.00951	4	0.01011	6	0.01211
		10	0.00741	7	0.00939	9	0.01089
		12	0.00793	8	0.00855	11	0.00971
		14	0.00745	16	0.00865	13	0.01532
		15	0.00780	18	0.00976	17	0.01029
		21	0.01025	23	0.00860	19	0.01179
		22	0.00925	27	0.00919	25	0.01050
		26	0.00775	28	0.01024	29	0.01380
		31	0.00616	30	0.00977	32	0.01350
		35	0.00872	33	0.01165	34	0.01614
10.22	D	1	0.00627	11	0.01255	2	0.01583
		4	0.00525	12	0.01163	3	0.01708
		6	0.00901	13	0.01238	5	0.01858
		9	0.00540	14	0.01015	7	0.01702
		16	0.00678	19	0.01093	8	0.01233
		17	0.01068	20	0.01141	15	0.01445
		18	0.00573	24	0.00952	21	0.01730
		22	0.00461	25	0.01076	27	0.01261
		30	0.00547	26	0.00950	29	0.01060
		32	0.00696	28	0.01024	33	0.01924
		35	0.00691	31	0.01107	34	0.01406

Table 5-1.3

The Heights Obtained for the Plants Involved in the First Statistical Analysis

Dose (Mrad)	Block No.	10 days		20 days		30 days	
		Pot No.	Height (cms)	Pot No.	Height (cms)	Pot No.	Height (cms)
0.00	A	3	5.6	2	5.9	1	9.1
		10	4.1	6	6.0	4	7.2
		11	3.0	7	3.4	8	7.4
		14	3.5	9	3.2	13	7.1
		16	4.9	12	4.5	15	6.3
		20	5.2	19	5.5	17	5.7
		24	3.9	21	5.4	18	7.3
		26	2.9	23	3.1	22	3.0
		27	4.9	28	7.4	25	7.8
		32	3.8	30	7.0	29	8.1
		33	3.6	35	7.4	31	7.2
0.00	B	7	2.5	1	3.3	8	7.2
		10	4.0	2	5.6	9	3.7
		12	4.0	3	6.8	18	5.4
		13	3.9	11	6.8	19	6.1
		14	4.3	15	6.4	20	4.5
		16	4.6	17	4.9	25	4.4
		24	3.2	21	4.2	29	7.8
		26	3.1	22	1.4	30	6.8
		27	5.2	23	6.2	33	5.4
		31	4.3	28	4.0	34	6.5
		32	3.3	4	4.5	35	5.6
0.00	C	3	4.4	1	6.1	2	6.9
		5	3.7	4	7.8	6	5.9
		10	3.6	7	6.5	9	7.0
		12	3.7	8	5.6	11	7.2
		14	3.8	16	5.9	13	7.2
		15	5.3	18	4.8	17	5.1
		21	4.3	23	7.6	19	6.8
		22	4.3	27	6.4	25	7.4
		26	3.7	28	6.3	29	6.1
		31	4.6	30	6.0	32	4.2
		35	4.7	33	7.0	34	4.3
0.00	D	1	3.7	11	7.7	2	6.2
		4	4.6	12	7.7	3	7.4
		6	4.2	13	4.3	5	5.8
		9	4.5	14	4.5	7	8.2
		16	5.2	19	5.0	8	9.3
		17	2.1	20	7.8	15	9.5
		18	4.7	24	6.9	21	7.5
		22	4.8	25	4.9	27	4.9
		30	4.0	26	4.6	29	14.3
		32	4.1	28	6.5	33	9.4
		35	4.2	31	6.6	34	9.2

/continued...

Table 5-1.3 continued

Dose (Mrad)	Block No.	10 days Pot No.	10 days Height (cms)	20 days Pot No.	20 days Height (cms)	30 days Pot No.	30 days Height (cms)
0.52	A	3	4.1	2	6.0	1	6.3
		10	3.9	6	6.8	4	6.4
		11	5.2	7	7.3	8	6.2
		14	6.2	9	8.0	13	7.4
		16	5.6	12	7.2	15	6.1
		20	4.4	19	5.9	17	8.7
		24	5.2	21	4.2	18	8.2
		26	4.4	23	5.0	22	6.2
		27	4.6	28	7.0	25	7.9
		32	5.4	30	5.9	29	5.9
		33	5.6	35	6.5	31	7.5
0.52	B	7	4.5	1	5.4	8	5.1
		10	4.2	2	7.8	9	7.2
		12	4.9	3	6.0	18	7.4
		13	5.3	11	5.1	19	5.8
		14	2.3	15	4.7	20	7.4
		16	4.3	17	7.0	25	8.3
		24	4.2	21	5.3	29	8.1
		26	4.4	22	4.6	30	6.2
		27	4.5	23	6.0	33	6.2
		31	4.0	28	6.5	34	3.0
		32	3.8	4	5.7	35	5.9
0.52	C	3	2.5	1	5.7	2	5.5
		5	4.6	4	4.9	6	8.4
		10	6.1	7	5.4	9	5.6
		12	6.4	8	5.1	11	7.4
		14	3.7	16	5.5	13	6.3
		15	1.8	18	5.5	17	10.9
		21	3.6	23	7.8	19	6.5
		22	4.8	27	5.4	25	9.6
		26	4.7	28	6.0	29	10. 1
		31	4.4	30	7.5	32	7.8
		35	4.2	33	4.3	34	7.7
0.52	D	1	4.7	11	7.0	2	12.5
		4	5.2	12	6.8	3	9.4
		6	4.3	13	5.5	5	11.2
		9	5.1	14	4.5	7	9.0
		16	5.1	19	5.0	8	6.0
		17	3.9	20	6.0	15	5.3
		18	4.7	24	7.3	21	6.0
		22	4.9	25	5.7	27	8.2
		30	4.1	26	3.6	29	8.5
		32	4.7	28	5.5	33	3.4
		35	3.9	31	4.9	34	7.5

/continued...

Table 5-1.3 continued

Dose (Mrad)	Block No.	10 days		20 days		30 days	
		Pot No.	Height (cms)	Pot No.	Height (cms)	Pot No.	Height (cms)
2.98	A	3	4.8	2	4.2	1	6.5
		10	4.2	6	5.5	4	6.8
		11	3.8	7	4.4	8	7.0
		14	3.9	9	5.3	13	5.3
		16	5.4	12	5.0	15	2.2
		20	4.9	19	5.0	17	7.2
		24	4.4	21	5.6	18	5.2
		26	5.6	23	5.7	22	5.0
		27	5.7	28	5.5	25	7.0
		32	3.6	30	5.4	29	5.8
		33	3.2	35	4.6	31	6.2
2.98	B	7	3.3	1	5.9	8	4.3
		10	3.4	2	6.2	9	8.3
		12	4.6	3	4.4	18	6.1
		13	3.6	11	7.2	19	4.5
		14	4.2	15	6.0	20	4.4
		16	4.0	17	4.9	25	7.9
		24	2.6	21	5.8	29	8.4
		26	4.1	22	5.9	30	6.0
		27	5.5	23	6.0	33	3.2
		31	3.4	28	4.3	34	2.5
		32	4.4	4	5.6	35	4.5
2.98	C	3	5.8	1	5.5	2	7.1
		5	4.6	4	6.5	6	5.6
		10	4.6	7	6.0	9	4.1
		12	4.0	8	4.4	11	4.5
		14	3.9	16	5.2	13	8.0
		15	4.3	18	6.1	17	4.5
		21	4.3	23	5.1	19	5.2
		22	2.0	27	6.1	25	9.1
		26	4.5	28	4.9	29	6.8
		31	4.3	30	4.9	32	9.3
		35	4.8	33	5.8	34	7.6
2.98	D	1	4.3	11	7.3	2	6.4
		4	4.8	12	5.5	3	9.1
		6	4.3	13	3.0	5	9.1
		9	4.8	14	7.0	7	9.8
		16	3.6	19	4.3	8	11.0
		17	4.7	20	6.5	15	7.1
		18	4.5	24	4.5	21	5.7
		22	4.2	25	5.4	27	8.0
		30	5.3	26	5.0	29	9.5
		32	3.0	28	4.0	33	9.5
		35	5.0	31	6.0	34	7.4

/continued...

Table 5-1.3 continued

Dose (Mrad)	Block No.	10 days Pot No.	10 days Height (cms)	20 days Pot No.	20 days Height (cms)	30 days Pot No.	30 days Height (cms)
10.22	A	3	4.5	2	6.9	1	6.1
		10	5.3	6	4.5	4	4.6
		11	4.6	7	4.6	8	4.2
		14	3.0	9	4.1	13	3.9
		16	6.1	12	5.3	15	5.7
		20	5.0	19	5.2	17	7.2
		24	3.6	21	5.1	18	3.8
		26	4.9	23	6.6	22	4.9
		27	5.0	28	6.1	25	6.2
		32	5.1	30	5.8	29	6.2
		33	4.1	35	5.4	31	6.3
10.22	B	7	4.3	1	5.2	8	5.2
		10	2.4	2	7.2	9	5.0
		12	6.5	3	4.6	18	5.4
		13	5.3	11	7.2	19	8.2
		14	3.9	15	7.1	20	7.2
		16	5.3	17	7.2	25	5.4
		24	4.4	21	6.5	29	6.5
		26	4.7	22	6.0	30	4.7
		27	4.6	23	4.3	33	4.3
		31	4.6	28	4.4	34	6.5
		32	3.5	4	5.3	35	5.7
10.22	C	3	5.4	1	6.9	2	4.8
		5	5.9	4	5.2	6	6.5
		10	4.0	7	6.2	9	7.3
		12	3.9	8	5.1	11	5.7
		14	5.0	16	6.1	13	7.2
		15	5.6	18	5.8	17	5.9
		21	6.4	23	5.7	19	7.4
		22	3.4	27	5.8	25	7.9
		26	3.9	28	5.0	29	6.9
		31	2.9	30	6.9	32	5.8
		35	3.5	33	6.1	34	8.6
10.22	D	1	3.4	11	6.8	2	9.5
		4	4.5	12	5.1	3	8.9
		6	5.1	13	6.9	5	7.9
		9	5.0	14	4.6	7	9.4
		16	3.9	19	7.9	8	7.6
		17	4.4	20	6.9	15	6.5
		18	4.4	24	6.1	21	8.6
		22	3.3	25	6.7	27	7.3
		30	3.3	26	5.0	29	6.2
		32	4.9	28	7.5	33	10.2
		35	5.8	31	6.4	34	7.9

Table 5-1.4 The Analysis of the Results

			Sum of Squares	Degrees Freedom
Between Lights	$\sum_{i=1}^{4} \left(\frac{T_{L_1}^2}{132} + \frac{T_{L_2}^2}{132} + \cdots \right) - \frac{T_G^2}{528}$	=	SS_L	3
Between Days	$\sum_{k=1}^{3} \left(\frac{T_{D_1}^2}{176} + \frac{T_{D_2}^2}{176} + \cdots \right) - \frac{T_G^2}{528}$	=	SS_D	2
Interactions Lights x Days	$\sum_{i=1}^{4} \sum_{k=1}^{3} \left(\frac{T_{L_1D_1}^2}{44} + \frac{T_{L_1D_2}^2}{44} + \cdots \right) - \frac{T_G^2}{528} - SS_L - SS_D$	=	SS_{LD}	6
Error (Days)	$\sum_{i=1}^{4} \sum_{k=1}^{3} \sum_{l=1}^{11} \left(\frac{T_{Pot_1}^2}{4} + \frac{T_{Pot_2}^2}{4} + \cdots \right) - \sum_{i=1}^{4} \sum_{k=1}^{3} \left(\frac{T_{L_1D_1}^2}{44} + \frac{T_{L_1D_2}^2}{44} + \cdots \right)$	=	$SS_{E(D)}$	120
Between Treatments (i.e. Doses)	$\sum_{j=1}^{4} \left(\frac{T_{T_1}^2}{132} + \frac{T_{T_2}^2}{132} + \cdots \right) - \frac{T_G^2}{528}$	=	SS_T	3
Interactions Treatments x Lights	$\sum_{j=1}^{4} \sum_{i=1}^{4} \left(\frac{T_{T_jL_i}^2}{33} + \cdots \right) - \frac{T_G^2}{528} - SS_T - SS_L$	=	SS_{TL}	9
Treatment x Days	$\sum_{j=1}^{4} \sum_{k=1}^{3} \left(\frac{T_{TD}^2}{44} + \frac{T_{TD}^2}{44} + \cdots \right) - \frac{T_G^2}{528} - SS_T - SS_D$	=	SS_{TD}	6

/continued...

Table 5-1.4 continued.

			Sum of Squares	Degrees Freedom
2nd Interaction Treatments x Lights x Days	$\sum_{j=1}^{4}\sum_{i=1}^{4}\sum_{k=1}^{3}\left(\frac{T^2_{TLD}}{11}+\frac{T^2_{TLD}}{11}+\ldots\right)-\frac{T^2_G}{528}-SS_{TL}$ $-SS_{TD}-SS_T-SS_L-SS_D$	$=$	SS_{TLD}	18
Error (Treatments)	$=\text{Total}-SS_L-SS_D-SS_{LD}-SS_{E(D)}-SS_T-SS_{TL}$ $-SS_{TD}-SS_{TLD}$	$=$	$SS_{E(T)}$	360
Total	$=\sum_{i=1}^{4}\sum_{j=1}^{4}\sum_{k=1}^{3}\sum_{l=1}^{11}Y^2_{ijkl}-\frac{T^2_G}{528}$			527

Table 5-1.5

The Analysis of Variance for the First Statistical Analysis of the Difference in Dry Weights Between the Plants

Sum of Squares		D.F.	Mean Square (Variance)	F (Variance Ratio)	
SS_L	0.00068469	3	0.00022823	$\frac{L}{LxD}$	2.2
SS_D	0.00095889	2	0.00047944	$\frac{D}{LxD}$	4.5
SS_{DL}	0.00063518	6	0.00010586	$\frac{LD}{E(D)}$	35.8
$SS_{E(D)}$	0.00035551	120	0.00000296		
SS_T	0.00003824	3	0.00001275		
SS_{TL}	0.00006028	9	0.00000670	$\frac{TL}{TDL}$	2.2
SS_{TD}	0.00001486	6	0.00000247	$\frac{TD}{TDL}$	0.8
SS_{TDL}	0.00005573	18	0.00000310	$\frac{TDL}{E(T)}$	0.7
$SS_{E(T)}$	0.00163111	360	0.00000453		
$\frac{SS_T}{Q^*}$	$\frac{0.00003824}{0.00176189}$	$\frac{3}{393}$	$\frac{0.00001275}{0.00000448}$		2.8**

Notes: * Q = Total interactions plus the error

** This value is significant at the 5% level of probability.

Table 5-1.6

The Analysis of Variance for the First Statistical Analysis of the Difference in Heights Between the Plants.

Sum of Squares		D.F.+	Mean Square (Variance)	F (Variance Ratio)	
SS_L	64.37709	3	21.45903	$\frac{L}{LxD}$	1.6
SS_D	528.30346	2	264.15173	$\frac{L}{LxD}$	19.5
SS_{DL}	81.42979	6	13.57163	$\frac{LD}{E(D)}$	8.4
$SS_{E(D)}$	194.26483	120	1.61887		
SS_T	16.69528	3	5.56509		
SS_{TL}	18.49789	9	2.05532	$\frac{TL}{TDL}$	1.1
SS_{TD}	11.77923	6	1.96321	$\frac{TD}{TDL}$	1.1
SS_{TDL}	33.52191	18	1.86223	$\frac{TDL}{E(T)}$	1.2
$SS_{E(T)}$	552.71277	360	1.53531		
$\frac{SS_T}{Q^*}$	$\frac{16.69528}{616.51180}$	$\frac{3}{393}$	$\frac{5.56509}{1.56873}$		3.5**

+ D.F. = Degrees of Freedom

*Q = Total Interactions plus Error

** This value is significant at the 1% level.

Table 5-1.7

The Total Dry Weights for Each Dose Group per Day of Analysis

Dose (Mrep)	Total Dry Weights (gms)			
	Day 1	Day 2	Day 3	Total
0.0	0.34144	0.40698	0.48680	1.23524
0.52	0.36033	0.43900	0.50166	1.30099
2.98	0.33250	0.41288	0.45983	1.20521
10.22	0.34006	0.41845	0.50683	1.26534

Table 5-1.8

The Total Heights for Each Dose Group per Day of Analysis

Dose (Mrep)	Total of Heights (cms)			
	Day 1	Day 2	Day 3	Total
0.0	180.0	249.4	299.4	728.8
0.52	198.4	258.8	320.2	777.4
2.98	188.2	237.4	288.7	714.3
10.22	198.6	259.3	287.2	745.1

Table 5-1.9

The Values of the Means for Dry Weight and Height for Each Dose

Dose (Mrep)	Mean Dry Weight (gms)	Mean Height (cms)
0.0	0.00935	5.50
0.52	0.00986	5.90
2.98	0.00915	5.40
10.22	0.00960	5.65

Table 5-1.10

Analysis of the Differences of the Means

	Dry Weight Analysis	Height Analysis	Significant
Mean square of $SS_{E(T)}$ = M.E(T)	0.00000414	1.535	
Population Standard Deviation (σ) = $\sqrt{M.E(T)}$	0.0021	1.24	
Differences Between the Means ($\bar{X}$)			
$\bar{X}_{0.52} - \bar{X}_{0.0}$	0.00051	0.4	
$\bar{X}_{2.98} - \bar{X}_{0.0}$	-0.00020	-0.1	
$\bar{X}_{10.22} - \bar{X}_{0.0}$	0.00025	0.15	
No. samples each group (N)	132	132	
* $Z_{0.52}$	2.80	3.81	Yes
$Z_{2.98}$	-1.09	-0.93	No
$Z_{10.22}$	1.37	1.39	Yes

$$* \; Z = \frac{\bar{X}_T - \bar{X}_{control}}{\sigma/\sqrt{N}}$$

5-2 The Second Statistical Analysis of Plant Growth

This second analysis was planned with a similar pattern to the first. It was intended to use longer time intervals and so each plant was grown in sterilized soil in a 6 in. diameter plastic Win Pot (from Winstones, of Auckland). For the purposes of the discussion, however, "Pot" refers to a set of four small pots each one corresponding to a different dose in a manner analogous to that in the first statistical analysis. The layout for the second is shown in Table 5-2.1, the order of numbers having been obtained by the use of a random number generator program for the Hewlit Packard Calculator The positions a, b, c and d were defined as clockwise positions beginning with a in the North-West corner. This layout was repeated in each of the four light groups used.

The three time intervals used for this experiment were 4 weeks, 8 weeks and 12 weeks. The dose groups used were 0.0, 0.52, 2.98 and 10.22 Mrep seeds. Eleven pots were selected from each Light Group for removal of plants at the end of each time period. The constant dry weight was determined for each plant and the results are shown in Table 5-2.2.

The results were analysed by computer using the same programme as for the first analysis. The Analysis of Variance is shown in Table 5-2.3. The difference between treatments is significant at the 0.1% level.

The differences between the means (shown in Table 5-2.4) were further analysed. This analysis is shown in Table 5-2.5. The variation between the means of each group of irradiated seeds and the non-irradiated controls is significant for all groups at a level greater than $P = 0.002$.

Table 5-2.1

The Layout used for the Second Statistical Analysis

Pot No.	a	b	c	d	Pot No.	a	b	c	d
1	2	1	3	4	22	1	3	4	2
2	1	3	4	2	23	1	4	3	2
3	4	2	1	3	24	4	3	2	1
4	3	1	2	4	25	4	2	1	3
5	3	4	1	2	26	3	1	2	4
6	3	1	2	4	27	4	1	2	3
7	2	1	3	4	28	4	2	3	1
8	2	3	1	4	29	2	1	4	3
9	1	2	3	4	30	1	2	3	4
10	3	1	4	2	31	1	2	3	4
11	3	1	4	2	32	4	3	1	2
12	4	3	2	1	33	1	3	4	2
13	3	1	4	2	34	3	1	2	4
14	1	3	2	4	35	2	4	1	3
15	2	1	4	3	36	1	3	2	4
16	2	4	1	3	37	3	1	2	4
17	3	1	4	2	38	3	2	4	1
18	1	2	4	3	39	3	1	4	2
19	3	4	1	2	40	4	3	2	1
20	3	4	2	1	41	2	4	1	3
21	1	2	4	3	42	1	3	4	2

Code	1	2	3	4
Dose (Mrep)	0.0	0.52	2.98	10.22

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21

22	23	24	25	26	27	28
29	30	31	32	33	34	35
36	37	38	39	40	41	42

The Pot Numbers shown for a Typical Light Block

Table 5-2.2

The Dry Weights Obtained for the Plants Involved in the Second Statistical Analysis

Dose (Mrad)	Light No.	Day One		Day Two		Day Three	
		Pot No.	Dry Weight	Pot No.	Dry Weight	Pot No.	Dry Weight
0.00	A	3	0.14615	2	0.13048	5	1.52944
		4	0.15120	6	0.17809	10	0.57122
		8	0.06825	12	0.42967	11	1.44111
		18	0.07303	14	0.39389	13	0.40271
		21	0.04332	16	0.68088	15	0.54302
		23	0.05847	24	0.26717	19	0.27283
		25	0.07724	28	0.18454	20	1.03745
		27	0.20137	31	0.22884	26	0.33624
		30	0.13956	32	0.33085	34	1.37087
		35	0.15331	39	0.23227	38	0.46057
		37	0.07270	41	0.09649	42	0.41881
0.00	B	3	0.03041	2	0.43944	5	1.01878
		4	0.14389	6	0.16281	10	0.45760
		8	0.04675	12	0.40518	11	0.95706
		18	0.08188	14	0.63754	13	0.76245
		21	0.02767	16	0.32177	15	1.17321
		23	0.10600	24	0.33861	19	0.38653
		25	0.04971	28	0.21695	20	0.78563
		27	0.08973	31	0.25746	26	0.26235
		30	0.08894	32	0.38029	34	0.54609
		35	0.06008	39	0.13885	38	0.11376
		37	0.22243	41	0.33046	42	0.22861
0.00	C	3	0.04150	2	0.46415	5	1.27348
		4	0.03798	6	0.34711	10	0.49009
		8	0.08195	12	0.32691	11	1.12640
		18	0.05155	14	0.12945	13	0.54227
		21	0.01505	16	0.36330	15	0.99079
		23	0.04881	24	0.11392	19	0.91673
		25	0.09739	28	0.26359	20	0.49791
		27	0.05794	31	0.33274	26	0.27326
		30	0.02859	32	0.19275	34	0.52336
		35	0.08257	39	0.29546	38	0.54697
		37	0.05482	41	0.24215	42	0.72221
0.00	D	3	0.04780	2	0.37990	5	1.00240
		4	0.04202	6	0.32574	10	0.80742
		8	0.06394	12	0.36158	11	0.74135
		18	0.05310	14	0.18016	13	0.28440
		21	0.08423	16	0.31204	15	1.00201
		23	0.05036	24	0.16054	19	0.28484
		25	0.05404	28	0.11308	20	0.57418
		27	0.09744	31	0.26203	26	0.66510
		30	0.03632	32	0.19782	34	0.43435
		35	0.03474	39	0.14971	38	0.63718
.	.	37	0.02935	41	0.30894	42	0.59905

/continued...

Table 5-2.2 continued

Dose (Mrad)	Light No.	Day One		Day Two		Day Three	
		Pot No.	Dry Weight	Pot No.	Dry Weight	Pot No.	Dry Weight
0.52	A	3	0.06660	2	0.34558	5	1.25712
		4	0.04137	6	0.39012	10	0.81300
		8	0.04602	12	0.80097	11	1.34893
		18	0.04926	14	0.42113	13	0.89037
		21	0.08074	16	0.46002	15	1.82041
		23	0.01033	24	0.22785	19	2.30813
		25	0.02679	28	0.34433	20	1.01455
		27	0.06481	31	0.20721	26	0.47946
		30	0.14156	32	0.22962	34	1.54878
		35	0.08402	39	0.21077	38	0.65999
		37	0.10782	41	0.33594	42	0.31711
0.52	B	3	0.15645	2	0.36588	5	1.66909
		4	0.15727	6	0.53954	10	0.72868
		8	0.05989	12	0.24501	11	1.31582
		18	0.00795	14	0.40934	13	0.83879
		21	0.00470	16	0.53243	15	0.65083
		23	0.13740	24	1.59874	19	0.59641
		25	0.10640	28	0.11894	20	0.30031
		27	0.07794	31	0.28719	26	0.17427
		30	0.14295	32	0.28000	34	1.12145
		35	0.17698	39	0.51576	38	0.59181
		37	0.09983	41	0.15623	42	0.54745
0.52	C	3	0.03222	2	0.41554	5	1.08285
		4	0.03397	6	0.90513	10	0.63251
		8	0.04223	12	0.52445	11	1.45727
		18	0.01969	14	0.21382	13	1.03055
		21	0.05934	16	0.19084	15	2.18066
		23	0.02770	24	0.28167	19	0.30023
		25	0.00907	28	0.31363	20	0.23891
		27	0.06217	31	0.33605	26	1.22561
		30	0.04890	32	0.32023	34	0.95783
		35	0.06647	39	0.15218	38	1.42892
		37	0.08318	41	0.28168	42	0.33181
0.52	D	3	0.03125	2	9.41835	5	1.47278
		4	0.02396	6	0.48718	10	0.50870
		8	0.03661	12	0.48543	11	0.42540
		18	0.02051	14	0.22873	13	0.49156
		21	0.00448	16	0.30924	15	2.19110
		23	0.06285	24	0.27999	19	0.75559
		25	0.04852	28	0.33044	20	1.16487
		27	0.06708	31	0.51916	26	0.81300
		30	0.05795	32	0.48503	34	0.26295
		35	0.01833	39	0.13609	38	0.99498
		37	0.03767	41	0.43358	42	0.43091

/continued...

Table 5-2.2 continued

Dose (Mrad)	Light No.	Day One		Day Two		Day Three	
		Pot No.	Dry Weight	Pot No.	Dry Weight	Pot No.	Dry Weight
2.98	A	3	0.04609	2	0.15495	5	1.46693
		4	0.01386	6	0.36222	10	0.64027
		8	0.007550	12	0.61024	11	1.79370
		18	0.08326	14	0.13585	13	1.53563
		21	0.01952	16	0.41884	15	0.97723
		23	0.03675	24	0.12550	19	0.16743
		25	0.03159	28	0.07149	20	0.92133
		27	0.08413	31	0.40660	26	0.49814
		30	0.08545	32	0.10125	34	0.94938
		35	0.09043	39	0.38440	38	0.57896
		37	0.06891	41	0.21105	42	0.50296
2.98	B	3	0.15360	2	0.27024	5	1.32511
		4	0.18205	6	0.33227	10	0.52494
		8	0.03350	12	0.56379	11	1.48446
		18	0.10944	14	0.11459	13	1.77017
		21	0.08211	16	0.10236	15	1.89638
		23	0.13289	24	0.49194	19	0.59789
		25	0.07750	28	0.17375	20	1.31596
		27	0.08141	31	0.38616	26	0.36488
		30	0.35736	32	0.23230	34	0.96953
		35	0.07288	39	0.38920	38	0.77027
		37	0.09524	41	0.04640	42	0.41745
2.98	C	3	0.01746	2	0.23391	5	0.94939
		4	0.02508	6	0.46862	10	0.64563
		8	0.01586	12	0.63162	11	0.83554
		18	0.02347	14	0.23834	13	1.41589
		21	0.03619	16	0.47496	15	1.76479
		23	0.00790	24	0.40407	19	0.35577
		25	0.02091	28	0.38500	20	0.56219
		27	0.07056	31	0.19201	26	0.31964
		30	0.03417	32	0.30515	34	1.05075
		35	0.05790	39	0.33576	38	0.29173
		37	0.13199	41	0.10036	42	0.55649
2.98	D	3	0.06115	2	0.45147	5	0.94390
		4	0.03732	6	0.51904	10	0.80136
		8	0.04618	12	0.37739	11	0.26436
		18	0.03055	14	0.27430	13	1.55051
		21	0.01930	16	0.59272	15	1.39494
		23	0.12245	24	0.46297	19	0.26381
		25	0.04234	28	0.28389	20	0.60509
		27	0.07030	31	0.15649	26	0.46457
		30	0.03981	32	0.69091	34	0.57173
		35	0.06856	39	0.40686	38	1.15744
		37	0.11205	41	0.44035	42	0.42238

/continued...

Table 5-2.2 continued

Dose (Mrad)	Light No.	Day One		Day Two		Day Three	
		Pot No.	Dry Weight	Pot No.	Dry Weight	Pot No.	Dry Weight
10.22	A	3	0.15594	2	0.25889	5	0.90769
		4	0.13446	6	0.22372	10	0.48953
		8	0.03581	12	0.43805	11	1.33556
		18	0.05958	14	0.05914	13	1.22486
		21	0.02006	16	0.15643	15	0.82099
		23	0.05035	24	0.25591	19	0.80285
		25	0.06330	28	0.15263	20	1.71280
		27	0.10240	31	0.35669	26	1.10766
		30	0.00527	32	0.53390	34	1.45317
		35	0.20557	39	0.39223	38	0.36926
		37	0.08790	41	0.31738	42	0.57543
10.22	B	3	0.03884	2	0.27236	5	0.96533
		4	0.10507	6	0.58763	10	0.94262
		8	0.07759	12	0.51141	11	1.43080
		18	0.05944	14	0.20131	13	1.77482
		21	0.02640	16	0.43106	15	1.26717
		23	0.07026	24	0.39814	19	0.61295
		25	0.04569	28	0.06779	20	1.36226
		27	0.10761	31	0.29013	26	0.23699
		30	0.06715	32	0.12230	34	0.77206
		35	0.09796	39	0.19459	38	0.62191
		37	0.03780	41	0.34041	42	0.44532
10.22	C	3	0.04242	2	0.53343	5	1.37821
		4	0.03488	6	0.73887	10	1.36255
		8	0.07748	12	0.79896	11	1.09731
		18	0.03940	14	0.20390	13	1.18822
		21	0.00846	16	0.29771	15	1.59986
		23	0.02360	24	0.43793	19	0.53372
		25	0.02120	28	0.38668	20	1.02723
		27	0.05262	31	0.17673	26	0.80537
		30	0.03398	32	0.23738	34	0.63449
		35	0.02708	39	0.15513	38	0.56479
		37	0.03332	41	0.37536	42	0.34362
10.22	D	3	0.03163	2	0.55757	5	1.17130
		4	0.04925	6	0.76800	10	0.37406
		8	0.08871	12	0.33466	11	1.67562
		18	0.02565	14	0.39268	13	1.59352
		21	0.01790	16	0.27116	15	1.40295
		23	0.06449	24	0.24979	19	0.29986
		25	0.03722	28	0.38183	20	0.58680
		27	0.05039	31	0.28763	26	0.74793
		30	0.05656	32	0.19174	34	0.96050
		35	0.04920	39	0.21549	38	0.99419
		37	0.04168	41	0.48570	42	0.42419

Table 5-2.3

The Analysis of Variance for the Second Statistical Analysis of Dry Weight of Plants.

Sum of Squares		D.F.	Mean square (Variance)	F (Variance ratio)	
SS_L	0.11581	3	0.038603	$\frac{L}{LxD}$	0.4
SS_D	57.87113	2	28.935505	$\frac{D}{LxD}$	321.3
SS_{DL}	0.54161	6	0.090268	$\frac{LxD}{E(D)}$	0.5
$SS_{E(D)}$	22.48355	120	0.187363		
SS_T	1.06700	3	0.355667		
SS_{TL}	0.28888	9	0.032097	$\frac{TL}{TDL}$	0.6
SS_{TD}	1.25265	6	0.208775	$\frac{TD}{TDL}$	3.7
SS_{TDL}	1.00971	18	0.056095	$\frac{TDL}{E(T)}$	1.1
$SS_{E(T)}$	18.53635	360	0.051489		
$\frac{SS_T}{Q^*}$	$\frac{1.06700}{21.08759}$	$\frac{3}{393}$	$\frac{0.355667}{0.053679}$		6.6^{**}

* Q = Total interactions plus error

** This value is significant at the 0.1% level.

Table 5-2.4

The Total Dry Weights and Mean Values for Each Dose Group

Dose (Mrep)	Total Dry Weights (gms)				
	Day 1	Day 2	Day 3	Total	Mean
0.0	3.32357	12.60559	30.01206	45.94124	0.34804
0.52	2.74122	17.07103	41.37172	61.18398	0.46351
2.98	3.10496	14.51157	38.65687	56.27341	0.42631
10.22	2.56156	15.04042	41.99829	59.60028	0.45152

Table 5-2.5

	Dry Weight analysis	Significant Level	
Mean square of $SS_{E(T)}$ (i.e. ME(T))	0.051489		
Population Standard Deviation (σ) $= \sqrt{ME(T)}$	0.2269		
Difference Between the Means ($\bar{X}$)			
$\bar{X}_{0.52} - \bar{X}_{0.0}$	0.11547		
$\bar{X}_{2.98} - \bar{X}_{0.0}$	0.07827		
$\bar{X}_{10.22} - \bar{X}_{0.0}$	0.10348		
No. samples each group (N)	132		
$^{*}Z_{0.52}$	5.86	Yes	0.002
$Z_{2.98}$	3.96	Yes	0.002
$Z_{10.22}$	5.24	Yes	0.002

* $$Z = \frac{\bar{X}_T - \bar{X}_{0.0}}{\sigma/\sqrt{N}}$$

5-3 Summary of Results

These statistical analyses show that the plants grown from irradiated but recovered seeds are significantly taller and contain more dry matter than do the plants grown from non-irradiated seeds.

During the earlier period of growth the difference between the groups is not so marked. In the first statistical analysis the growth exhibited by the 2.98 Mrep group was in fact less than that of the control group but the difference was not statistically significant. In the second analysis the control group had the highest total dry weight after 4 weeks growth but was very closely followed by the 2.98 Mrep group. By day 2 and day 3, however, the 0.52 Mrep group was 33-42% heavier than the controls and all the irradiated groups were significantly heavier than the controls.

The 0.52 Mrep group had begun flowering by Day 2 (8 weeks) whereas the control group had barely begun to flower at the conclusion of this experiment (12 weeks).

6. THE EFFECTS OF GAMMA RADIATION ON THE GERMINATION OF MANY SEED SPECIES

6-0 Introduction

It was decided to investigate the effects of gamma-radiation on a large range of seed species to determine whether or not the data obtained for mustard seeds was similar to that for other seeds. Using seedling growth as a criteria for radiosensitivity, it has normally been accepted that mustard seeds are the most radioresistant seed species. Lettuce, pea and broad bean seeds in particular, are normally regarded as the more radiosensitive seeds.

6-1 Experimental Details

6-1.1 Irradiation Procedure

Seeds in paper envelopes were equilibrated overnight at 75% relative humidity, above a slurry of sodium chloride and water in a desiccator. The seeds were then irradiated with various doses of gamma-radiation from a cobalt-60 source at the Institute of Nuclear Science, Wellington.

6-1.2 Germination Studies

For the germination tests a random selection of one hundred seeds of each dose group were placed on two 11 cm No.5 Whatman filter papers in Petri dishes. Distilled water was added, ensuring that each seed was moistened and the seeds were separated from one another using a spatula. The Petri dishes were kept in an incubator at 30°C. The germinated seeds were counted at regular intervals of 24 hours, taking the protrusion of the radicle as the criterion for germination.

6-2 Germination Studies

Seeds of the following species and varieties were exposed to various doses of Co-60 gamma-rays:

Squash (var. "Butternut")

Water melon (var. "Black Diamond Florida Giant")

Wheat

Oat ("Algerian")

Pea

Broad Bean

Lettuce ("Webbs Wonderful" and "Great Lakes")

Onion ("White Silverskin")

Cucumber ("Heinz Gherkin")

Egg Plant ("New York Purple")

Spinach ("Long Round Standing Viking")

Parsnip ("Hollow Crown Select")

Tomato ("Frost Resistant" and "Beefsteak")

White mustard.

The germination of these seeds was examined following the irradiation procedures. The results are shown in Tables 6-2.1 to 6-2.15, inclusive and in the corresponding graphs (contained in the Appendix).

Table 6-2.16 is a summary of the results obtained for the total germination expressed as a percentage of that of the control, for the dose groups and seeds used in these experiments. The absolute dose for each group of seeds can be found by referring back to the appropriate table. For all the seeds examined after having received a dose below 0.27 Mrad the effect on the total germination was negligible. A dose of 0.33-0.34 Mrad slightly supressed the germination percentage of broad bean, had a

Table 6-2.16

Summary of the Effects of Gamma-Radiation on the Total Germination of Seeds (Expressed as % of Control)

Dose Range (Mrad)	0.0	0.10-0.17	0.265	0.33-0.34	0.49-0.52	0.672	0.98-1.4	2.0	3.3-3.5	5.0-5.14	10.4-10.8	30.7-32.3
Squash	100				84					0		
Water melon	100				89					0		
Wheat	100				90					0		
Oat	100				99					0		
Pea	100	94		90	21		19		0	0		
Broad bean	100	96		79	4				0	0		
Lettuce	100	100		98	95	96	86	0	0	0		0
Onion	100				88		87	0		0		0
Cucumber	100				100					0		
Egg plant	100				72					0		
Spinach	100				61					0		
Parsnip	100				0					0		
Tomato	100				81 & 89					0		
Mustard	100	100	99		100		30	0	0	0	0	0
Ryegrass	100				66			0		0	0	
White clover	100				91			0		0	0	

smaller effect on pea seeds and a negligible effect on lettuce seeds.

The responses of seeds to a dose in the range of 0.49-0.51 Mrads were extremely varied for the seeds examined. Mustard and cucumber were totally unaffected (100% of the control). The effect on the germination of oat (99%) and lettuce (95%) was negligible. The germination of white clover (91%), wheat (90%), water melon (89%), tomato variety "Frost Resistant" (89%), onion (88%) and squash (84%) was slightly suppressed. Tomato variety "Beefsteak" (81%), egg plant (72%), ryegrass (66%) and spinach (61%) seeds were more adversely affected but still showed over 50% of the germination of the non-irradiated seeds. Hence the LD_{50} value for all the seeds mentioned so far is greater than 0.5 Mrads. In the dose range of 0.49-0.51 Mrads the germination of pea seeds (21%), broad bean (4%), and parsnip (0%) was seriously affected. The LD_{50} for these seeds must be less than 0.5 Mrads.

The experimental LD_{100} for the seeds examined lies between 1.14 Mrad and 2.0, 3.3 or 5.0 Mrads. All seeds examined after having received a dose of 2.0 Mrads, although the more radioresistant based on their response to 0.5 Mrad, lacked the ability to germinate.

The LD_{50} values for the more radiosensitive seeds examined seems to lie between 0.33 and 0.5 Mrad (pea and broad bean) and for the more radioresistant seeds between 0.5 Mrad and 2.0 Mrad.

From the graphs it can be seen that in general the "Percentage Germination" versus "Time" curves differ for the various doses of gamma radiation received by the seeds of each type examined. The series of graphs designated "a", of "Rate % germination" versus "time" are, in effect, the first derivative of the corresponding "Percentage Germination" versus "time" graph. For most seeds examined the maximum rate of germination decreased and became progressively later with increasing dose. In some

seeds, however, e.g. water melon, a small percentage of the irradiated (0.51 Mrad) seeds germinated more quickly than the non-irradiated seeds and this was followed by the pattern normally observed, i.e. delayed germination of the remaining portion.

The effects of gamma-radiation on mustard seeds have been examined at several dose rates. For these seeds, at least, the total dose appears to be the important factor regulating the germination response and not the dose-rate as is thought to be true for seedling-height response.

6-3 Summary of Results

The LD_{50} for germination of the majority of seeds examined is greater than 0.5 Megarads (i.e. 5×10^5 rads). This value is much higher than that suspected before these experiments were begun.

Gamma-radiation of seeds, without storage, at high doses, causes a decrease in the total percent germination with increasing dose as well as a decrease in the maximum rate of germination which occurs later with increasing dose.

7. THE EFFECTS OF STORAGE UNDER VARIOUS CONDITIONS ON THE GERMINATION OF IRRADIATED SEEDS.

7-0 Introduction

From the results obtained with gamma-irradiated Sinapis alba seeds reported in section four it was concluded that the relative humidity of storage was an extremely important factor controlling the effects of gamma-radiation on seeds. It was therefore decided to carry out an extensive experiment with a variety of seeds stored at various relative humidities and the effects of the storage on the germination studied. In addition to storing many seeds at fixed relative humidities others were also stored at room humidities. It must be stressed, however, that the room humidities mentioned here were in fact different to those mentioned earlier. The first few months were similar to those earlier in that the seeds were stored in boxes on the 2nd floor of the Chemistry Department at Victoria University. After this period they were transferred to Waikato University into a building centrally heated by natural gas, and hence the conditions differed to that of the earlier period.

7-1 Experimental Details

7-1.1 Storage

Seeds reported as stored "in air" were kept in paper envelopes inside cardboard boxes on shelves in the relevant laboratory. The other seeds were stored in Agee Jars on platforms above slurries of salt solutions to give the required relative humidity.

7-1.2 Germination Studies

The germination of the seeds used in this experiment before storage have been reported in section six. The methods used for the germination procedures were identical to those detailed in section 6-1.2.

7-2 Relationship Between Relative Humidity of Storage and Water Content of Seeds

The relative humidities of atmospheres in equilibrium with various concentrated salt solutions are shown in Table 7-2.1. Slurries of potassium sulphate, potassium nitrate, potassium chloride, sodium chloride, sodium nitrate, sodium dichromate, magnesium chloride, potassium acetate, potassium hydroxide and calcium chloride were prepared and carefully placed in the bottom of a series of Agee jars. Small metal stands were placed above the salt solutions, covered with 2 layers of cotton wool and the seeds placed on this platform. Nonirradiated mustard seeds were stored in each jar and at room humidity for one week to ensure equilibrium. The seeds were then quickly removed and weighed, dried to constant weight (this took over a week at 100°C) and reweighed. The results obtained are shown in Table 7-2.2

If the water content is plotted on the Y-axis and the relative humidity of storage on the X-axis of semi-log paper a straight line should be obtained, the slope of this line differing for each kind of seed.[326]

Using the data obtained for mustard seeds such a graph of water content versus relative humidity was plotted. (Graph 7-2.2). The lower region of this graph gave the theoretical straight line but the upper portion corresponded more closely to a second straight line of a different slope. The water content of seeds stored at room humidities was 9.26% and hence lay on the lower straight line portion. The relative humidity corresponding to such a water content is 47.5%.

The water contents for mustard seeds at the various relative humidities used for storage are shown in the table. Those for other seed species would differ. When stored at 76% R.H. at 20°C the water content of lettuce seeds is 11.1%, of onion 17.1% and of tomato 15.9%.[327] These values

Table 7-2.1

The Relative Humidities of Saturated Salt Solutions[339]

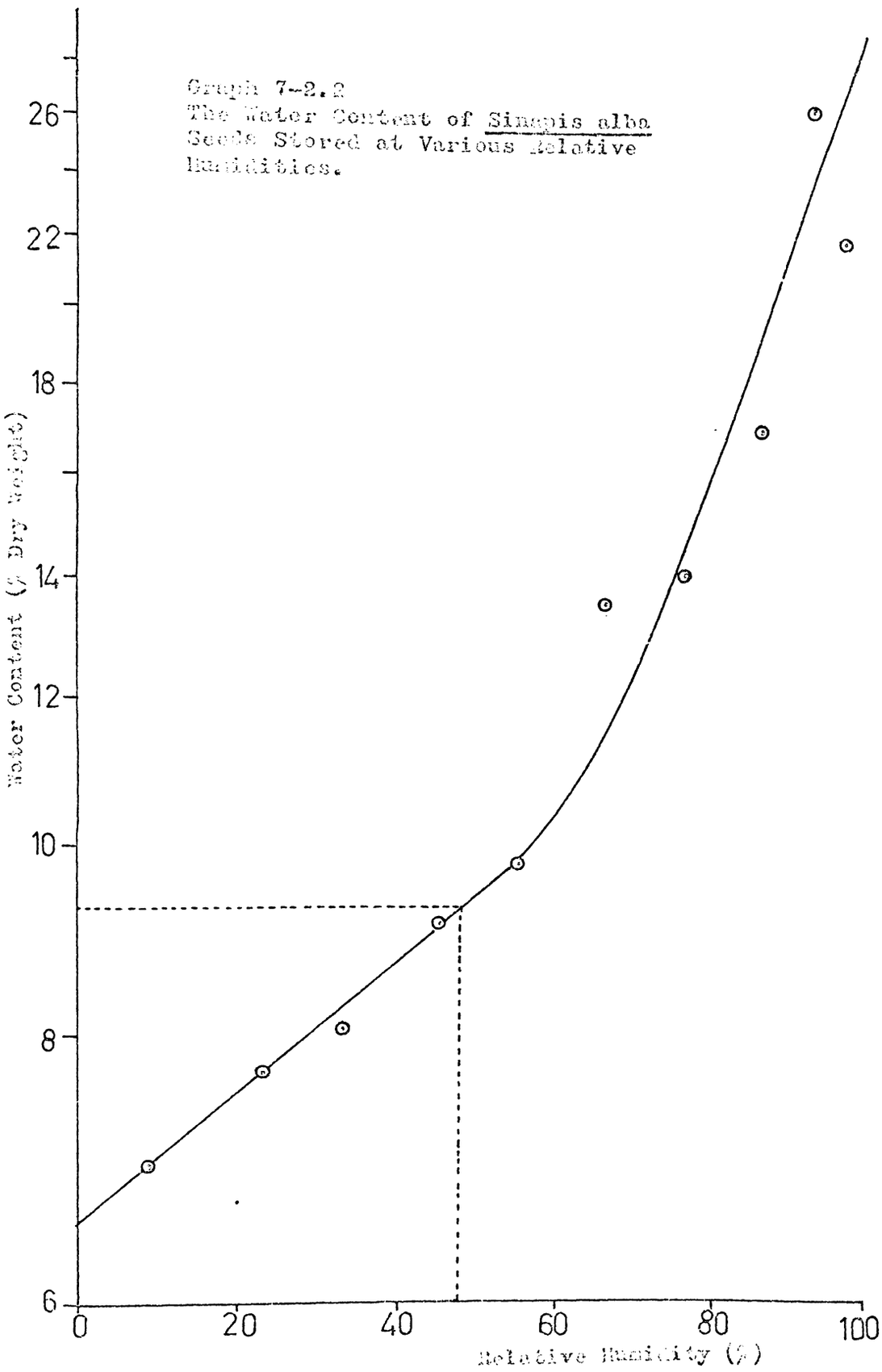
Solution	Temperature											Solns. Used
	0	5	10	15	20	25	30	35	40	50	60	
K_2SO_4	99	98	98	97	97	97	96	96	96	96	96	*
KNO_3	97	96	95	94	93	92	91	89	88	85	82	*
KCl	89	88	88	87	86	85	85	84	82	81	80	*
$(NH_4)_2SO_4$	83	82	82	81	81	80	80	80	79	79	78	
NaCl	76	76	76	76	76	75	75	75	75	75	75	*
$NaNO_3$	-	-	-	-	66	65	63	62	62	59	59	*
NH_4NO_3	77	74	72	69	65	62	59	55	53	47	42	
$Na_2Cr_2O_7$	60	59	58	56	55	54	52	51	50	47	-	*
$Mg(NO_3)_2$	60	58	57	56	55	53	53	52	50	49	46	
K_2CO_3	-	-	47	44	44	43	43	43	42	-	-	
$MgCl_2$	35	34	34	34	33	33	33	32	32	31	30	*
CH_3COOK	25	24	24	23	23	22	22	21	20	-	-	*
LiCl	15	14	14	13	12	12	12	12	11	11	11	
KOH	-	14	13	10	9	8	7	6	6	6	-	*
$CaCl_2$	45.4											*
LiBr	11											
$Ca(NO_3)_2$	56											

Table 7-2.2

The Water Content of Sinapis alba Seeds Stored at Various Relative Humidities

Relative Humidity	Dry Weight	Wet Weight	Water Content	% Water by Dry Weight
9%	14.530	15.539	1.009	6.94
23%	9.986	10.751	0.765	7.66
33%	19.673	21.254	1.581	8.04
45%	16.862	18.395	1.533	9.09
55%	16.539	18.154	1.615	9.76
66%	17.455	19.809	2.354	13.49
76%	17.979	20.497	2.518	14.01
86%	18.580	21.749	3.169	17.02
93%	10.746	13.583	2.837	26.40
97%	14.400	17.564	3.164	21.97
Room	29.729	32.483	2.754	9.26

Graph 7-2.2
The Water Content of Sinapis alba Seeds Stored at Various Relative Humidities.



are also equivalent to the water contents of the seeds before irradiation was carried out as they were equilibrated at 76% R.H. above a slurry of sodium chloride.

7-3 The Effects of Storage of Gamma-irradiated Seeds

At various time intervals the germination of seeds which had been stored at constant or room relative humidities was examined. The results obtained are shown in Tables 7-3.1 to 7-3.75 inclusive (in the Appendix). Broad bean seeds which had received a dose of 0.1 Mrad showed a slight improvement in the total % germination after storage at room humidities for up to 15 months but the rate remained similar to that without storage. After 2 months' storage at 2% R.H. the rate of germination of seeds which had received a dose of 0.33 Mrad much more closely resembled that for the control seeds than did the rate before storage. During the next 10 months these seeds underwent damage during storage and would no longer germinate. Seeds which had received a dose of 0.33 Mrad and were stored at 93% R.H. had lost their germinating ability within 8 months. Broad bean seeds showed 4% of the control germination immediately after receiving a dose of 1.0 Mrad but these seeds lost their ability to germinate after 15 months' storage in air. Seeds which had received 3.3 Mrad (stored at room humidities) and 5.1 Mrad (stored at 33% and 86%) did not germinate either immediately after irradiation or during storage.

Cucumber seeds after a dose of 0.51 Mrad showed slight improvement in the rate of germination after 2 months' storage at 33% R.H. but then deteriorated slightly during storage so that after 12 months' storage the rate of germination was once again similar to that of the control. Storage at 93% relative humidity reduced the germination of these seeds to 85% within 2 months and to 0% within 12 months. Cucumber seeds which had been

exposed to 5.1 Mrads did not germinate at any stage of the experiment.

Storage at 9% R.H. reduced the total % germination and caused a deterioration in the rate throughout the period studied for egg plant seeds which had received a dose of 0.51 Mrads. Storage of these seeds at 5% R.H. caused a loss in total % germination but did not alter the rate of germination. Storage at 97% caused a rapid loss of germinating ability but those seeds which did germinate did so more quickly than the controls. After 12 months' storage at 97% the germination had been reduced to zero. Storage at 33% and 93% R.H. of seeds after a dose of 5.0 Mrad led to appreciable recovery of these seeds. Before storage no germination occurred but after storage up to 6% germination was found.

Storage of oat seeds (0.51 Mrad) at 33% R.H. for 2 months caused a greater loss in total % germination than did storage at 76% R.H. but the seeds at the latter humidity soon deteriorated and lost the ability to germinate. Those stored at 33% R.H. did not, however, and after 17 months' storage showed a marked improvement in the rate of germination and, in terms of % of the control germination, a marked improvement in the total % germination but had not completely recovered to the value without storage. Oat seeds which had received a dose of 5.1 Mrad did not germinate with or without storage.

Lettuce seeds (variety "Webbs Wonderful") after a dose of 0.51 Mrad showed a decrease in total % germination after 2 months' storage at 9%, 23%, 45%, 57%, 76% and 97% relative humidities. This decrease had a minimum value for storage at 23% and 97%. After 8 months' storage at 9%, 23%, 45% and 57% these seeds had recovered but those stored at 76% and 97% continued to deteriorate, giving 0% germination at 8 months and thereafter. Lettuce seeds stored at room humidities after a dose of 0.51 Mrad showed rapid deterioration, from 95% to 4% germination, after 15 months' storage. A similar result was

obtained for 0.98 Mrad seeds, for which the germination fell from 86% to 0%. Two Megarad and 5.0 Mrad seeds stored at room humidities did not germinate at any stage of the experiment. After 2 months' storage at 23% and 76% R.H., the 5.0 Mrad seeds showed slight recovery in their ability to germinate (3% and 1% respectively). After a further period of storage at 55%, 76% and 97% no germination of these seeds occurred. However, slight recovery continued with the seeds stored at 43%, 23% and 9% but at no stage did all of these seeds recover their ability to germinate. Of the seeds which had received a dose of 32.3 Megarads, no germination was found except for a result of 1% after 2 months' storage at 66% R.H.

Lettuce seeds, variety "Great Lakes", were examined after receiving doses of 0.49 and 0.9 Mrad giving, without storage germination percents of 98 and 88 respectively. These seeds rapidly deteriorated on storage at room humidities and did not germinate after 15 months. Similar results were obtained for lettuce seeds, variety "White Cos". Germination of the "Great Lakes" variety after a dose of 32.3 Mrad was not obtained either before or after storage at various relative humidities.

Mustard seeds which had received a dose of 0.118 Mrad and which were stored at room humidities displayed neither deterioration nor recovery. Those which had received a dose of 0.265 Mrad showed an increase in their rate of germination on storage. After a dose of 0.5 Mrad, mustard seeds showed a greater % germination than the control when stored in the room. When stored at 9%, 23%, 33%, 43%, 55%, 66%, 76% and 86% R.H. for 2 months these seeds showed a marked increase in their rate of germination. Storage at 93% and 97% did not alter the maximum rate of germination but slightly depressed the total germination. Further storage of these seeds at humidities below 55% led to a marked

increase in their rate of germination and increased germination % totals and after 12 months' storage these were significantly greater than that for the nonirradiated seeds (e.g. 45% R.H. gave 116% germination of the control). Storage at 66% and 76% R.H. for prolonged time periods led to a decrease in the total % germination. Above these values, for the humidity of storage, deterioration proceeded rapidly reducing the germination to zero within 8 months. Mustard seeds which had received a dose of 1.14 Megarads gave only 30% germination immediately after irradiation but on storage in air for 8 months this had increased to 56% and a marked increase in the rate of germination had occurred.

White mustard seeds which had been exposed to doses of 2 Mrad or above did not germinate immediately after irradiation. When the irradiation was followed by 15 months' storage at room humidities some recovery did occur (3% germination). Storage of 4.38 Mrad seeds at 33% and 97% for 2 months led to 1% germination. Further storage at relative humidities below 45% gave further slight recovery (e.g. 4% germination after 8 months at 23% R.H.). Seeds which had received doses of 4.5, 5.0, 9.0 and 30.7 Mrads did not possess the ability to germinate even after prolonged storage at room humidities. Those which had received 10.4 and 10.8 Mrads gave 1% and 2% germination respectively after 8 months' storage in air. A dose of 32.3 Mrad reduced germination of mustard seeds to zero. Germination (2%) of these seeds was only observed after 14 months' storage at 23% R.H. and not after storage at 45%, 66% or 93% for various time periods.

Little change was observed for irradiated onion seeds on storage. Doses of 0.49 and 0.672 Mrad and storage at room humidities led to a marked increase in the rate of germination but slight depression in the total % germination. Storage at 76% R.H. of 0.49 Mrad seeds led to deterioration, reducing the germination to zero after 12 months.

Parsnip seeds which were exposed to 0.51 and 5.1 Megarads did not germinate immediately after irradiation nor after storage.

Storage of 0.1 and 0.33 Mrad pea seeds at room humidities for 15 months led to loss of germinating ability (from above 90% to 10%) but for the 0.51 Mrad seeds recovery occurred (21% to 95% at 15 months). Storage of irradiated pea seeds at 97% R.H. led to rapid deterioration. The germination of 0.51 Mrad seeds was reduced from 21% to 0% after only 2 months' storage.

Rye grass seeds exposed to a dose of 0.52 Mrad and stored at room humidities, recovered in terms of total % germination and in rate of germination. Those which had received doses of 2.0, 5.0 and 10.0 Mrad did not germinate.

Storage of 0.51 Mrad spinach seeds at 86% reduced the germination to zero within 2 months and at 33% within 12 months. The more heavily irradiated seeds did not recover on storage at 33% and 86%.

Storage at 86% R.H. rapidly reduced the germination of irradiated squash and tomato seeds to zero. Water melon seeds which had received a dose of 0.51 Mrad were adversely affected by storage at 55% and 86% R.H. At the latter value the germination was zero after 12 months' storage.

Wheat seeds gave 90% germination immediately after irradiation. After 2 months' storage at 9%, 23%, 45%, 66%, 76%, 86% and 97% relative humidities the germination expressed as % of the control was 103%, 95%, 96%, 87%, 88%, 56% and 52% respectively. A marked increase in the rate of germination of these seeds also occurred. After a further 10 months' storage no germination was found for those seeds stored at 66% R.H. or above. Those kept at 45%, 23% and 9% continued to show recovery. No germination was observed for seeds which had received a dose of 5.14 Mrads and stored at various relative humidities.

White clover seeds were exposed to doses of 0.52, 2.0, 5.0 and 10.0 Mrads and then stored at room humidities. Recovery of the 0.52 Mrad group occurred on storage, in terms of both total % germination and rate of germination. After 8 months' storage in air 10% germination was obtained for the 2.0 Mrad group. The 5.0 and 10.0 Mrad groups did not germinate at any stage.

After the germination of the seeds examined above the subsequent growth of the plants for the first 2 weeks approximately was observed. After 2 months' storage of 0.5 Mrad mustard seeds the total % germination was barely effected but the growth of the plants formed was. The seeds which had been stored at 9% R.H. germinated normally but the plants did not develop, and those at 23% R.H. gave the poor growth typical of freshly irradiated seeds. Those which had been stored at 33% and 45% showed excellent plant growth and were at least as tall as the control group. Storage at 55% and 66% gave poor plant growth where/as storage at 76% R.H. gave excellent plant growth. The growth of plants from seeds stored at 86%, 93% and 97% was also similar to that from nonirradiated seeds. The tallest plants from the 0.0 and 0.52 Mrad groups after 2 months' storage corresponded to 0.52 Mrad plants stored at 45% and 76%. It is interesting that these values also correspond to the room humidity at Victoria University in which full recovery of the seeds discussed in Section 4 occurred and the R.H. of the irradiation procedure.

Seeds which germinated on storage after receiving doses of 2.0 Mrad and above tended to give plants which appeared normal. Those from lower doses gave varied responses. Some plants would show little development whilst others appeared comparable to the control, but in general storage at 45% gave plants closely resembling those from non-irradiated seeds and storage at 9% gave retarded plants.

7-4 Discussion

At no stage of this experiment was 100% recovery similar to that described in Sections 4 and 5 observed, although in various instances some form of recovery was observed. These experiments do, however, show that relative humidities of storage control to a large extent the effects of irradiation on seeds.

Both 0.5 Mrad mustard and 0.51 Mrad lettuce seeds (var. "Webbs Wonderful") showed depression of their total % germination after 2 months' storage at most humidities and this was followed by rapid recovery for storage below 55% R.H. The depression after 2 months was less marked for medium humidities of storage. Such results could arise from increased damage occurring during this first 2 months of storage due to the long lived free radicals present in the seeds after irradiation. At medium water contents they can be removed by reaction with the water molecules at centres further away from important zones of the cells. At low water content the water is preferentially attracted to the DNA of the cell and in this situation chromosomal damage could occur if free radicals were formed in this water. At high relative humidities the seed metabolism or associated fungi or mould causes rapid loss of germinating ability.

It is possible from these results that minimum damage would occur to irradiated seeds if they are stored at high relative humidities for 1 month approximately to dissipate the long-lived free radicals rapidly and then at medium humidities to allow the recovery processes to occur but not deterioration of the seed. The seeds in these experiments were stored at constant humidities or room humidities only.

8. THE EFFECT OF ADDED SUBSTANCES ON THE GERMINATION OF IRRADIATED AND NONIRRADIATED SINAPIS ALBA SEEDS

8-0 Introduction

The effect of the addition of various compounds on the germination of seeds was investigated. Haber and Luippold[267] reported that addition of gibberellic acid caused the germination of lettuce seeds which had received a dose of 1.0 Mrad and which reportedly would not germinate in distilled water only. He only reported a time interval of 7 days for germination whereas the workers in the laboratory have shown that lettuce seeds of varieties "Webbs Wonderful", "Great Lakes", and "White Cos" will germinate after receiving between 1.0 and 1.5 Mrads although no germination occurs within the first 7 days. Further reports[70] state that added citric acid can lead to high germination percentages for nonviable seeds (although these had not been produced by irradiation).

Irradiated seeds which lacked the ability to germinate in distilled water were allowed to imbibe in solutions of various substances to study the effect on germination. The solutions used were sodium chloride, gibberellic acid, citric acid, thiourea, aspartic acid, glutamine, succinic acid, sucrose, lactic acid, glutamic acid, alanine, ferrous sulphate and a mixture of all these substances.*

8-1 Experimental Methods

The solutions of required concentration were prepared in distilled water. For the metabolic acids examined the acid itself was used, not the sodium or potassium salts of the acid. The germination studies were carried out in the manner previously described except that only the specified solution was added and not distilled water.

*Chosen because gibberellic acid, citric acid, thiourea - germination promoters; sodium chloride to check for ion effects; ferrous sulphate scavenger of free radicles; others labelled in tritiated water expts.

8-2 Experimental Results

The results are tabulated in Tables 8-2.1 to 8-2.13 inclusive and for each table a graph of percent germination versus time has been drawn.

In the first table the effect of added sodium chloride is shown. The seeds, which were irradiated in 1968 and which had completely recovered in both germination and plant growth, had been stored over a slurry of sodium chloride during equilibration and for 24 hours after irradiation. It was thought possible that the seeds may have come into contact with the slurry during the return voyage from Gracefield following irradiation. No germination occurred in the 4.9 Mrad dose group, either in water or in salt solution. The salt solution had little effect on the control seeds and those which had received 0.98 Mrad of gamma-rays although it does seem to prevent the initial slow onset of germination causing a higher percentage of seeds to germinate within the period of maximum rate (between 24-48 hours for the control). These results show that although the ions of the solution appear to penetrate the seed coat they have little effect on the damage caused by irradiation.

Added gibberellic acid caused a large increase in the initial rate of germination of nonirradiated seeds (77%, 76% in the first 24 hours as compared to 41% for distilled water). In 10^{-4} Molar gibberellic acid 100% germination was obtained although only 90% germination occurred in 10^{-2}M solution. No germination of the irradiated 3.5 Mrad seeds was observed in either gibberellic acid solution or distilled water. Hence this germination promoter does not cause the germination of irradiated, non-viable seeds.

Citric acid had the greatest effect of the solutions examined on the germination of mustard seeds which had received a dose of 3.5 Mrads.

A total of 3% was obtained in a 1.0 Molar solution whereas no germination occurred for nonirradiated seeds at this concentration. At concentrations of 10^{-2} Molar and 10^{-4} Molar, 5% and 1% germination respectively were obtained. Citric acid increased the rate of germination of nonirradiated seeds to a greater extent than did added gibberellic acid. Within the first 24 hrs, 83% of the seeds in 10^{-4} molar citric acid germinated, 76% in 10^{-4} molar gibberellic acid and only 41% in distilled water. These results show that citric acid must play an extremely important part during germination and be involved, at least in part, in metabolic cycles damaged by irradiation, leading to loss of germinating ability.

At the lower concentrations used thiourea solution had a negligible effect on the germination of nonirradiated seeds. No germination occurred in the one molar solution. The irradiated seeds did not germinate in 1.0 or 10^{-2} M solutions but 2% did at 10^{-4} molar thiourea. Added aspartic acid caused a slight retardation in the rate and total % germination at concentrations of 10^{-2} and 10^{-4} molar. A 1.0 molar solution only gave 24% germination of the non-irradiated seeds, possibly due to osmotic effects. The irradiated seeds did not germinate at concentrations of 1.0 and 10^{-2} molar but gave 4% germination in a 10^{-4} molar solution.

Glutamine had no effect on nonirradiated seeds at lower concentrations but a 1.0 Molar solution reduced germination to 11%. With irradiated (3.5 Mrad) seeds, 3% germination was obtained for 10^{-2} molar and 1% for 10^{-4} molar, glutamine.

Succinic acid reduced germination to zero at a concentration of one molar. The 10^{-2} M solution reduced the per cent germination during the first 24 hrs of non-irradiated seeds by 75% while the 10^{-4} molar caused a very slight increase in the rate of germination. For the irradiated seeds germination was obtained in only a 1.0 Molar solution.

Very similar results were obtained for added sucrose solution as shown in Table 8-2.8.

The results using lactic acid itself (reported in Table 8-2.9) and not its salt as was used in previously published work,[329] were extremely interesting. The addition of lactic acid at concentrations of 10^{-2} and 10^{-4} molar led to an extremely marked increase in the rate of germination compared to that of the control. A 10^{-4} M solution of lactic acid gave 92% germination of control seeds in only 48 hrs whereas this value was not reached until 192 hrs had elapsed for seeds germinating in distilled water. In fact the results are similar to those obtained for gibberellic acid, a well known promoter of germination and as well as those obtained for citric acid. This experiment was repeated giving the same result and hence it seems most unlikely that accumulation of lactic acid was the reason for the nonviability of seeds under anaerobic atmospheres as reported.[329] It is much more likely that it plays an extremely important role in germination and the results under anaerobic atmospheres relate to damage to the metabolism of this compound. The accumulation of lactic acid itself would not be lethal but the effect of "blocking" the food resources in the form of lactic acid could be. Of all the pure solutions examined lactic acid had the greatest effect on germination of control seeds in terms of rate and total % germination. Of the irradiated seeds, germination was only obtained in 1.0M lactic acid (1%).

Added glutamic acid solution had very little effect on germination at 10^{-4}M. At 10^{-2}M slight inhibition occurred in the total % germination and at 1.0 M, germination was seriously inhibited. Germination of irradiated seeds was not obtained in the glutamic acid solutions used.

The results (table 8-2.11) for added alanine to control seeds were similar to those for glutamic acid. With the irradiated seeds, however,

Table 8-2.1

The Effect of Added Sodium Chloride on the Germination of Irradiated and Non-irradiated Sinapis alba Seeds

Dose (Mrad)	0.0		0.0		0.98		0.98		4.9		4.9	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	0		10^{-1}		0		10^{-1}		0		10^{-1}	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	7	7	0	0	0	0	0	0	0	0	0	0
2	64	71	79	79	1	1	0	0	0	0	0	0
3	15	86	10	89	18	19	20	20	0	0	0	0
4	3	89	5	94	10	29	12	32	0	0	0	0
5	2	91	2	96	10	39	10	42	0	0	0	0
6	2	93	1	97	13	52	9	51	0	0	0	0
7	2	95	1	98	12	64	11	62	0	0	0	0
8	0	95	0	98	9	73	10	72	0	0	0	0
9	0	95	0	98	6	79	5	77	0	0	0	0
10	1	96	0	98	4	83	2	79	0	0	0	0
11	1	97	0	98	2	85	2	81	0	0	0	0
12	1	98	0	98	2	87	3	84	0	0	0	0
13	0	98	0	98	1	88	1	85	0	0	0	0
14	1	99	0	98	2	90	0	85	0	0	0	0
15	0	99	0	98	0	90	2	87	0	0	0	0
16	0	99	0	98	1	91	2	89	0	0	0	0
17	0	99	0	98	0	91	0	89	0	0	0	0
18	0	99	0	98	0	91	0	89	0	0	0	0
19	0	99	0	98	0	91	0	89	0	0	0	0
20	0	99	0	98	0	91	0	89	0	0	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 8-2.1 The Effect of Added Sodium Chloride on the Germination of Irradiated Sinapis alba Seeds.

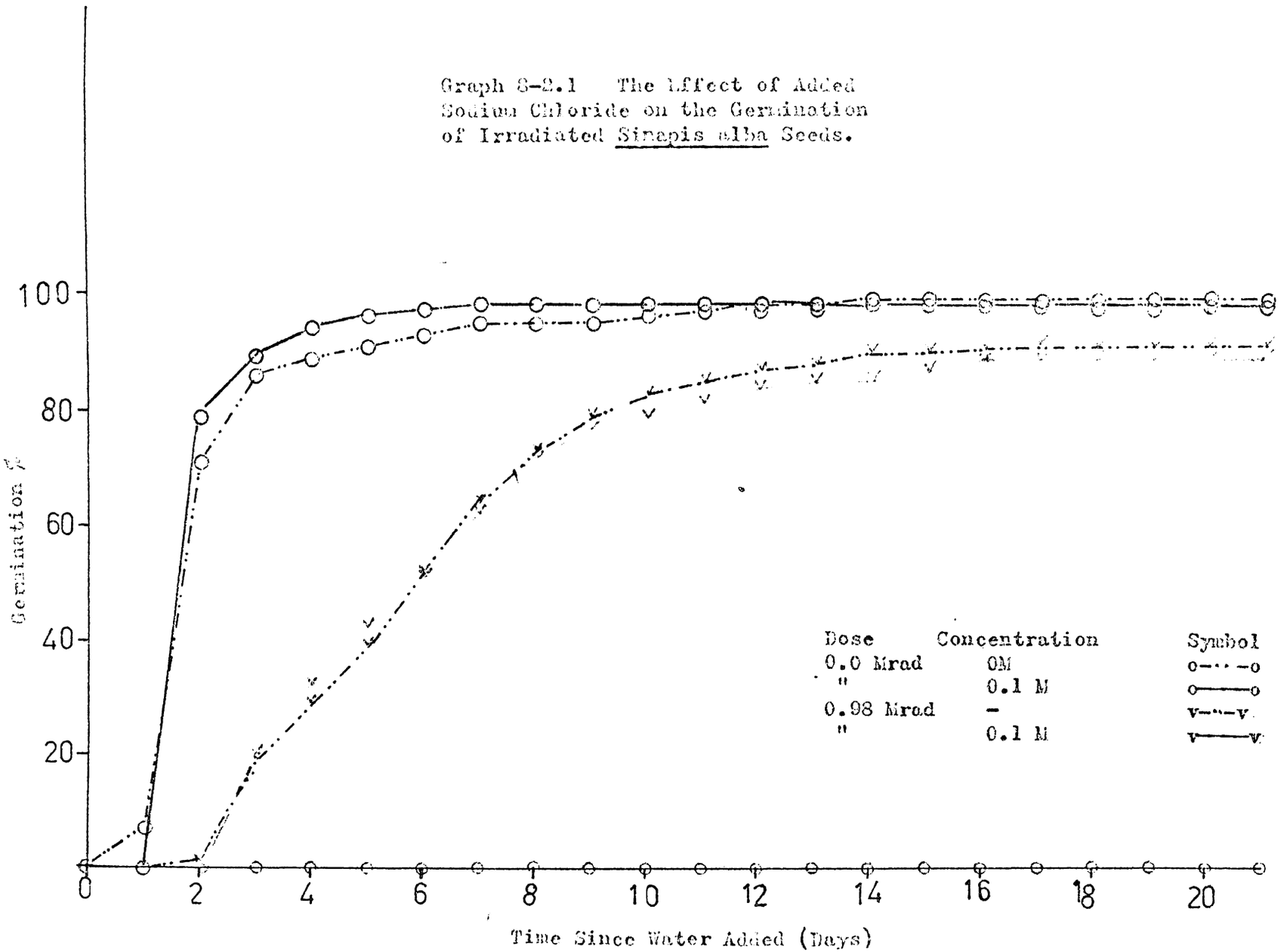


Table 8-2.2

The Effect of Added Gibberellic Acid on the Germination of *Sinapis alba* Seeds

Dose (Mrad)	0.0		0.0		0.0		3.5		3.5		3.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	10^{-2}		10^{-4}		-**		10^{-2}		10^{-4}		-**	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	77	77	76	76	41	41	0	0	0	0	0	0
2	6	83	15	91	40	81	0	0	0	0	0	0
3	0	83	0	91	4	85	0	0	0	0	0	0
4	0	83	1	92	3	88	0	0	0	0	0	0
5	0	83	1	93	2	90	0	0	0	0	0	0
6	2	85	2	95	1	91	0	0	0	0	0	0
7	2	87	1	96	0	91	0	0	0	0	0	0
8	2	89	0	96	2	93	0	0	0	0	0	0
9	0	89	0	96	0	93	0	0	0	0	0	0
10	0	89	1	97	0	93	0	0	0	0	0	0
11	0	89	1	98	1	94	0	0	0	0	0	0
12	0	89	0	98	1	95	0	0	0	0	0	0
13	1	90	0	98	0	95	0	0	0	0	0	0
14	0	90	0	98	0	95	0	0	0	0	0	0
15	0	90	1	99	0	95	0	0	0	0	0	0
16	0	90	1	100	0	95	0	0	0	0	0	0
17	0	90	-	100	1	96	0	0	0	0	0	0
18	0	90	-	100	0	96	0	0	0	0	0	0
19	0	90	-	100	0	96	0	0	0	0	0	0
20	0	90	-	100	0	96	0	0	0	0	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

** Note: These two samples with only distilled water added were the control germination tests for Tables 8-2.2 to 8-2.14 (inclusive)

Graph 8-2.2

The Effect of Added Gibberellic Acid on the Germination of Irradiated *Sinapis alba* Seeds.

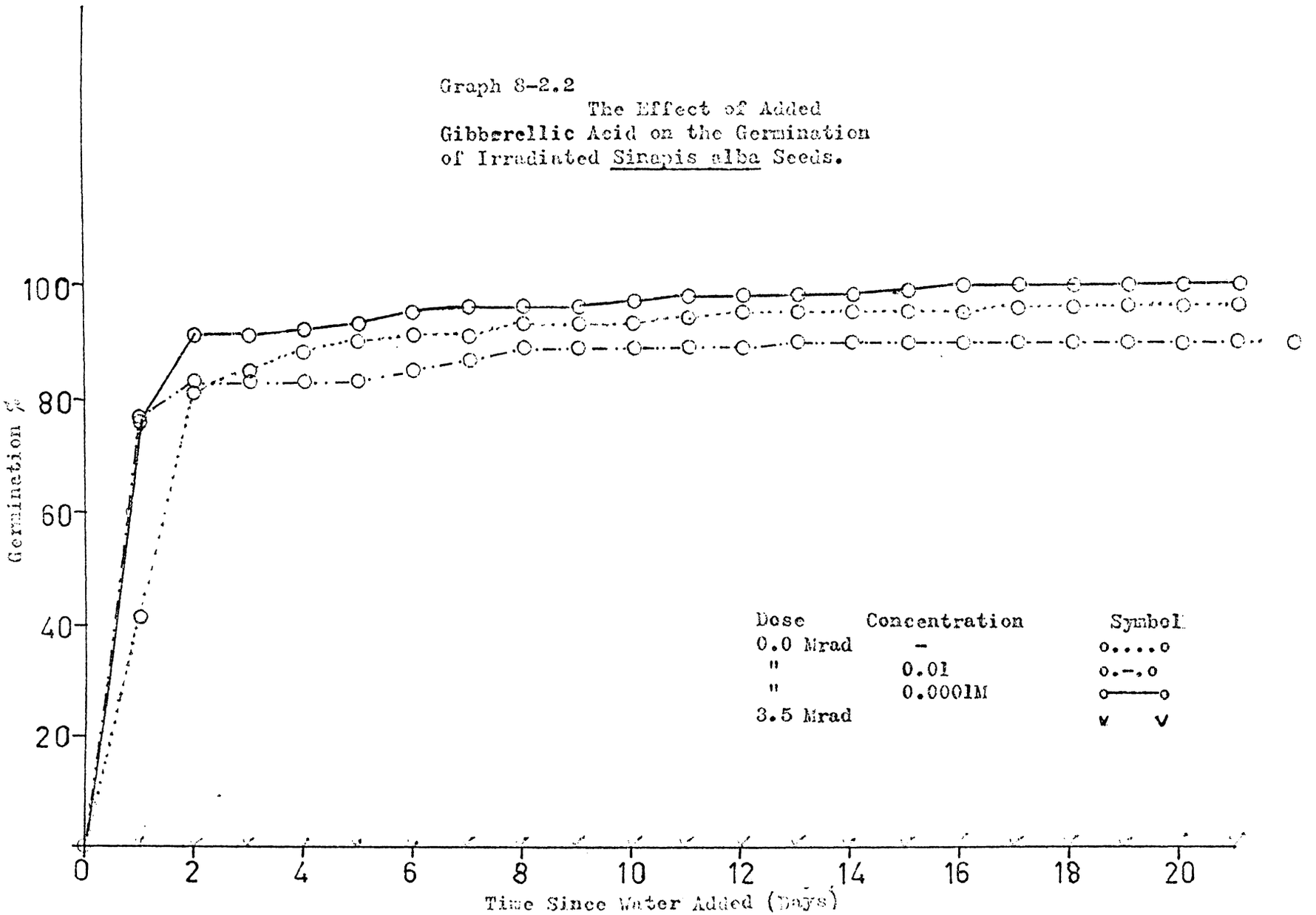


Table 8-2.3

The Effect of Added Citric Acid Solution on the Germination of *Sinapis alba* Seeds

Dose (Mrad)	0.0		0.0		0.0		3.5		3.5		3.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	1.0		10^{-2}		10^{-4}		1.0		10^{-2}		10^{-4}	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	70	70	83	83	1	1	3	3	1	1
2	0	0	11	81	5	88	1	2	1	4	0	1
3	0	0	2	83	0	88	0	2	1	5	0	1
4	0	0	3	86	0	88	0	2	0	5	0	1
5	0	0	2	88	3	91	0	2	0	5	0	1
6	0	0	2	90	3	94	1	3	0	5	0	1
7	0	0	2	92	2	96	0	3	0	5	0	1
8	0	0	3	95	4	100	0	3	0	5	0	1
9	0	0	1	96	-	100	0	3	0	5	0	1
10	0	0	0	96	-	100	0	3	0	5	0	1
11	0	0	2	98	-	100	0	3	0	5	0	1
12	0	0	0	98	-	100	0	3	0	5	0	1
13	0	0	1	99	-	100	0	3	0	5	0	1
14	0	0	0	99	-	100	0	3	0	5	0	1
15	0	0	0	99	-	100	0	3	0	5	0	1
16	0	0	0	99	-	100	0	3	0	5	0	1
17	0	0	1	100	-	100	0	3	0	5	0	1
18	0	0	-	100	-	100	0	3	0	5	0	1
19	0	0	-	100	-	100	0	3	0	5	0	1
20	0	0	-	100	-	100	0	3	0	5	0	1

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Note: Values for *Sinapis alba* seeds plus distilled water shown in Table 8-2.2

Graph 8-2.3

The Effect of Added Citric Acid on the Germination of Irradiated Sinapis alba Seeds.

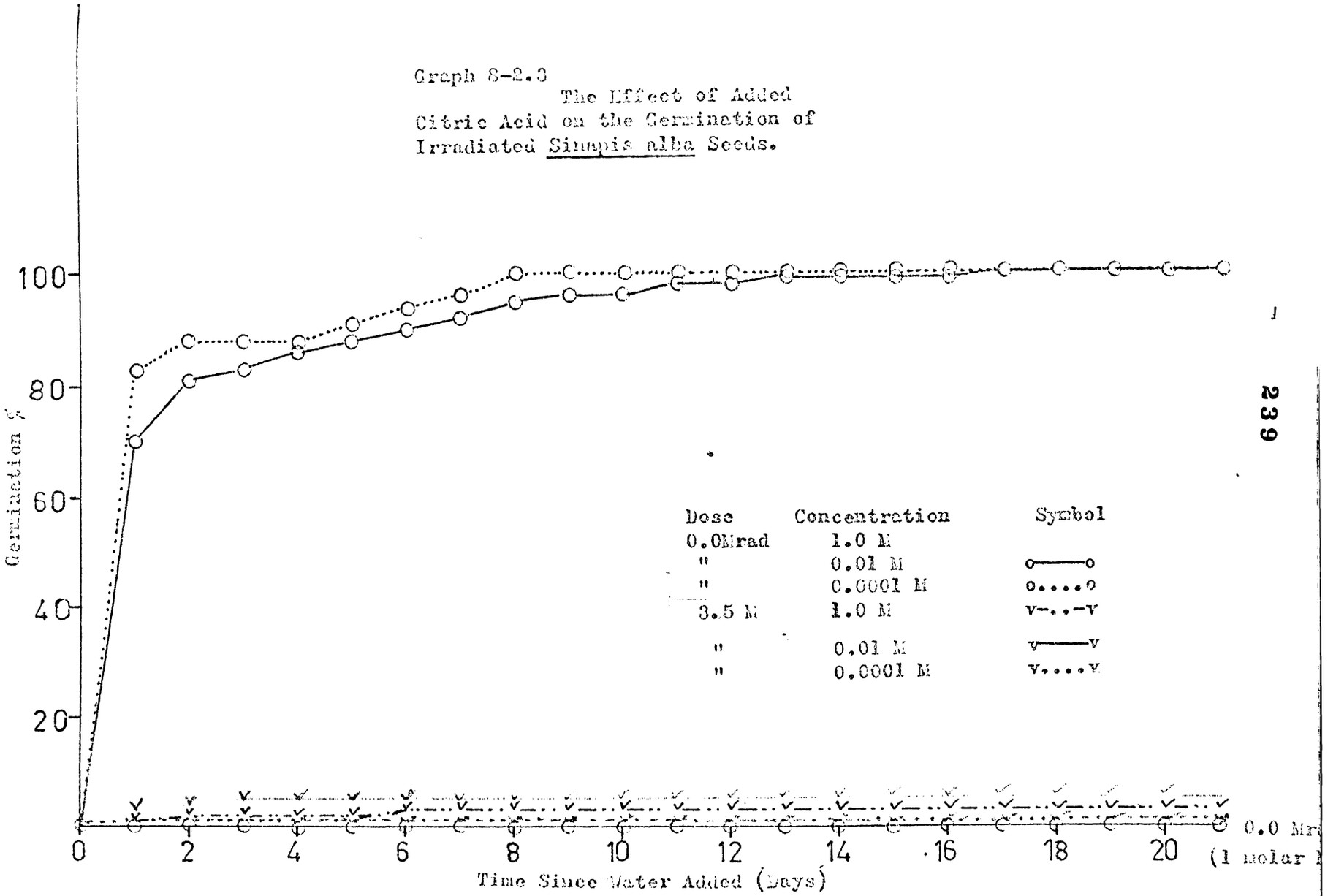


Table 8-2.4

The Effect of added Thiourea Solution on the Germination of Sinapis alba Seeds

Dose (Mrad)	0.0		0.0		0.0		3.5		3.5		3.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	1.0		10^{-2}		10^{-4}		1.0		10^{-2}		10^{-4}	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	38	38	46	46	0	0	0	0	1	1
2	0	0	46	84	41	87	0	0	0	0	0	1
3	0	0	5	89	2	89	0	0	0	0	0	1
4	0	0	3	92	1	90	0	0	0	0	0	1
5	0	0	3	95	3	93	0	0	0	0	1	2
6	0	0	2	97	2	95	0	0	0	0	0	2
7	0	0	1	98	2	97	0	0	0	0	0	2
8	0	0	0	98	0	97	0	0	0	0	0	2
9	0	0	0	98	1	98	0	0	0	0	0	2
10	0	0	1	99	0	98	0	0	0	0	0	2
11	0	0	0	99	0	98	0	0	0	0	0	2
12	0	0	0	99	0	98	0	0	0	0	0	2
13	0	0	0	99	0	98	0	0	0	0	0	2
14	0	0	0	99	0	98	0	0	0	0	0	2
15	0	0	0	99	0	98	0	0	0	0	0	2
16	0	0	0	99	1	99	0	0	0	0	0	2
17	0	0	0	99	0	99	0	0	0	0	0	2
18	0	0	0	99	0	99	0	0	0	0	0	2
19	0	0	0	99	0	99	0	0	0	0	0	2
20	0	0	0	99	0	99	0	0	0	0	0	2

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Note: Values for Sinapis alba seeds plus distilled water shown in Table 8-2.2.

Graph 8-2.4

The Effect of Added Thiourea on the Germination of Irradiated Sinapis alba Seeds.

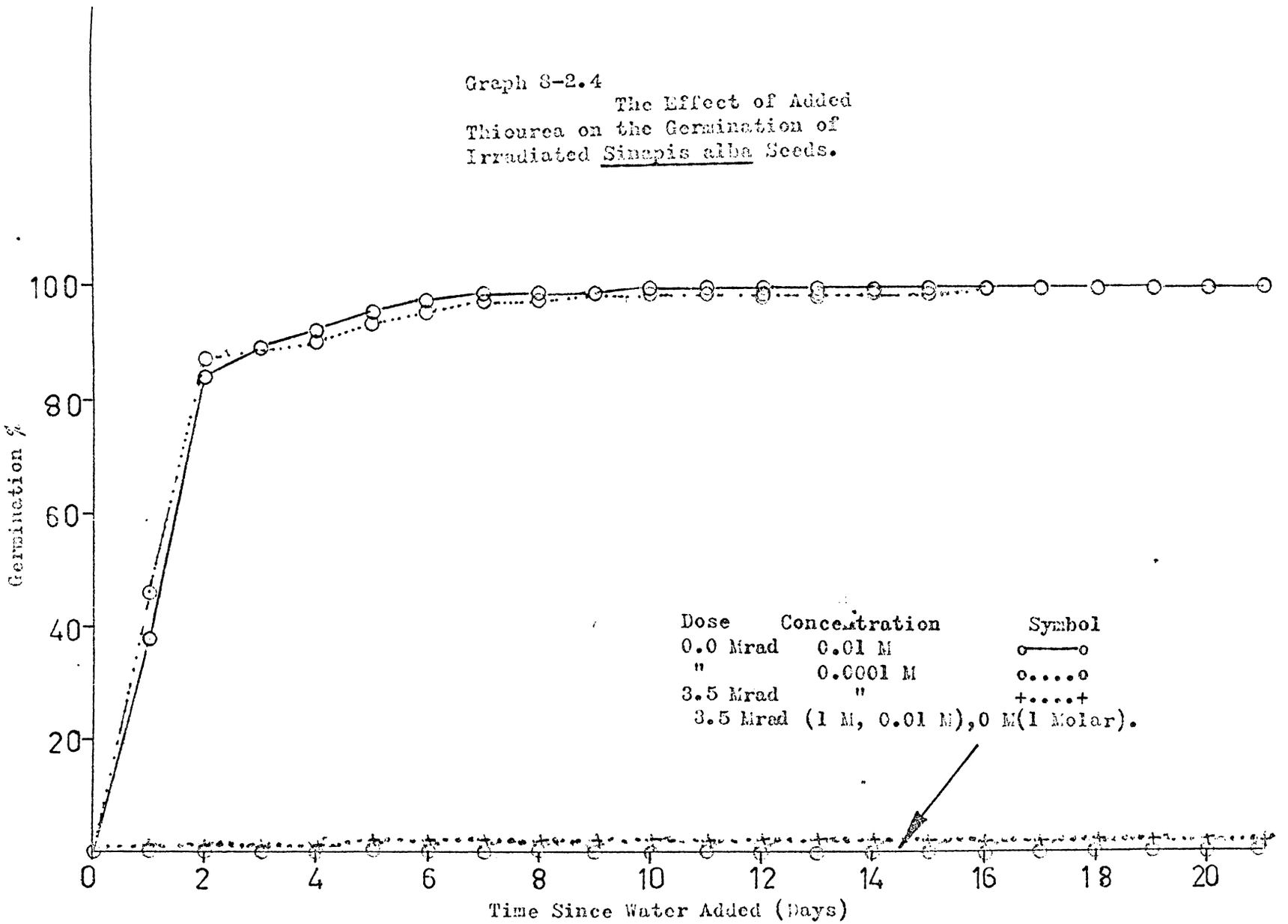


Table 8-2.5

The Effect of Added Aspartic Acid Solution on the Germination of Sinapis alba Seeds

Dose (Mrad)	0.0		0.0		0.0		3.5		3.5		3.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	1.0		10^{-2}		10^{-4}		1.0		10^{-2}		10^{-4}	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	12	12	33	33	36	36	0	0	0	0	2	2
2	11	23	39	72	42	78	0	0	0	0	0	2
3	0	23	5	77	3	81	0	0	0	0	0	2
4	0	23	3	80	3	84	0	0	0	0	0	2
5	0	23	2	82	1	85	0	0	0	0	0	2
6	1	24	2	84	3	88	0	0	0	0	0	2
7	0	24	2	86	3	91	0	0	0	0	0	2
8	0	24	0	86	1	92	0	0	0	0	0	2
9	0	24	1	87	0	92	0	0	0	0	0	2
10	0	24	2	89	1	93	0	0	0	0	0	2
11	0	24	1	90	0	93	0	0	0	0	0	2
12	0	24	1	91	0	93	0	0	0	0	0	2
13	0	24	0	91	0	93	0	0	0	0	0	2
14	0	24	0	91	0	93	0	0	0	0	0	2
15	0	24	0	91	0	93	0	0	0	0	0	2
16	0	24	0	91	0	93	0	0	0	0	0	2
17	0	24	0	91	0	93	0	0	0	0	0	2
18	0	24	0	91	0	93	0	0	0	0	0	2
19	0	24	0	91	0	93	0	0	0	0	0	2
20	0	24	0	91	0	93	0	0	0	0	0	2

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Note: Values for Sinapis alba seeds plus distilled water shown in Table 8-2.2

Graph 8-2.5.
The Effect of Added Aspartic Acid on the Germination of Irradiated *Sinapis alba* Seeds.

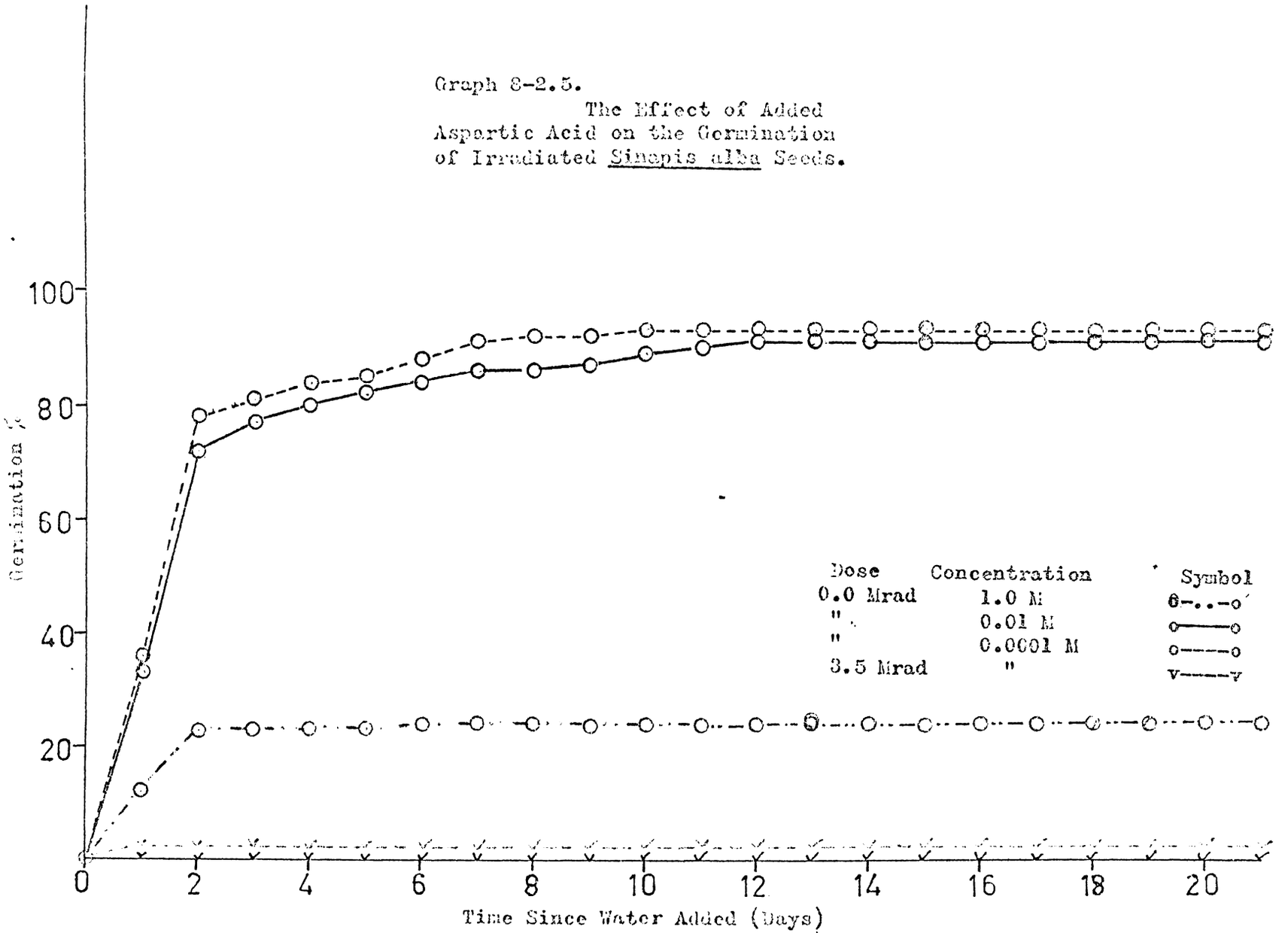


Table 8-2.6

The Effect of Added Glutamine Solution on the Germination of *Sinapis alba* Seeds.

Dose (Mrad)	0.0		0.0		0.0		3.5		3.5		3.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	1.0		10^{-2}		10^{-4}		1.0		10^{-2}		10^{-4}	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	43	43	40	40	0	0	1	1	1	1
2	0	0	34	77	44	84	0	0	0	1	0	1
3	0	0	18	95	4	88	0	0	1	2	0	1
4	0	0	3	98	2	90	0	0	0	2	0	1
5	2	2	1	99	4	94	0	0	0	2	0	1
6	3	5	0	99	2	96	0	0	0	2	0	1
7	3	8	0	99	0	96	0	0	0	2	0	1
8	1	9	0	99	1	97	0	0	1	3	0	1
9	2	11	0	99	1	98	0	0	0	3	0	1
10	0	11	0	99	0	98	0	0	0	3	0	1
11	0	11	0	99	1	99	0	0	0	3	0	1
12	0	11	1	100	0	99	0	0	0	3	0	1
13	0	11	-	100	0	99	0	0	0	3	0	1
14	0	11	-	100	0	99	0	0	0	3	0	1
15	0	11	-	100	0	99	0	0	0	3	0	1
16	0	11	-	100	0	99	0	0	0	3	0	1
17	0	11	-	100	0	99	0	0	0	3	0	1
18	0	11	-	100	0	99	0	0	0	3	0	1
19	0	11	-	100	0	99	0	0	0	3	0	1
20	0	11	-	100	0	99	0	0	0	3	0	1

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Note: Values for *Sinapis alba* Seeds plus distilled water shown in Table 8-2.2.

Graph 8-2.6

The Effect of Added Glutamine on the Germination of Irradiated Sinapis alba Seeds.

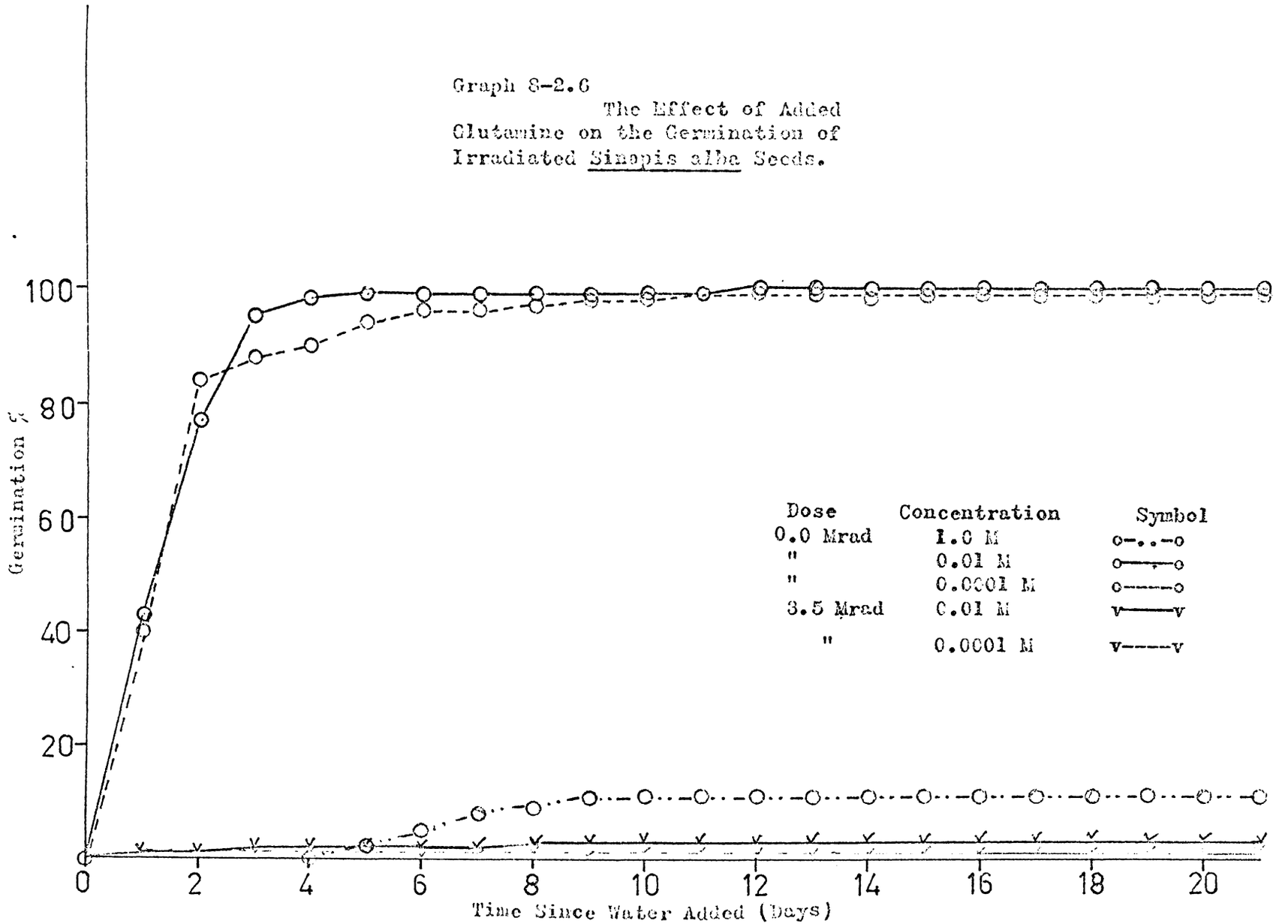


Table 8-2.7

The Effect of Added Succinic Acid on the Germination of Sinapis alba Seeds.

Dose (Mrad)	0.0		0.0		0.0		3.5		3.5		3.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	1.0		10^{-2}		10^{-4}		1.0		10^{-2}		10^{-4}	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	11	11	54	54	0	0	0	0	0	0
2	0	0	61	72	29	83	1	1	0	0	0	0
3	0	0	4	76	5	88	0	1	0	0	0	0
4	0	0	2	78	1	89	0	1	0	0	0	0
5	0	0	1	79	1	90	0	1	0	0	0	0
6	0	0	0	79	0	90	0	1	0	0	0	0
7	0	0	0	79	0	90	0	1	0	0	0	0
8	0	0	0	79	1	91	0	1	0	0	0	0
9	0	0	0	79	0	91	0	1	0	0	0	0
10	0	0	0	79	0	91	0	1	0	0	0	0
11	0	0	0	79	0	91	0	1	0	0	0	0
12	0	0	0	79	0	91	0	1	0	0	0	0
13	0	0	0	79	0	91	0	1	0	0	0	0
14	0	0	0	79	0	91	0	1	0	0	0	0
15	0	0	0	79	0	91	0	1	0	0	0	0
16	0	0	0	79	0	91	0	1	0	0	0	0
17	0	0	0	79	0	91	0	1	0	0	0	0
18	0	0	0	79	0	91	0	1	0	0	0	0
19	0	0	0	79	0	91	0	1	0	0	0	0
20	0	0	0	79	0	91	0	1	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Note: Values for Sinapis alba Seeds plus distilled water shown in Table 8-2.2

Graph 8-2.7

The Effect of Added Succinic Acid on the Germination of Irradiated Sinapis alba Seeds.

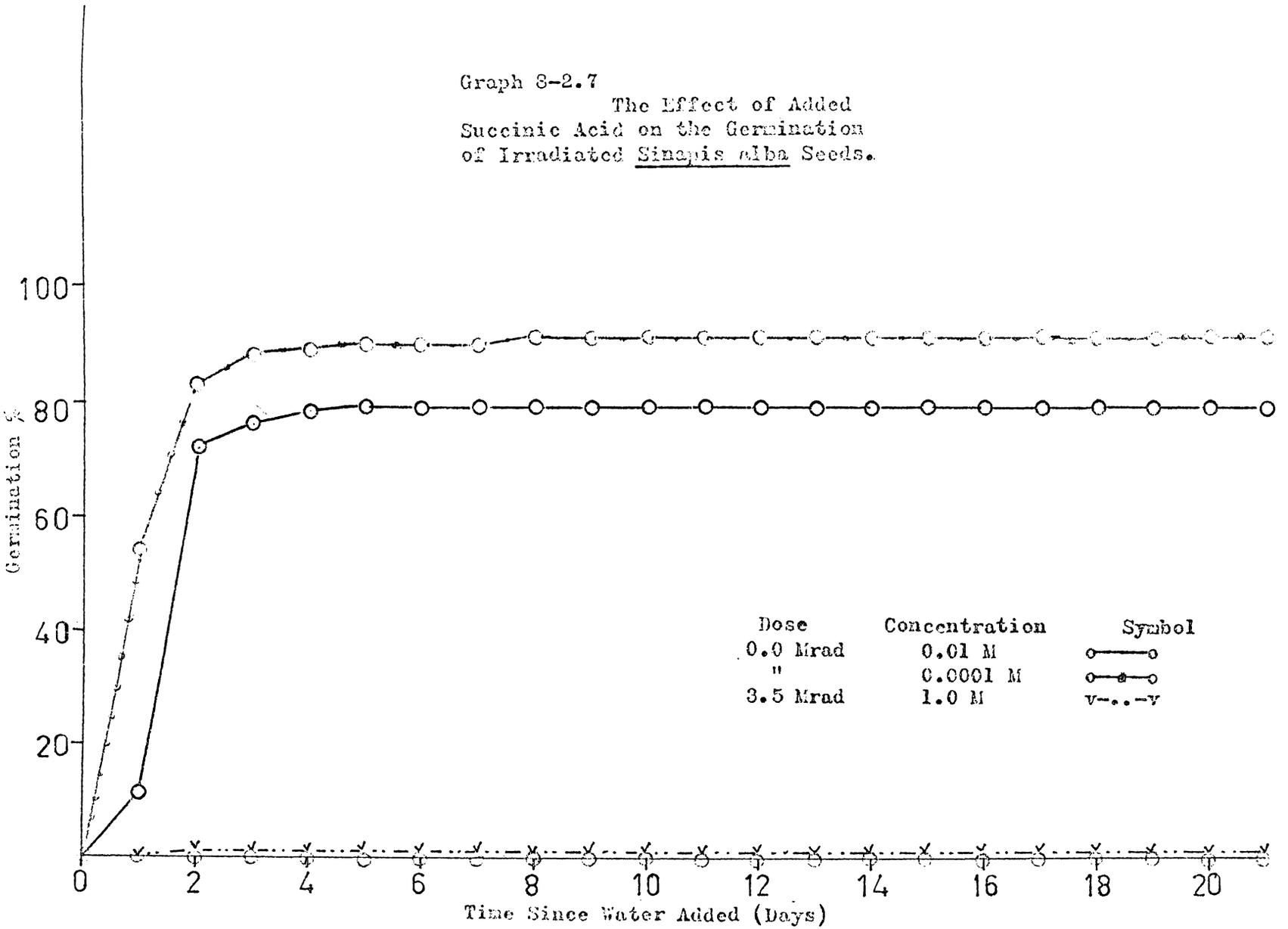


Table 8-2.8

The Effect of Added Sucrose on the Germination of Sinapis alba Seeds

Dose (Mrad)	0.0		0.0		0.0		3.5		3.5		3.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	1.0		10^{-2}		10^{-4}		1.0		10^{-2}		10^{-4}	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	7	7	59	59	1	1	0	0	0	0
2	0	0	25	32	28	87	0	1	0	0	0	0
3	0	0	30	62	5	92	0	1	0	0	0	0
4	0	0	8	70	3	95	0	1	0	0	0	0
5	0	0	1	71	1	96	0	1	0	0	0	0
6	0	0	2	73	0	96	0	1	0	0	0	0
7	0	0	0	73	1	97	0	1	0	0	0	0
8	0	0	1	74	2	99	0	1	0	0	0	0
9	0	0	1	75	0	99	0	1	0	0	0	0
10	0	0	0	75	0	99	0	1	0	0	0	0
11	0	0	0	75	0	99	0	1	0	0	0	0
12	0	0	0	75	0	99	0	1	0	0	0	0
13	0	0	0	75	1	100	0	1	0	0	0	0
14	0	0	1	76	-	100	0	1	0	0	0	0
15	0	0	1	77	-	100	0	1	0	0	0	0
16	0	0	0	77	-	100	0	1	0	0	0	0
17	0	0	0	77	-	100	0	1	0	0	0	0
18	0	0	1	78	-	100	0	1	0	0	0	0
19	0	0	0	78	-	100	0	1	0	0	0	0
20	0	0	1	79	-	100	0	1	0	0	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Note: Values for Sinapis alba seeds plus distilled water shown in Table 8-2.2.

Graph 8-2.8

The Effect of Added Sucrose on the Germination of Irradiated *Sinapis alba* Seeds.

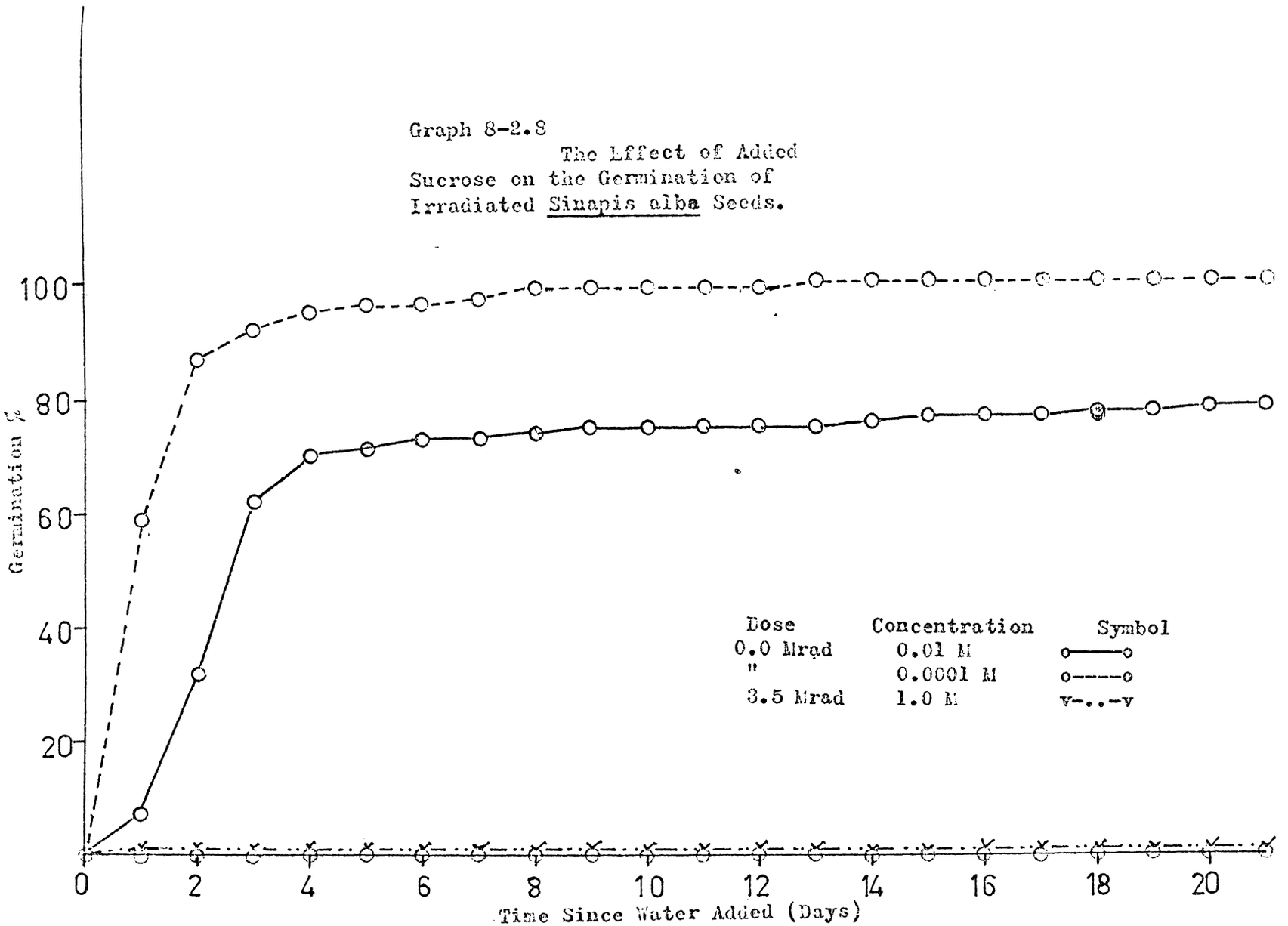


Table 8-2.9

The Effect of Added Lactic Acid on the Germination of *Sinapis alba* Seeds

Dose (Mrad)	0.0		0.0		0.0		3.5		3.5		3.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	1.0		10^{-2}		10^{-4}		1.0		10^{-2}		10^{-4}	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	60	60	78	78	1	1	0	0	0	0
2	0	0	20	80	14	92	0	1	0	0	0	0
3	0	0	3	83	1	93	0	1	0	0	0	0
4	0	0	4	87	2	95	0	1	0	0	0	0
5	0	0	8	95	1	96	0	1	0	0	0	0
6	0	0	3	98	0	96	0	1	0	0	0	0
7	0	0	1	99	2	98	0	1	0	0	0	0
8	0	0	0	99	1	99	0	1	0	0	0	0
9	0	0	1	100	0	99	0	1	0	0	0	0
10	0	0	-	100	0	99	0	1	0	0	0	0
11	0	0	-	100	0	99	0	1	0	0	0	0
12	0	0	-	100	0	99	0	1	0	0	0	0
13	0	0	-	100	0	99	0	1	0	0	0	0
14	0	0	-	100	0	99	0	1	0	0	0	0
15	0	0	-	100	0	99	0	1	0	0	0	0
16	0	0	-	100	0	99	0	1	0	0	0	0
17	0	0	-	100	0	99	0	1	0	0	0	0
18	0	0	-	100	0	99	0	1	0	0	0	0
19	0	0	-	100	0	99	0	1	0	0	0	0
20	0	0	-	100	0	99	0	1	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Note: Values for *Sinapis alba* seeds plus distilled water shown in Table 8-2.2.

Graph S-2.9

The Effect of Added Lactic Acid on the Germination of Irradiated Sinapis alba Seeds.

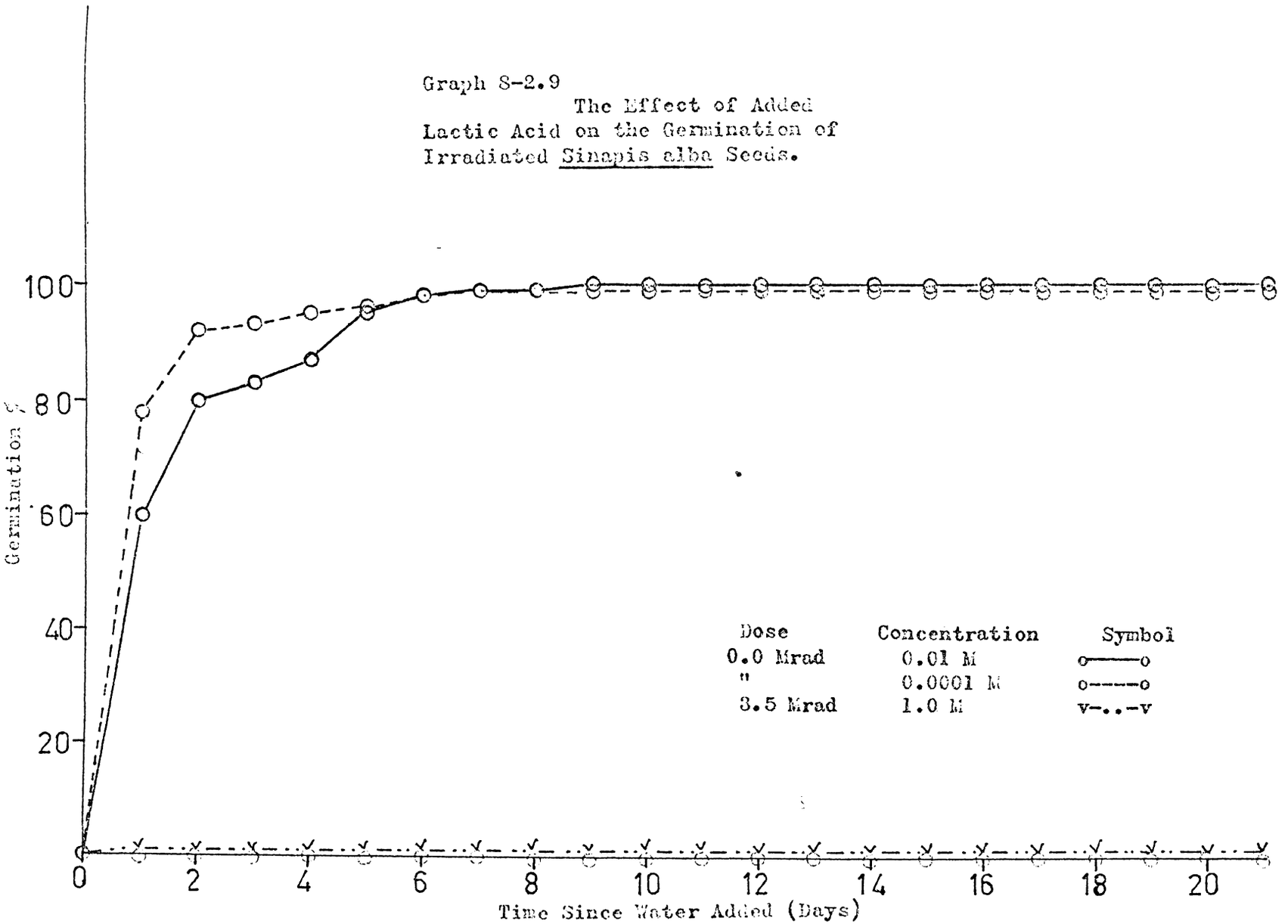


Table 8-2.10

The Effect of Added Glutamic Acid Solution on the Germination of Sinapis alba Seeds

Dose (Mrad)	0.0		0.0		0.0		3.5		3.5		3.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	1.0		10^{-2}		10^{-4}		1.0		10^{-2}		10^{-4}	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	2	2	40	40	54	54	0	0	0	0	0	0
2	6	8	39	79	25	79	0	0	0	0	0	0
3	0	8	1	80	4	83	0	0	0	0	0	0
4	0	8	1	81	3	86	0	0	0	0	0	0
5	0	8	0	81	2	88	0	0	0	0	0	0
6	0	8	1	82	2	90	0	0	0	0	0	0
7	0	8	2	84	0	90	0	0	0	0	0	0
8	0	8	1	85	2	92	0	0	0	0	0	0
9	0	8	0	85	1	93	0	0	0	0	0	0
10	0	8	0	85	1	94	0	0	0	0	0	0
11	0	8	0	85	0	94	0	0	0	0	0	0
12	0	8	0	85	1	95	0	0	0	0	0	0
13	0	8	0	85	2	97	0	0	0	0	0	0
14	0	8	0	85	0	97	0	0	0	0	0	0
15	0	8	0	85	0	97	0	0	0	0	0	0
16	0	8	0	85	1	98	0	0	0	0	0	0
17	0	8	0	85	0	98	0	0	0	0	0	0
18	0	8	0	85	0	98	0	0	0	0	0	0
19	0	8	0	85	0	98	0	0	0	0	0	0
20	0	8	0	85	0	98	0	0	0	0	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Note: Values for Sinapis alba seeds plus distilled water shown in Table 8-2.2.

Graph 8-2.10
The Effect of Added Glutamic Acid on the Germination of Irradiated Sinapis alba Seeds

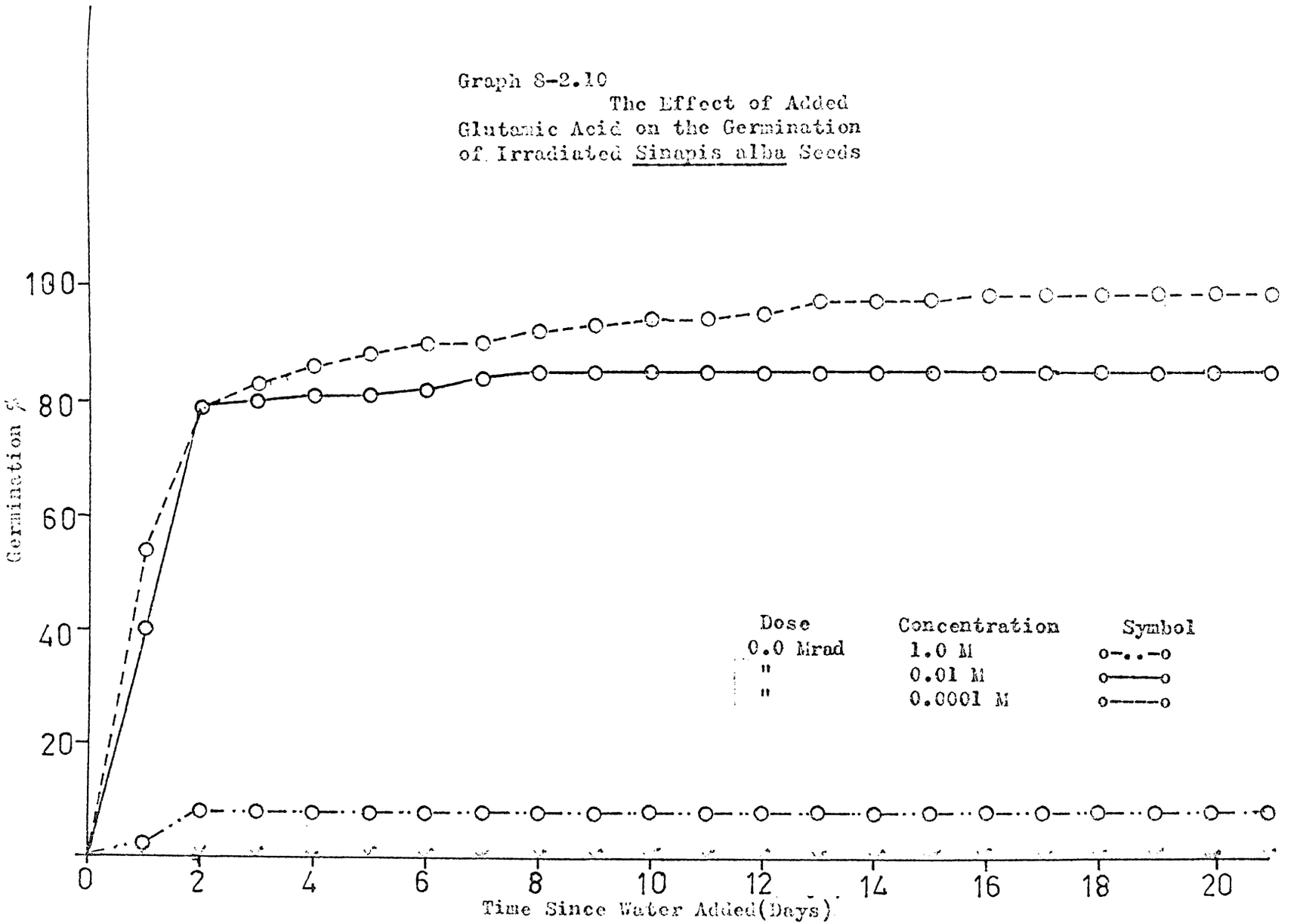


Table 8-2.11

The Effect of Added Alanine on the Germination of Sinapis alba Seeds.

Dose (Mrad)	0.0		0.0		0.0		3.5		3.5		3.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	1.0		10^{-2}		10^{-4}		1.0		10^{-2}		10^{-4}	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	33	33	54	54	0	0	1	1	0	0
2	1	1	33	66	26	80	0	0	0	1	0	0
3	0	1	9	75	2	82	0	0	0	1	0	0
4	0	1	2	77	2	84	0	0	0	1	0	0
5	0	1	1	78	3	87	1	1	0	1	0	0
6	0	1	2	80	2	89	0	1	0	1	0	0
7	0	1	2	82	3	92	1	2	0	1	0	0
8	0	1	1	83	1	93	0	2	0	1	0	0
9	0	1	0	83	0	93	0	2	0	1	0	0
10	0	1	1	84	0	93	0	2	0	1	0	0
11	0	1	1	85	1	94	0	2	0	1	0	0
12	0	1	1	86	0	94	0	2	0	1	0	0
13	0	1	1	87	0	94	0	2	0	1	0	0
14	0	1	0	87	0	94	0	2	0	1	0	0
15	0	1	0	87	0	94	0	2	0	1	0	0
16	0	1	1	88	0	94	0	2	0	1	0	0
17	0	1	1	89	1	95	0	2	0	1	0	0
18	0	1	0	89	0	95	0	2	0	1	0	0
19	0	1	0	89	0	95	0	2	0	1	0	0
20	0	1	0	89	0	95	0	2	0	1	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Note: Values for Sinapis alba seeds plus distilled water shown in Table 8-2.2.

Graph 8-2.11

The Effect of Added Alanine on the Germination of Irradiated Sinapis alba Seeds.

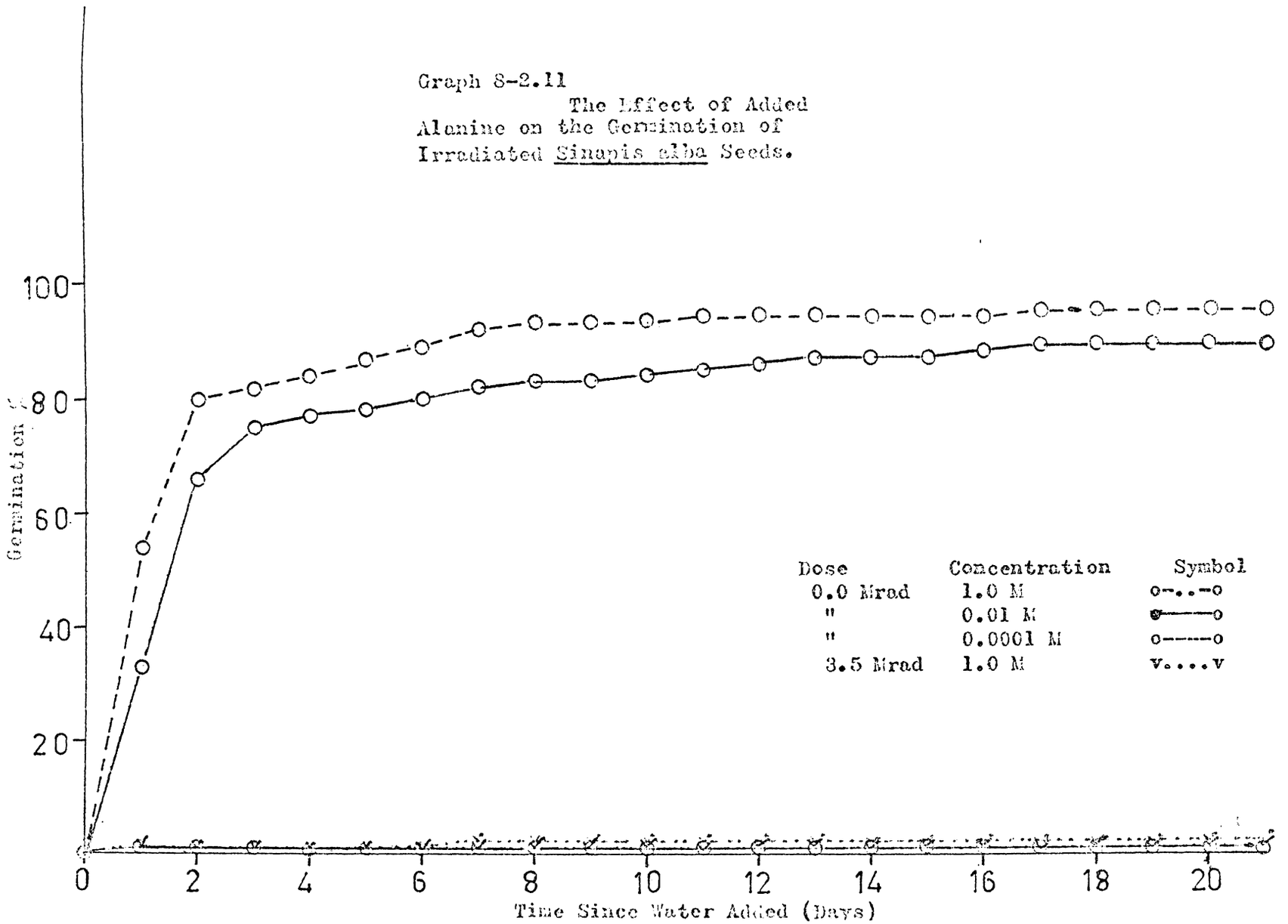


Table 8-2.12

The Effect of Added Ferrous Sulphate Solution on the Germination of Sinapis alba Seeds.

Dose (Mrad)	0.0		0.0		0.0		3.5		3.5		3.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	1.0		10^{-2}		10^{-4}		1.0		10^{-2}		10^{-4}	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	4	4	74	74	0	0	0	0	3	3
2	0	0	4	8	18	92	0	0	1	1	0	3
3	0	0	0	8	1	93	0	0	0	1	0	3
4	0	0	0	8	0	93	0	0	0	1	1	4
5	0	0	0	8	0	93	0	0	0	1	0	4
6	0	0	1	9	1	94	0	0	1	2	0	4
7	0	0	2	11	2	96	0	0	0	2	0	4
8	0	0	0	11	1	97	0	0	0	2	0	4
9	0	0	0	11	0	97	0	0	0	2	0	4
10	0	0	0	11	0	97	0	0	0	2	0	4
11	0	0	0	11	0	97	0	0	0	2	0	4
12	0	0	0	11	0	97	0	0	0	2	0	4
13	0	0	0	11	0	97	0	0	0	2	0	4
14	0	0	0	11	0	97	0	0	0	2	0	4
15	0	0	0	11	0	97	0	0	0	2	0	4
16	0	0	0	11	0	97	0	0	0	2	0	4
17	0	0	0	11	0	97	0	0	0	2	0	4
18	0	0	0	11	0	97	0	0	0	2	0	4
19	0	0	0	11	0	97	0	0	0	2	0	4
20	0	0	0	11	0	97	0	0	0	2	0	4

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Note: Values for Sinapis alba seeds plus distilled water shown in Table 8-2.2.

Graph S-2.12
The Effect of Added
Ferrous Sulphate on the Germination
of Irradiated Sinapis alba Seeds.

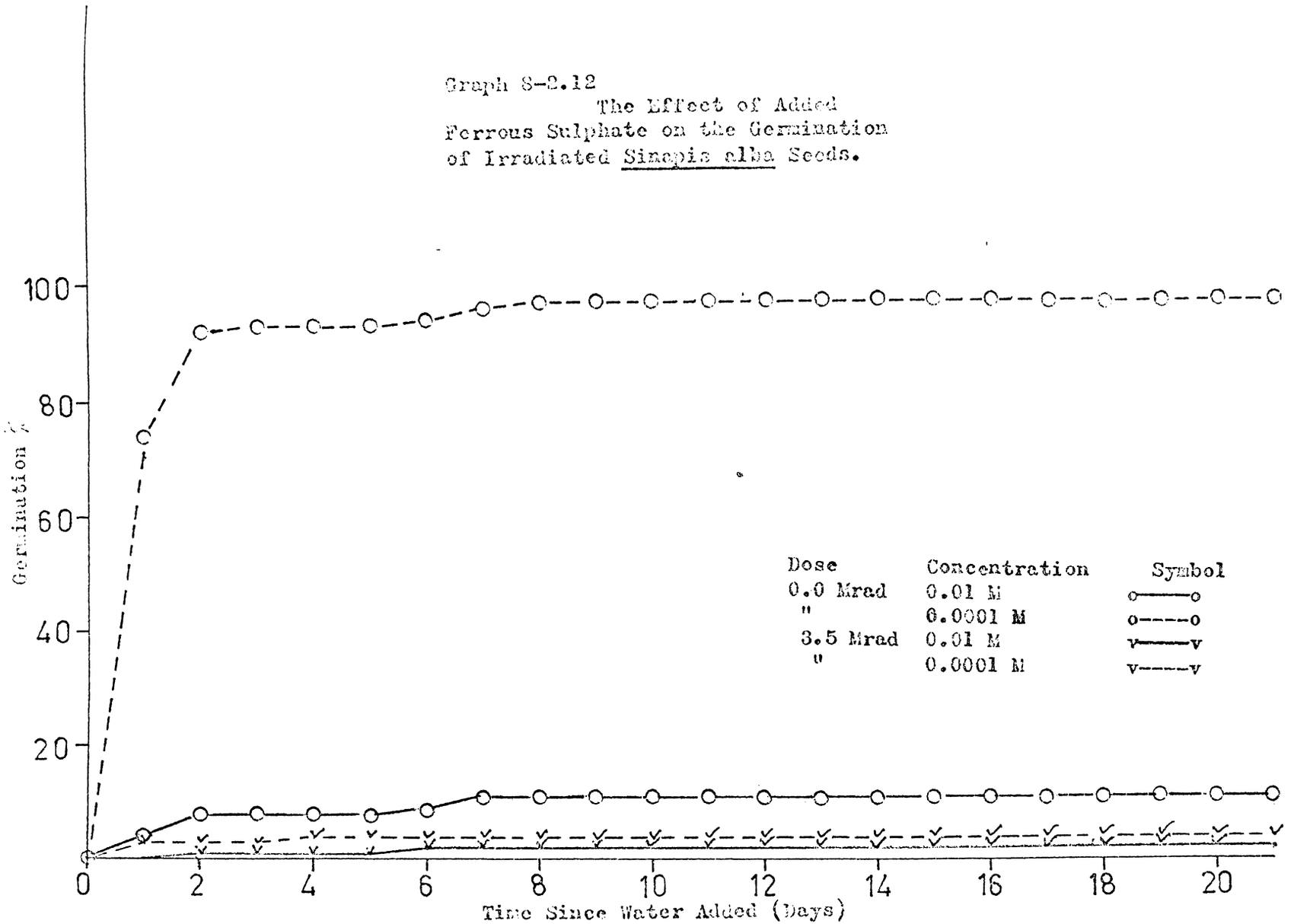


Table 8-2.13

The Effect of an Added Solution Containing Lactic Acid, Alanine, Sucrose, Ferrous Sulphate, Succinic Acid, Glutamine, Aspartic Acid, Thiourea, Citric Acid, and Gibberellic Acid, & Glutamic Acid.

Dose (Mrad)	0.0		0.0		0.0		3.5		3.5		3.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M	10^{-1}		10^{-3}		10^{-5}		10^{-1}		10^{-3}		10^{-5}	
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	74	74	81	81	0	0	1	1	0	0
2	1	1	16	90	8	89	0	0	0	1	0	0
3	0	1	1	91	0	89	0	0	0	1	0	0
4	0	1	1	92	2	91	0	0	0	1	0	0
5	0	1	1	93	1	92	0	0	0	1	0	0
6	0	1	2	95	1	93	0	0	1	2	0	0
7	0	1	1	96	0	93	0	0	0	2	0	0
8	0	1	0	96	1	94	0	0	0	2	0	0
9	0	1	1	97	3	97	0	0	0	2	0	0
10	0	1	1	98	2	99	0	0	0	2	0	0
11	0	1	1	99	0	99	0	0	0	2	0	0
12	0	1	0	99	0	99	0	0	0	2	0	0
13	0	1	0	99	0	99	0	0	0	2	0	0
14	0	1	0	99	0	99	0	0	0	2	0	0
15	0	1	0	99	0	99	0	0	0	2	0	0
16	0	1	0	99	1	100	0	0	0	2	0	0
17	0	1	0	99	-	100	0	0	0	2	0	0
18	0	1	0	99	-	100	0	0	0	2	0	0
19	0	1	1	100	-	100	0	0	0	2	0	0
20	0	1	-	100	-	100	0	0	0	2	0	0

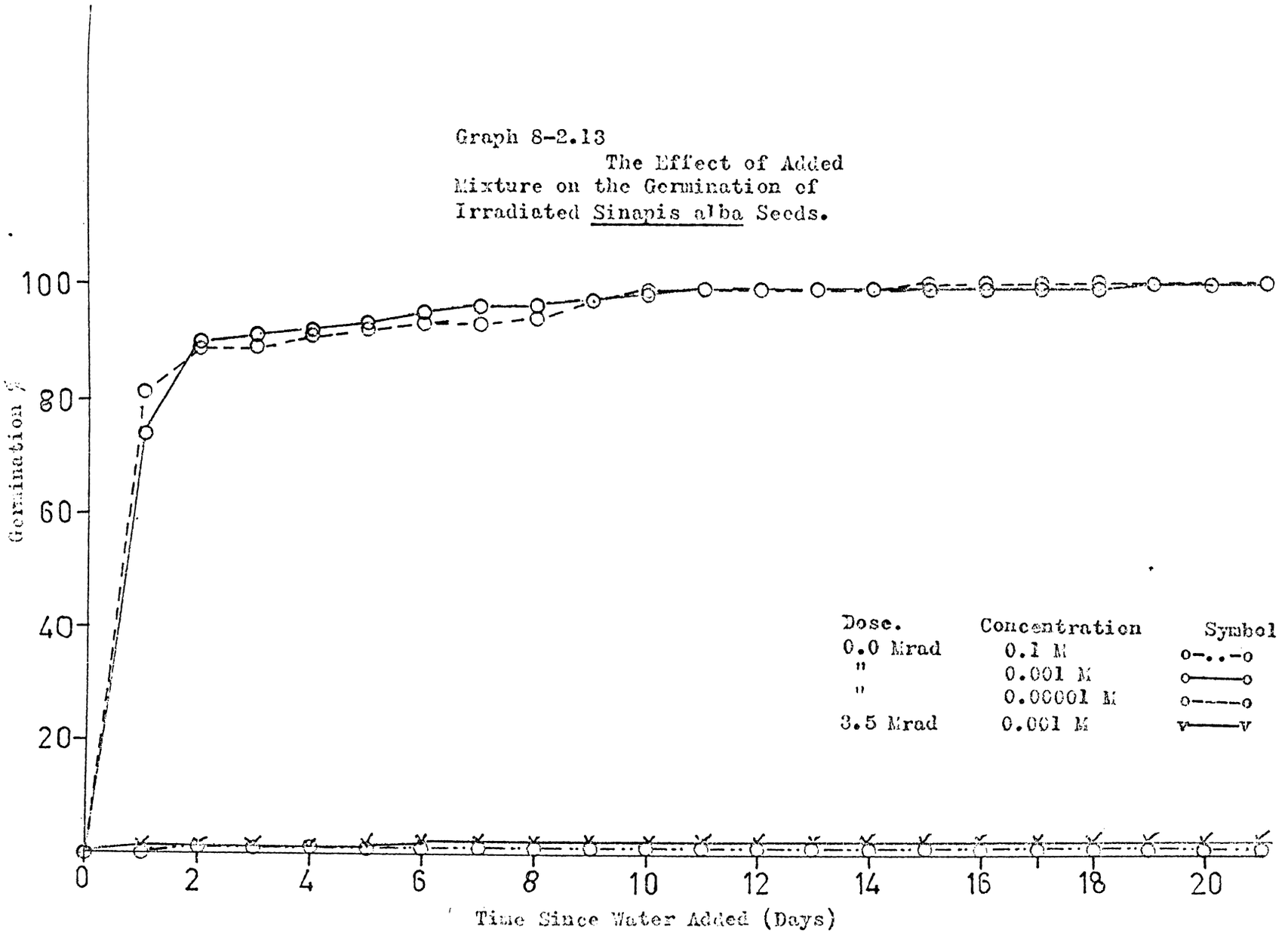
* (a) % Germinated previous 24 hours

* (b) Total % germination.

Note: Values for Sinapis alba seeds plus distilled water shown in Table 8-2.2.

Graph 8-2.13

The Effect of Added Mixture on the Germination of Irradiated Sinapis alba Seeds.



2% germination was obtained in a 1.0M solution, and 1% in a 10^{-2}M solution.

The effects of ferrous sulphate on irradiated seeds was investigated as it was thought that it could act as a scavenger of free radicals and hence prevent any radiation damage which occurs during the early stages of imbibition due to the presence of long-lived free radicals within the seeds. The effects of this compound at a concentration of 0.0001M on the irradiated seeds, leading to 4% germination was greater, than that obtained for any other solutions at this concentration. Ferrous sulphate seriously inhibited the germination of control seeds at 1.0 and 10^{-2} molar concentrations but a 10^{-4} molar solution caused a rapid increase in the rate of germination, the result being similar to that obtained for lactic acid. It is noteworthy that both of these substances could act as reducing agents.

The final results in this section are reported in Table 8-2.13, and shown in the corresponding graph. The solution added in this experiment contained all of the compounds examined in the previous tables each at concentrations of 10^{-1}, 10^{-3} and 10^{-5} molar. An increased rate of germination occurred for the control seeds when 10^{-3} and 10^{-5} molar concentrations were used but 10^{-1} M inhibited germination. Two percent germination of irradiated seeds occurred at 10^{-3}M concentrations but not at the other values used.

8-3 Discussion of Results

All solutions examined at concentrations of 1.0M severely inhibited germination of control seeds and yet for many compounds this was the only concentration leading to germination of irradiated seeds. In plant cells the cell-wall does not act as the semipermeable membrane involved in osmosis. The surface of the cytoplasm forms an extremely thin and delicate membrane known as the ectoplasm and it is this

membrane which is differentially permeable.[7] After the turgorpressure has been exceeded in normal cells osmosis stops and for the cells of nonirradiated seeds this occurs for 1.0M solutions of the substances examined. With the irradiated seeds germination often occurred at this concentration and the majority of the seeds showed the swelling typical of imbibition whether or not they finally germinated. It is therefore obvious that part of the damage caused by irradiation is located in the ectoplasm, giving rise to differences in permeability between irradiated and non-irradiated seeds. If diffusion of the solute can occur then increased intracellular concentrations would occur at the higher concentrations leading, for important compounds, to the germination observed.

Citric acid, gibberellic acid, lactic acid and ferrous sulphate caused marked improvement in the germination of control seeds whereas virtually no effects were observed for sodium chloride, thiourea, aspartic acid, glutamine, succinic acid, sucrose or glutamic acid, although these substances are thought to be important during the early stages of germination.

These results suggest that the clue to the germination mechanism lies in the roles played by lactic acid (or reducing substances) and citric acid.

Table 9-2.1

The Effect of Immersion in Liquid Air on the Germination of Non-irradiated Sinapis alba seeds.

Time Immersed in Liquid Air	-		10mins.		30mins.		1hr.		1½hrs		4 hrs	
Days Since Water Added	a*	b*	a	b	a	b	a	b	a	b	a	b
1	66	66	67	67	60	60	69	69	66	66	89	89
2	17	83	19	86	28	88	14	83	17	83	4	93
3	10	93	9	95	6	94	12	95	5	88	2	95
4	2	95	4	99	2	96	2	97	5	93	0	95
5	0	95	0	99	1	97	0	97	4	97	0	95
6	1	96	0	99	0	97	2	99	1	98	0	95
7	2	98	1	100	0	97	0	99	0	98	0	95
8	0	98	-	100	1	98	0	99	1	99	0	95
9	0	98	-	100	0	98	0	99	1	100	0	95
10	1	99	-	100	1	99	0	99	-	100	0	95
11	0	99	-	100	0	99	0	99	-	100	2	97
12	0	99	-	100	0	99	1	100	-	100	0	97
13	0	99	-	100	0	99	-	100	-	100	0	97
14	0	99	-	100	0	99	-	100	-	100	0	97
15	1	100	-	100	0	99	-	100	-	100	1	98
16	-	100	-	100	0	99	-	100	-	100	0	98
17	-	100	-	100	0	99	-	100	-	100	0	98
18	-	100	-	100	0	99	-	100	-	100	0	98
19	-	100	-	100	0	99	-	100	-	100	0	98
20	-	100	-	100	0	99	-	100	-	100	1	99
21	-	100	-	100	0	99	-	100	-	100	0	99

* a % germinated previous 24 hrs.

b Total % germination.

Table 9-2.2

The Effects of Prolonged Periods at 100°C on the Germination of *Sinapis alba* Seeds

Time at 100°C	½hr		6hrs		12hrs		24hrs		30hrs		48hrs		-	
Days Since Water Added	a*	b*	a	b	a	b	a	b	a	b	a	b	a	b
1	68	68	60	60	70	70	59	59	57	57	54	54	66	66
2	15	83	17	77	10	80	20	79	10	67	12	66	20	86
3	9	92	10	87	6	86	8	87	9	76	10	76	8	94
4	4	96	9	96	6	92	1	88	8	84	9	85	3	97
5	2	98	1	97	4	96	3	91	2	86	3	88	0	97
6	0	98	0	97	1	97	2	93	2	88	2	90	2	99
7	0	98	1	98	1	98	3	96	1	89	1	91	0	99
8	0	98	0	98	1	99	1	97	1	90	1	92	0	99
9	0	98	0	98	0	99	0	97	2	92	2	94	0	99
10	0	98	0	98	0	99	1	98	1	93	0	94	0	99
11	0	98	0	98	1	100	0	98	1	94	1	95	0	99
12	0	98	0	98	-	100	0	98	1	95	1	96	0	99
13	1	99	0	98	-	100	0	98	1	96	0	96	1	100
14	0	99	0	98	-	100	1	99	1	97	1	97	-	100
15	0	99	0	98	-	100	0	99	0	97	0	97	-	100
16	0	99	1	99	-	100	0	99	0	97	0	97	-	100
17	0	99	0	99	-	100	0	99	0	97	2	99	-	100
18	0	99	0	99	-	100	0	99	0	97	0	99	-	100
19	0	99	1	100	-	100	0	99	0	97	0	99	-	100
20	0	99	0	100	-	100	0	99	0	97	0	99	-	100
21	0	99	0	100	-	100	0	99	0	97	0	99	-	100

* a %germinated previous 24 hrs.

b Total % germination.

9. THE EFFECTS ON GERMINATION OF VERY LOW AND HIGH TEMPERATURES, APPLIED TO DRY SEEDS

9-0 Introduction

As it was intended to freeze seeds in liquid air to facilitate the formation of a powder suitable for enzyme extraction it was decided to test the effect of the freezing process on enzyme systems by examining the ability of seeds to germinate after having been frozen for several periods of time.

The results concerning the effects of high temperatures were discovered initially while drying seeds to constant weight and were later investigated further.

9-1 Experimental Methods

The seeds were plunged into liquid air contained in a vacuum flask and the flask kept filled with liquid air. For the shorter time intervals the seeds were placed in a large earthenware mortar and kept well-covered with liquid air throughout the time period.

In the high temperature experiments the seeds were stored in an evaporating dish in an oven at 100°C and left there for the specified time.

The germination studies were carried out in the manner described earlier.

9-2 Experimental Results

Results concerning the germination of seeds which had been immersed in liquid air for time periods of 10 mins, 30 mins, 1 hour, 1½ hours and 4 hours are shown in Table 9-2.1. These show that immersion

in liquid air has very little effect on the germination of mustard seeds, except for an apparent stimulation in the rate of germination in the first 24 hours for seeds which had been immersed for 4 hours. The method of grinding seeds in liquid air as used in future sections of this thesis, will not damage the seed enzyme systems by temperature affects alone as germination of whole seeds was normal after such treatment. Any damage caused would therefore be similar to that of biochemical analyses and arise from destruction of the cell structures such as membranes, causing loss of the organisation of the system.

The results of keeping seeds at a temperature of 100°C for $\frac{1}{2}$ hr, 6 hrs, 12 hrs, 24 hrs, 30 hrs and 48 hrs are shown in Table 9-2.2. Keeping the seeds at 100°C for 48 hrs had a slight effect on the rate of germination but not on the total percent germination.

These results show that the enzyme systems or potential enzyme systems of dry seeds are extremely temperature stable due to their dehydrated state. The germination of mustard seeds was not affected by either extremely low or high temperatures.

10. INVESTIGATIONS OF THE AMINO ACID METABOLISM OF SEEDS

10-0 Introduction

Earlier theories on the metabolism of germinating seeds considered that the endogenous amino acids in the dry resting seed were an effective means of storing the more unstable keto-acids of the tricarboxylic acid cycle. It was thought that this cycle would be initiated soon after the beginning of imbibition to supply the seed with the energy required in the form of ATP. Amino acids can be converted to their corresponding keto acids by transamination or deamination. In this section *in vitro* investigations of the amino acid metabolism of germinating seeds are described.

10-1. Experimental Methods

10-1.1 Chromatographic Methods

Method (a). *Sinapis alba* seeds were frozen in liquid air and ground in a mortar and pestle, under a layer of liquid air to ensure that they remained frozen. The "Liquid Air Powder" (i.e. "LAP") formed was kept in a desiccator for periods of up to a week. One gram of this powder was mixed with 10 mls of doubly distilled water or phosphate buffer pH 7.4, to form the "Enzyme Solution". For the incubation period, 2 mls of this solution were incubated with 50 mgs of the specified metabolite and without it for the blank samples. After 30 mins or two hours, the proteins were precipitated by the addition of trichloroacetic acid, the tubes were spun then stored in an ice bath and 10 λ of the supernatant spotted onto the origin of a chromatogram. Each 1-D chromatogram contained spots of at least two analyses, spots corresponding to enzyme solution which had been incubated without substrate and spots

corresponding to standard solutions of pure metabolites. The chromatograms were run in the various solvents and identical chromatograms examined by the sprays for various types of compounds.

Method (b). A glycerol extract of dried seeds, prepared as described in Bergmeyer[330] was prepared and used as the enzyme extract.

To prepare this extract 5 gms of seeds were ground in a mortar and pestle with 2 mls of toluene, 10 gm purified sand and a little 20% glycerol solution. The mixture was then transferred to a 100 ml volumetric flask using 20% glycerol solution and 1 ml toluene added. The flask was shaken for one hour then diluted to the mark with 20% glycerol, mixed thoroughly and filtered through a fluted paper. The extract can be stored in the refrigerator for several days.

For the analysis one ml of this extract was incubated at 20, 30 or 37°C with 20 mg of the required substrate (s) and 2 mls of pH 7.4 phosphate buffer (made by making 87 mls of 0.2M Na_2HPO_4 plus 13 mls of 0.2 NaH_2PO_4 up to 200 mls with doubly distilled water). The protein was precipitated with trichloroacetic acid, the tubes centrifuged and 10 λ of the supernatant spotted onto the origins of 1-D chromatograms as described in the first method.

Another spray for amino acids was found to be extremely useful for this section. The isatin spray was prepared as follows:

1 gm isatin

1.5 gm zinc acetate

1 ml pyridine

100 ml isopropanol.

The zinc acetate and isatin are dissolved in the iso-propanol by warming in a water-bath at 80°C after which the solution must be kept cool. After spraying, the paper is heated to 80-85°C for 30 mins.

The ferric chloride reagent (2 gm $FeCl_3$ in 100 ml H_2O and 1 ml 2N HCl), diluted five times in water for use was found useful for the detection of phenyl pyruvic acid. Many acids especially hydroxy acids react with this reagent yielding yellow or brown spots. The sensitivity of the reagent is poor but it gives a distinctive green colour for phenyl pyruvic acid.

10-1.2 Spectrophotometric Analysis

The method used was the colorimetric determination with 2,4-dinitro phenylhydrazine as described by Bergmeyer.[330] The solutions used were:

(A) Substrate-buffer solution (0.1M K_2HPO_4/KH_2PO_4 buffer (pH 7.4), 0.1M L-aspartate (or alanine), $2x10^{-3}$M alpha-ketoglutarate).

(B) Ketone Reagent (10^{-3}M 2,4-dinitrophenylhydrazine in 1N HCl.

(C) Enzyme Solution, two grams of the LAP were weighed in a 50 ml beaker and then it was placed in an ice bath. Ten mls of doubly distilled water were added and the mixture stirred gently for 5 minutes after which it was centrifuged. The supernatant was carefully decanted into a clean test tube and returned to the ice bath.

Method: For samples mix (by inversion) 1 ml substrate buffer solution and 0.2 mls of enzyme solution. Incubate for exactly 1 hour. Add 1 ml of the ketone reagent.

For the blank 1 ml of the ketone reagent is added to 1 ml of the buffer-substrate solution and finally the enzyme solution is added. These tubes are not incubated.

After the ketone reagent has been added 10 mls of 0.4N sodium hydroxide are added, the tubes stood at room temperature for 5 minutes and read at a wavelength of 546 mu on a Beckman DB spectrophotometer.

10-1.3 Partial Purification of Transaminases from Sinapis alba Seeds

The two samples used consisted of Sinapis alba seeds in the dry resting stage and seeds which had been germinating for one hour prior to the experiment.

Ten grams of the sample were ground in a mortar with sand and 50 mls of pH 7.4 phosphate buffer. Transaminases tend to be thermostable, unlike most enzymes. The crude enzyme preparation was held in a 70°C water bath until the temperature of the sample reached 50°C. It was then rapidly transferred to a water bath held at 50°C. Half of the sample was removed after 10 mins and the remainder after 20 mins.

The samples were then centrifuged and the supernatant retained. Due to the instability of most proteins at 50°C the supernatant should contain mainly transaminases and perhaps amylases. Using the Nomogram[332] for obtaining amounts of ammonium sulphate to be added to give the required % saturation of the solution the solutions were brought to 25%. The samples were allowed to stand for 15 mins. and then centrifuged at 10,000 to 15,000 rpm in an International refrigerated centrifuge (0-2°C). The supernatants were carefully removed into a clean tube and the precipitates of protein dissolved in one millilitre of buffer. This procedure of increasing the saturation and precipitating the protein was repeated to yield fractions corresponding to 0-25%, 25-50%, 50-75% and 75-100% for all samples.

Each precipitate was redissolved in buffer and then examined by the Biuret reaction (for protein) and for transaminase activity.

10-1.4 Preparation and Purification of Keto acid-DNP-Hydrazones Standard Solutions[331]

About 0.5 gms of the keto acid is dissolved in 10 ml of water. A slight excess of a saturated solution of DNP-hydrazine in 2N HCl

(approx. 0.5% soln.) is added. The DNP-hydrazones begin to crystallize out almost immediately, the reaction being complete in 20-30 mins. at 37°C. The crystals are removed by filtration and recrystallized from ethanol, water or ethyl acetate.

Purification from Plant Material[331]

As DNP-hydrazine reacts with a variety of ketones other than the keto acids a lengthy purification procedure is required. This separation based on the acidic nature of the keto-acids results in a final extract containing the DNP-hydrazones of ketoacids only. The method is as follows:

1. Add 20 ml 1% DNP-hydrazine in 5N H_2SO_4 to the extract. Stand for 20 mins. at 37°C.
2. Extract DNP-hydrazones with $3x\frac{1}{3}$ vol. of peroxide-free ether.
3. Shake the ether extract with a slight excess of saturated $NaHCO_3$ solution such that the pH remains definitely alkaline (8.4); about 40 mls may be required. A second extract with 15 ml of $NaHCO_3$ solution is made and emulsions which may form can be separated by centrifugation.
4. The combined bicarbonate extract is acidified to pH 2 with 3N H_2SO_4 and extracted with $3x\frac{1}{2}$ volume of chloroform containing 15% (v/v) ether. The organic extract is taken to dryness and stored in the refrigerator.

10-2 Results of Paper Chromatographic Analyses

The enzyme extracts were incubated with the substrates: GABA, glutamate, aspartate or alanine, as well as with mixtures of these and alpha-KG.

Using method (a), the high endogenous amino acid content, of the seeds, tended to make the interpretation of results extremely difficult.

The use of a buffer for the incubation caused severe streaking of the chromatograms so this was discontinued. The pH of the enzyme solution as prepared was usually just slightly acidic.

Using method (a) and 30°C or 37°C a spot which exhibited green-blue fluorescence and had an R_F of 0.65 in Butanol:Propionic Acid:Water (BPW) was always found in the GABA incubation mixture, only in the glutamate mixture after 1 hour incubation and not $\frac{1}{2}$ hour, and never in the blank incubation mixture. The compound (called UVGB) appeared to be definitely formed from GABA. It did not respond to any of the sprays used, i.e. ninhydrin, pyridine isatin reagent, Rydon Smith sulphanilimide, mercurochrome, aniline-phthalic acid reagent or xylose-aniline reagent. Hence it is not an amino acid, a reducing sugar, an organic acid or 2-pyrrolidone-5-carboxylic acid. The second unknown (called YPN) for which higher concentrations were associated with added GABA or glutamate had an R_F of 0.7 in BPW and 0.5 in phenol:water. This compound responded to ninhydrin, giving a yellow colour which turned purple in 12-24 hours. Glutamate was rapidly converted to GABA and in the longer time interval to UVGB and YPN. The conversion to the latter two compounds appeared to be via GABA. Glutamate did not form alpha-KG or glutamine. Arginine or proline were also not detected on the chromatograms. At 20°C GABA was converted to YPN but little other activity was observed. The ninhydrin positive compounds in the blank run were present in very low quantities at 20°C as compared to 30 or 37°C, only YPN being present in approximately the same quantity in all three runs. These results imply that total amino acid content is increasing at the higher temperatures during the $\frac{1}{2}$ to 1 hour incubation period but not at 20°C.

No transaminase activity was detected for the amino acids studied, using this paper chromatographic method.

An investigation of transamination of aspartate and alanine with alpha-KG using the glycerol extract also gave little evidence for transamination. Glutamate could not be detected in any aspartate/alpha-KG samples after chromatography. Aspartate was, however, converted to alanine and GABA at 30° or 37°C; and at 20°C only to GABA. Added alanine was associated with the appearance of GABA. In the blank runs the concentration of YPN was high for all temperatures and no conclusions could be made regarding this compound. Other than this compound the blank sample contained very small amounts of alanine and aspartate (barely detectable by ninhydrin) but no ninhydrin detectable glutamate. A mixture of alanine and alpha-KG gave very faint bands corresponding to glutamate and so GOT activity is possible. With the aspartate run it is possible that the activity of GPT was too low for detection of the glutamate formed.

These results are summarized in Table 10-2.1.

The Pyridine-Isatin reagent was found extremely useful in this section but no complete list of the colours given by the amino acids could be found. Therefore solutions of each amino acid were spotted onto chromatography paper and the colours noted. The results are shown in Table 10-2.2. Although this reagent is not as sensitive as ninhydrin the colours developed are an extremely useful aid for identifying amino acids, in particular proline and hydroxyproline. The unknown YPN did not develop a colour with this spray. The colours developed do depend on the concentration of the amino acid but this is most often merely a slight difference in shade.

TABLE 10-2.1

The Conversion of Added Substrates by Enzymes from Sinapis alba Seeds

Substance Added	Substances Formed
Glutamate	GABA, UVGB,* YPN*
GABA	UVGB, YPN
Aspartate	Alanine, GABA
Alanine	GABA
Alanine/alpha KG	GABA, Glu

* These compounds are unknowns whose properties are described in the text.

TABLE 10-2.2

The Colours Developed wity Pyridine-Isatin Reagent

Amino Acid	Colour
α-alanine	purple
β-alanine	purple
α-amino-n-butyric acid	pink
GABA	pink
α-amino-isobutyric acid	pink
α-amino-octamic acid	pink
L-arginine	pink
L-asparagine	brown-pink
L-aspartate	purple
L-citrulline	pink
L-cystine	pale pink-purple
L-cysteic acid	pale pink
3,5-di-iodo-L-tyrosine	light brown
Ethanolamine	pink
L-glutamate	bright pink
L-glutamine	pale pink
Glycine	pale brown
Histamine	red-brown
Histidine	pink
Homocystine	pink
Hydroxy-proline	very pale green
iso-leucine	pale pink
Leucine	pink
Lysine	pink
Methionine	light brown
Methylhistidine	light brown
Norvaline	pink
Ornithine	mauve-purple
Phenylalanine	red
Proline	dark blue
Sarcosine	pale yellow-green
Serine	browny-pink
Taurine	pale pink
Threonine	brown
Tryptophane	red-brown
Tyrosine	yellow-brown
Valine	red

10-3 Investigation of Transaminase Reactions by Spectrophotometry

The analysis for GOT activity was set up as previously described. The incubation was carried out at 23°C (room temperature) and 37°C. The results shown in Table 10-3.1 were obtained by reading the tubes against the corresponding blank. A similar experiment using alanine as the substrate is shown in the same table.

TABLE 10-3.1

Investigation of GOT and GPT Activity of Sinapis alba Seeds

Amino Acid	Amount Enzyme Solution (mls)	Temp. (°C)	O.D. (546mu)	Units/ml
L-Aspartate	0.2	23	0.135	56
"	0.2	37	0.256	161
L-Alanine	0.2	23	0.105	27
"	0.2	37	0.115	30

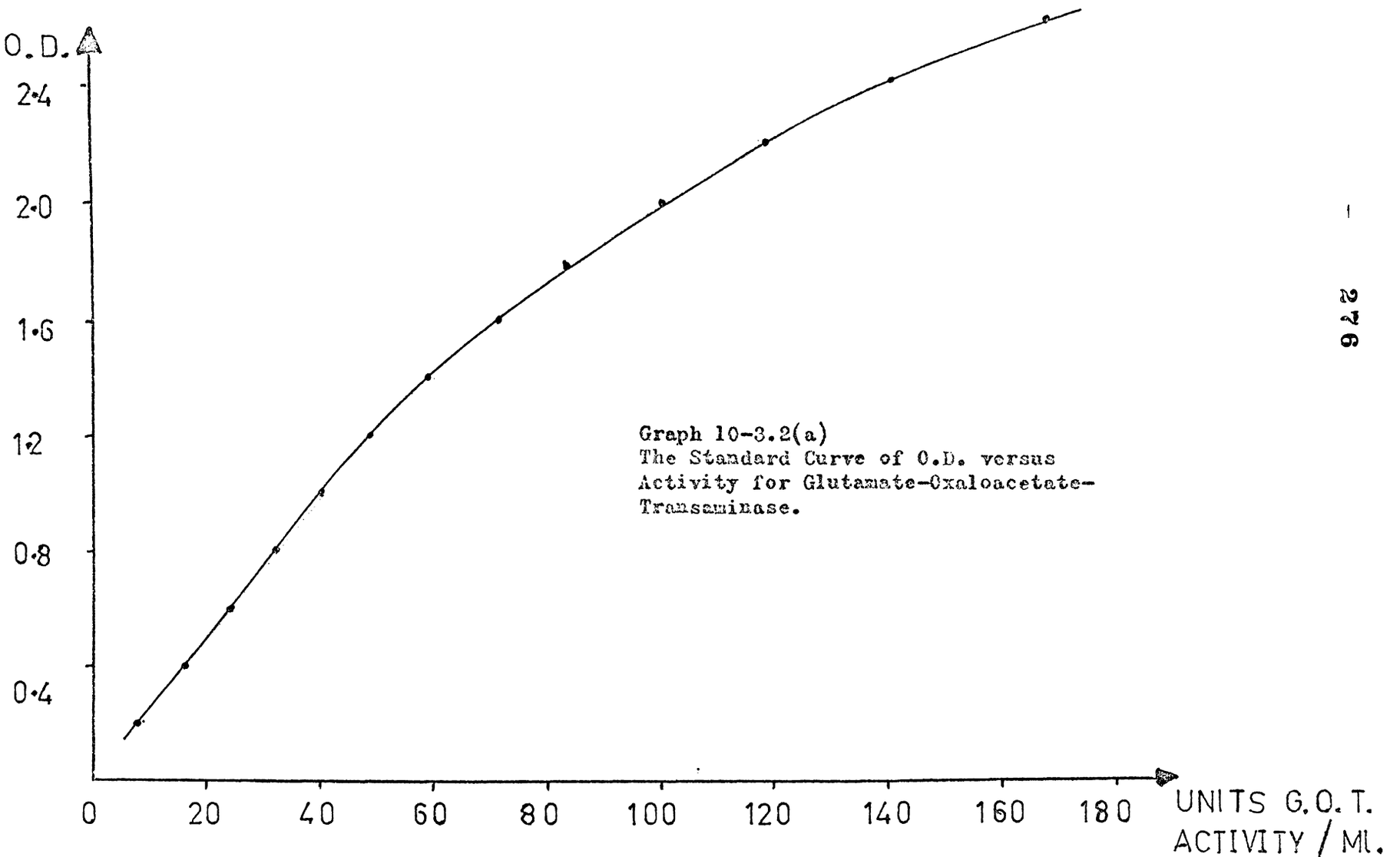
The standard curves (Graphs 10-3.2(a) and 10-3.2(b) were drawn using the values shown in Table 10-3.2. From these curves the values of 56 units of GOT/ml (23°C) 161 units of GOT/ml (37°C), 27 units of GPT/ml (23°C) and 30 units GPT/ml (37°C) were obtained.

The most interesting result is the high optimal temperature (37°C) under the conditions investigated. If transamination reactions are important during germination it could be expected that the optimum would correspond more closely to the temperature of germination (i.e. room temperature).

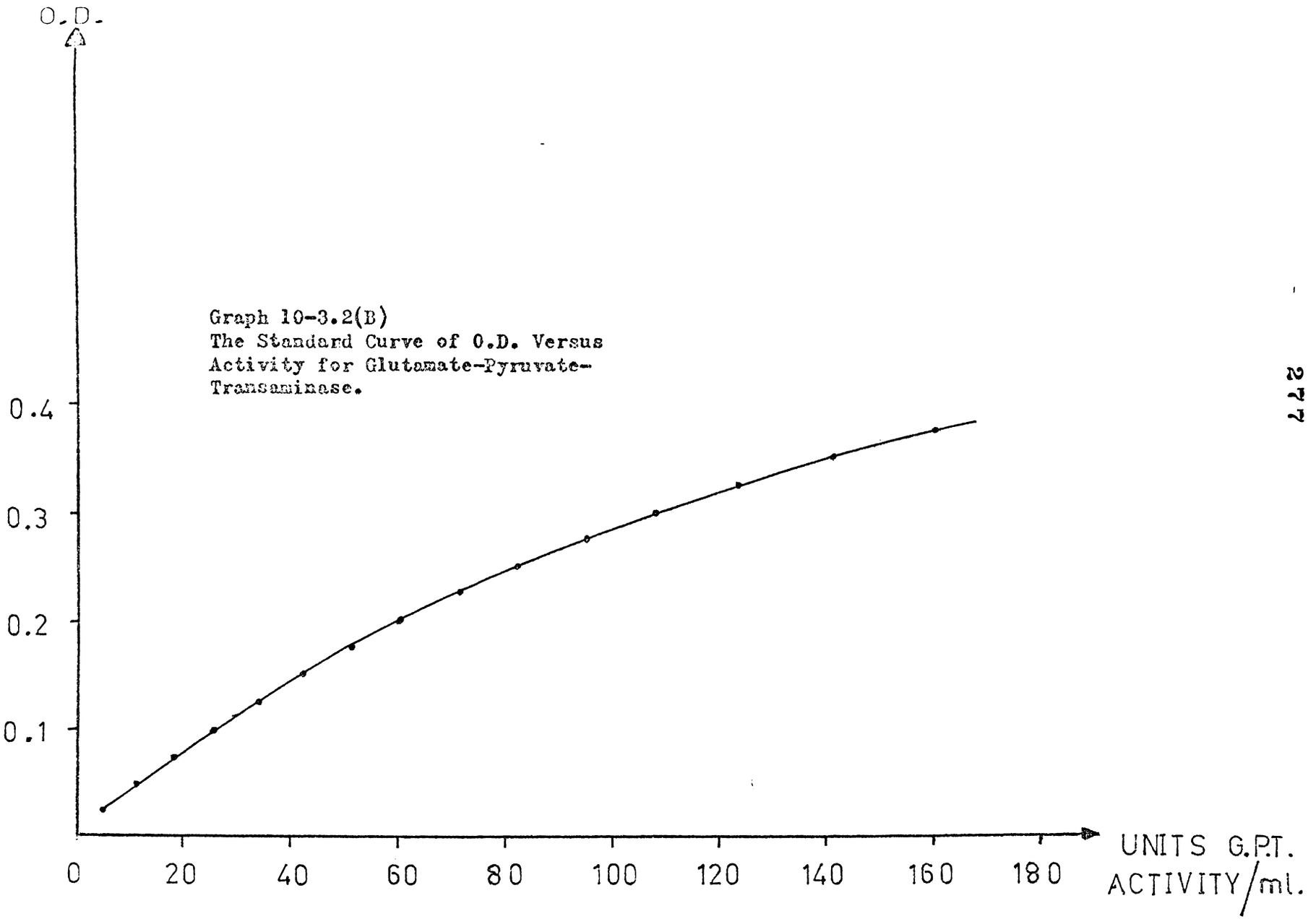
TABLE 10-3.2

Derivation of the Standard Curvesfor Investigations of the Transaminase Activity[330].

Glutamate/oxaloacetate		Glutamate/pyruvate	
O.D. (546mu)	GOT units/ml	O.D. (546mu)	GPT units/ml
0.02	8	0.025	5
0.04	16	0.050	11
0.06	24	0.075	18
0.08	32	0.100	25
0.10	40	0.125	34
0.12	49	0.150	42
0.14	59	0.170	51
0.16	71	0.200	60
0.18	83	0.225	71
0.20	100	0.250	82
0.22	118	0.275	95
0.24	140	0.300	108
0.26	167	0.325	123
		0.350	141
		0.375	160



Graph 10-3.2(a)
The Standard Curve of O.D. versus Activity for Glutamate-Oxaloacetate-Transaminase.



Graph 10-3.2(B)
The Standard Curve of O.D. Versus Activity for Glutamate-Pyruvate-Transaminase.

10-4. Partial Purification of Transaminases from Sinapis alba Seeds

The precipitates of protein corresponding to 0-25%, 25-50%, 50-75% and 75-100% were obtained in the manner previously described and examined for transaminase activity using the 2,4-dinitrophenyl hydrazones of the keto acids.

The results for dry seeds are shown in Table 10-4.1 and for germinating seeds (1 hr of imbibition) in Table 10-4.2. The histograms shown in graphs 10-4(a) and 10-4(b) were drawn from these results. It should be stressed that under the experimental procedure used, activation of zymogens in the dry seeds could have taken place during the purification procedure. It took approximately 15 mins to grind the seeds and bring the temperature of the sample to 50°C. For the 10 minute sample the results could correspond to those in the whole seed after 20 mins of germination and for the 20 minute sample, depending on how quickly the other enzymes or activating processes were denatured by 50°C the pattern could correspond to between 20 and 30 mins germination. After the initial centrifugation it is unlikely that any further activation of transaminases would occur.

A comparison of the 10 min and 20 min. samples for "dry" seeds shows that increased activity was found in the 50-75% saturation precipitate for GOT activity and in both 25-50 and 75-100% precipitates for GPT activity. It is therefore likely that activation of zymogens of transaminases does occur during the very early periods of germination and this activation process appears to be relatively stable to heat. Comparison of the precipitates from dry seeds to those which had been germinating for one hour prior to the extraction processes shows that transaminases are rapidly destroyed during this second phase of germination. The activity of GPT had virtually disappeared and that of

TABLE 10-4.1

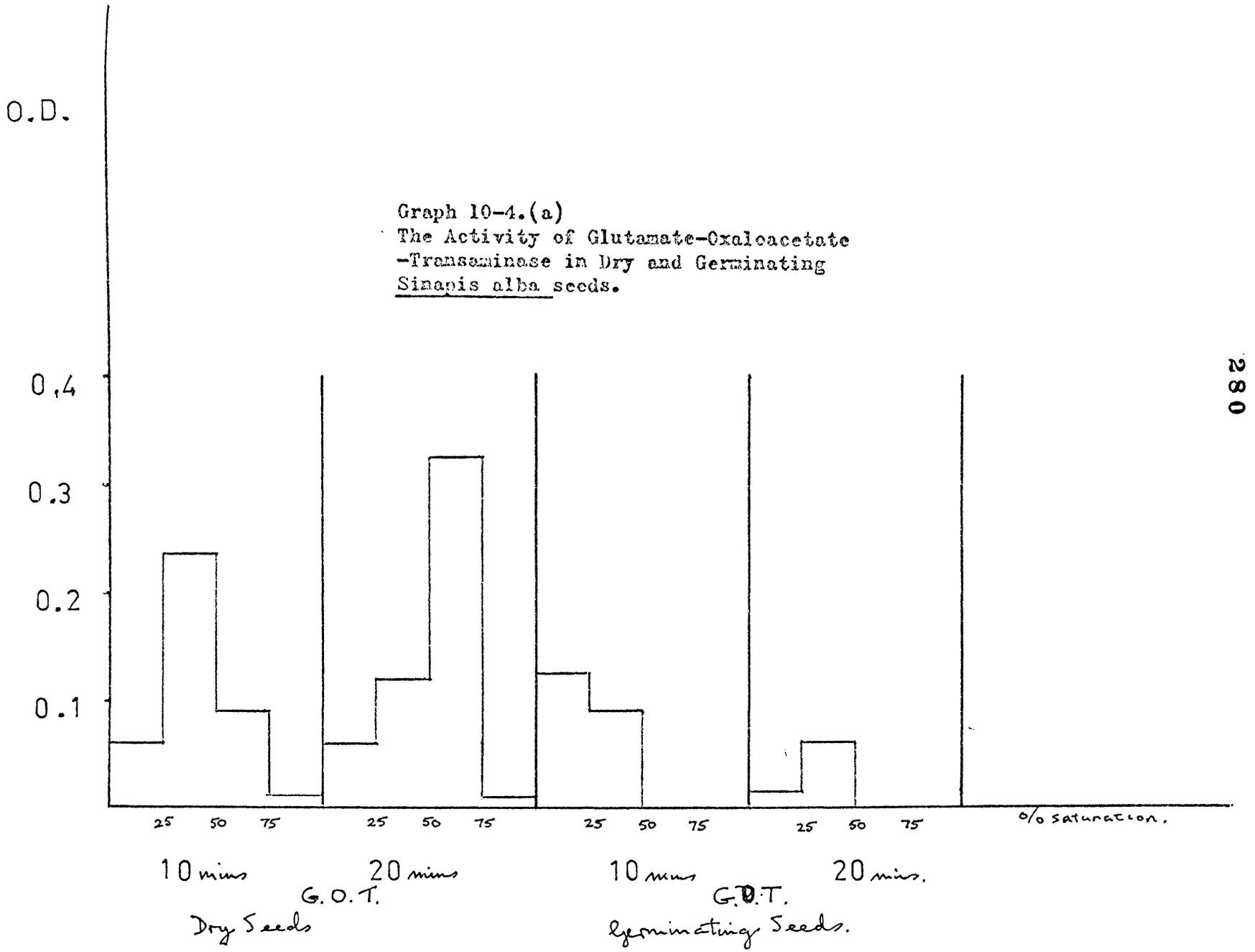
The GOT and GPT activities as measured by the optical density at 546 mu for dry Sinapis alba seeds (7.5% water content).

% Saturation	Time 50°C (mins)	O.D. (GOT) 546 mu	O.D. (GPT) 546 mu	Biuret
Crude (0%)	10	0.240	0.004	+++
	20	0.055	0.060	+
0-25	10	0.060	0.031	+
	20	0.060	0.020	+
25-50	10	0.236	0.047	++
	20	0.141	0.251	++
50-75	10	0.090	0.060	+?
	20	0.328	0.039	+?
75-100	10	0.018	0.026	+?
	20	0.005	0.125	+?
Supernatant	10	0	0	-
	20	0	0	-

TABLE 10-4.2

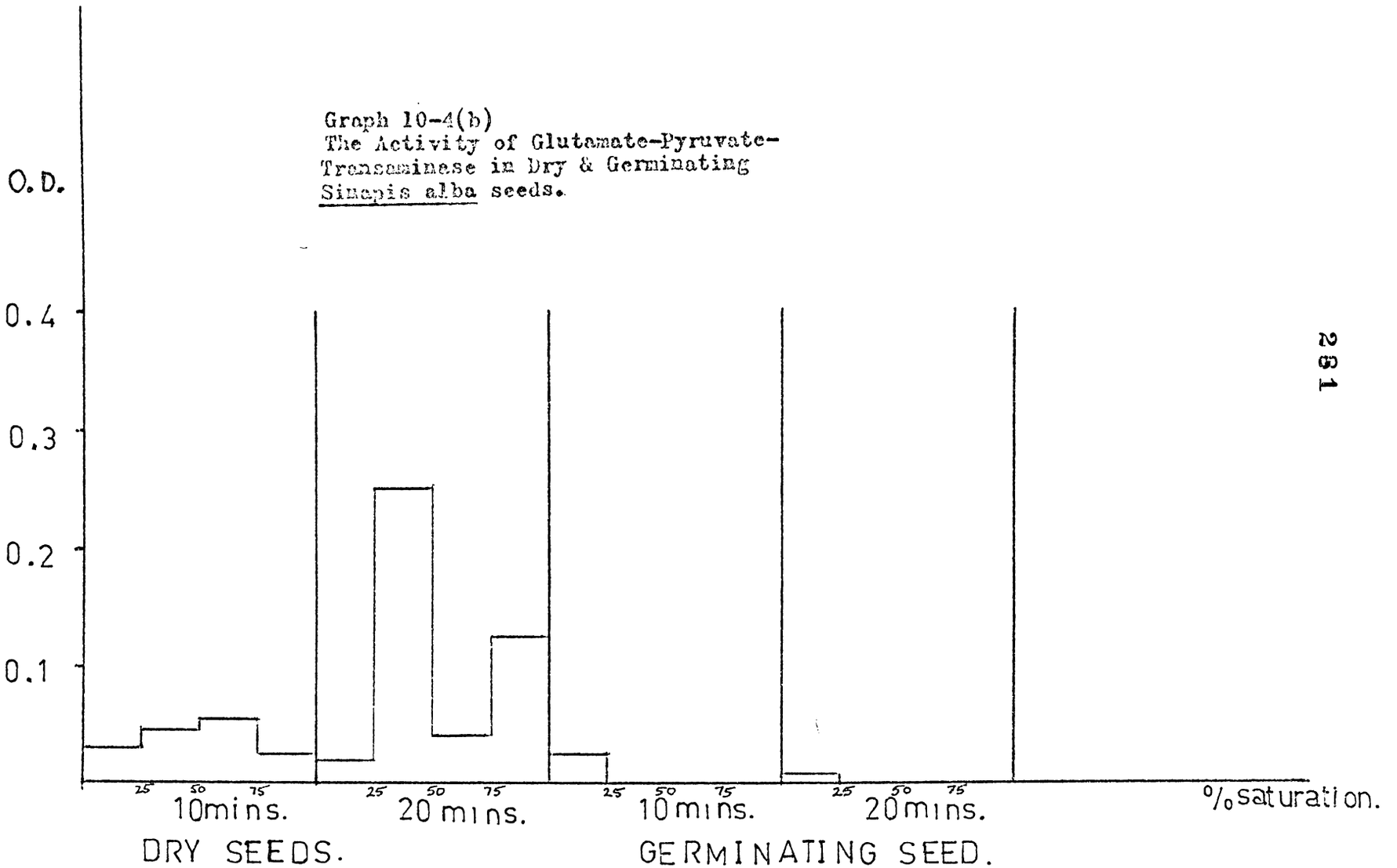
The GOT and GPT activities as measured by the optical density at 546 mu for Sinapis alba seeds which had been germinating for 1 hour previous to the separation.

% Saturation	Time 50°C (mins)	O.D. (GOT 546mu	O.D. (GPT) 546mu	Biuret
Crude (0%)	10	0.190	0.005	+
	20	0.055	0.040	+
0-25	10	0.125	0.025	+
	20	0.006	0.002	+
25-50	10	0.090	0	++
	20	0.060	0	+++
50-75	10	0	0	+
	20	0	0	+
75-100	10	0	0	+
	20	0	0	+
Supernatant	10	0	0	-
	20	0	0	-



Graph 10-4.(a)
The Activity of Glutamate-Oxaloacetate -Transaminase in Dry and Germinating Sinapis alba seeds.

Graph 10-4(b)
The Activity of Glutamate-Pyruvate-Transaminase in Dry & Germinating Sinapis alba seeds.



GOT was only present in the 0-25% and 0-50% precipitates.

These results imply that activation of transaminases occurs during the first 0-30 mins of germination but this is rapidly followed by inactivation of these enzymes, and within 1½ hrs germination their activity is extremely reduced.

10-5. X-ray Analyses of the DNP-Hydrazones of the Keto Acids Formed During the Investigation of Transaminase Activity

The enzyme analyses for GOT and GPT were carried out as previously described but using proportionately larger quantities such that 100 mls of each solution were obtained after the final addition. The DNP-hydrazones of the keto acids were then prepared as described in section 10-1.4 and allowed to crystallize. Pure DNP-hydrazones of pyruvate, oxaloacetate and alpha-KG were also prepared.

The reactions being studied were as follows:

GPT Alanine + alpha-KG $\rightleftharpoons$ glutamate + pyruvate

GOT Aspartate + alpha-KG $\rightleftharpoons$ glutamate + oxaloacetate.

The substrates used were either alanine or aspartate and alpha-KG. Hence if transaminases were not present in the seeds it would be expected that the alpha-KG used in the enzyme analysis would not be metabolised and would be extracted in the form of its DNP-hydrazone. Whereas, if transaminases are present the extracted hydrazones of keto acids would contain a predominance of either oxaloacetate or pyruvate DNP-hydrazone.

The samples were analysed in a Phillips Powder X-ray Diffractometer using the Cu "$K_{(\alpha)}$" line and plots of 2θ versus intensity obtained. For a mixture of compounds the pattern obtained should be a super-imposition of the patterns for each individual compound unless a solid

state reaction has occurred. The "d" values were calculated from the Bragg equation $n = 2d \sin 0$ for the four most intense lines of each spectrum. These values are quoted in Table 10-5.1. Unfortunately, although several references were found concerning the diffraction patterns of the 2,4-dinitrophenylhydrazones of aldehydes and ketones, the authors did not include alpha-keto acids in their analyses.

Neither the blanks nor the samples from the enzyme analyses had peaks between values of 2 and 26 for 2θ, an area in which several large peaks occurred for 2,4-dinitrophenylhydrazine itself. This shows that the excess DNP hydrazine had indeed been removed by the extraction procedure.

The results obtained for the presence of oxaloacetate, pyruvate or alpha-KG hydrazones in the samples extracted were equivocal. The X-ray diffraction patterns of these compounds contained several intense peaks for values of σ below $2\sigma = 27^{o}$ but these were absent from the extracted samples. If the added alpha-KG was not metabolised then its hydrazone would be expected to occur in both the blank and the sample for the enzyme analyses. If it was being metabolised then the diffraction bands would be expected to occur in the blanks and not in the samples. In the latter case bands corresponding to the appropriate keto acid formed, i.e. oxalo-acetate or pyruvate would be expected to appear.

In the experiment such changes were not found. No major variations in the peaks occurred. The patterns for all 4 extracted samples were extremely similar (see Table 10-5.1) and the differences between samples and blanks consisted of relative changes in peak intensity. These changes are summarized in Table 10-5.2 in which the "d" value of the peak and its intensity in the sample and corresponding blank are given.

Two of the peaks which changed did not correspond to any peaks for the standard hydrazones. Decreases in peaks corresponding to alpha-KG for both the GOT and GPT analyses are shown as well as increases in peaks

for pyruvate-2,4-dinitrophenylhydrazone.

TABLE 10-5.1

"d" Values of Most Intense Lines of the X-ray Diffraction Pattern of the Keto-acid-2,4-dinitrophenylhydrazones (in order of decreasing intensity).

Keto-Acid Sample	d (Å)			
	I	II	III	IV
pyruvic acid	2.81	4.96	3.41	3.37
alpha-KG	4.41	7.92	2.96	3.16
oxaloacetic acid	4.41	13.18	2.83	2.76
Blank GOT analysis	2.52	1.78	1.27	2.89
GOT analysis	2.52	1.78	1.27	2.89
Blank GPT analysis	2.51	1.80	1.27	2.89
GPT analysis	2.52	1.79	1.27	2.89
2:4 DNPH	5.17	1.60	3.29	2.83

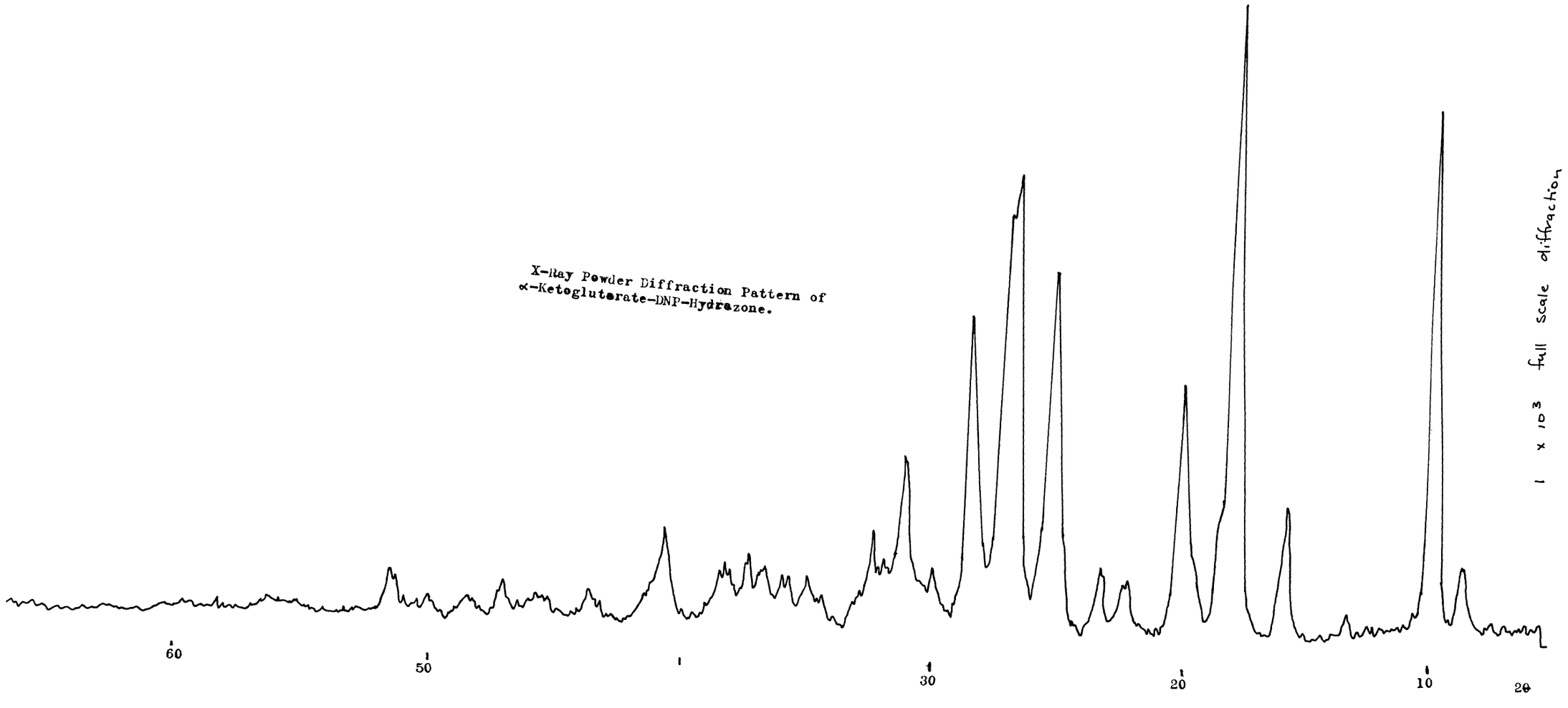
TABLE 10-5.2

The "d" Values of Lines which Changed in Intensity Between the Enzyme Blanks and Samples (Intensity Values given in Brackets).

Sample	"d" Values				
GOT Blank	1.27 (2)	2.89 (28)	2.80 (9)	1.52 (8)	1.99 (4)
GOT Sample	" (36)	" (24)	" (4)	" (6)	" (7)
GPT Blank	" (43)	" (31)	" (9)	" (7)	-
GPT Sample	" (32)	" (30)	" (15)	" (5)	-
Corres. Lines*	none	alpha-KG	alpha-KG pyruvate	None	alpha-KG pyruvate

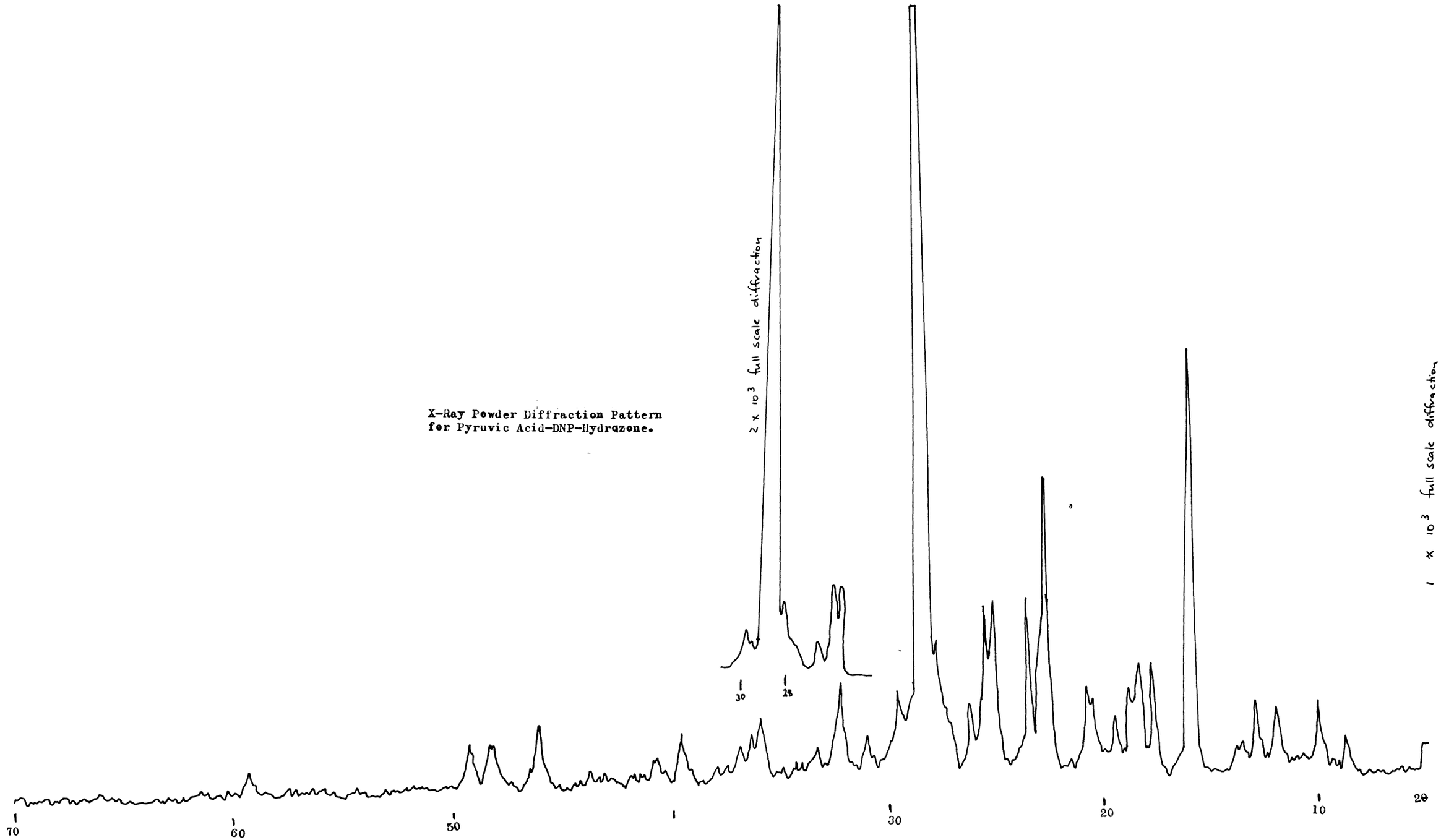
*This denotes lines which were found in hydrazones of the three keto acids studied.

X-Ray Powder Diffraction Pattern of
Oxaloacetate-DNP-Hydrazone.

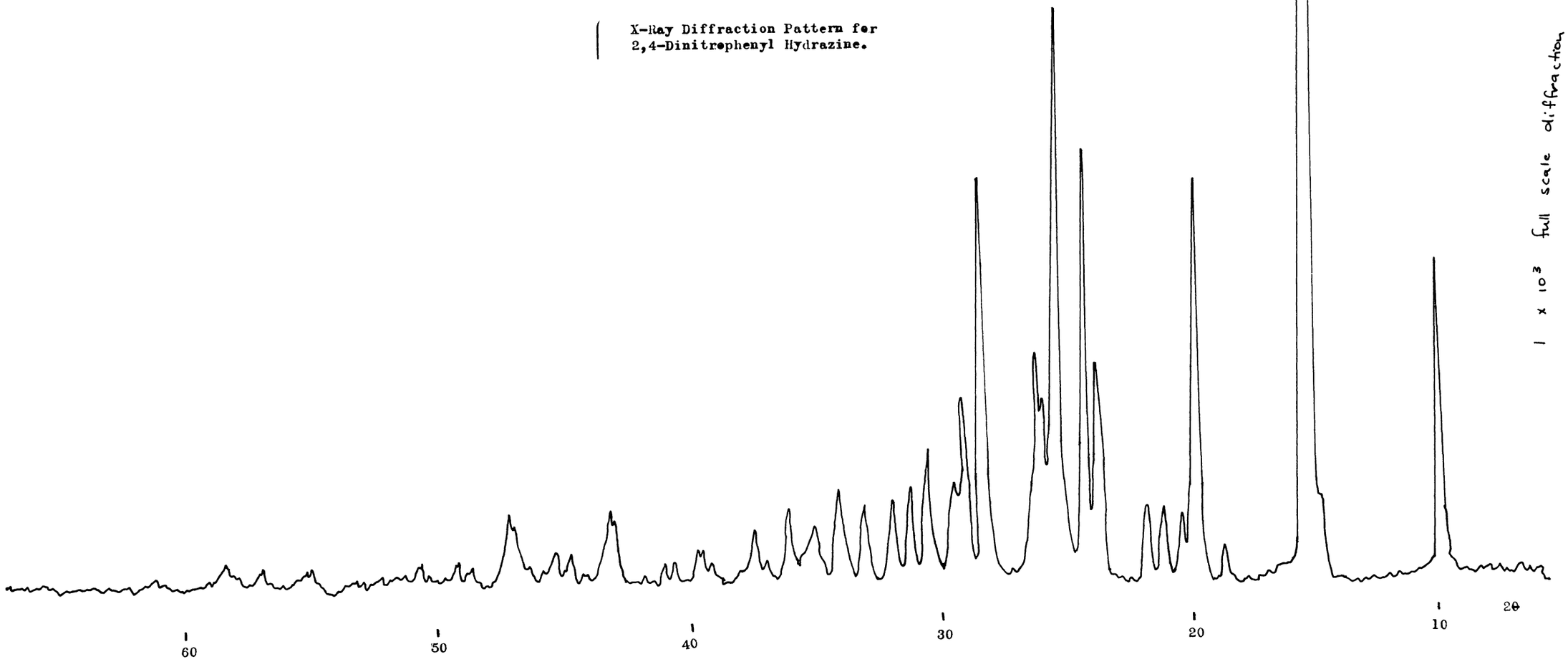


X-Ray Powder Diffraction Pattern of
∝-Ketoglutarate-DNP-Hydrazone.

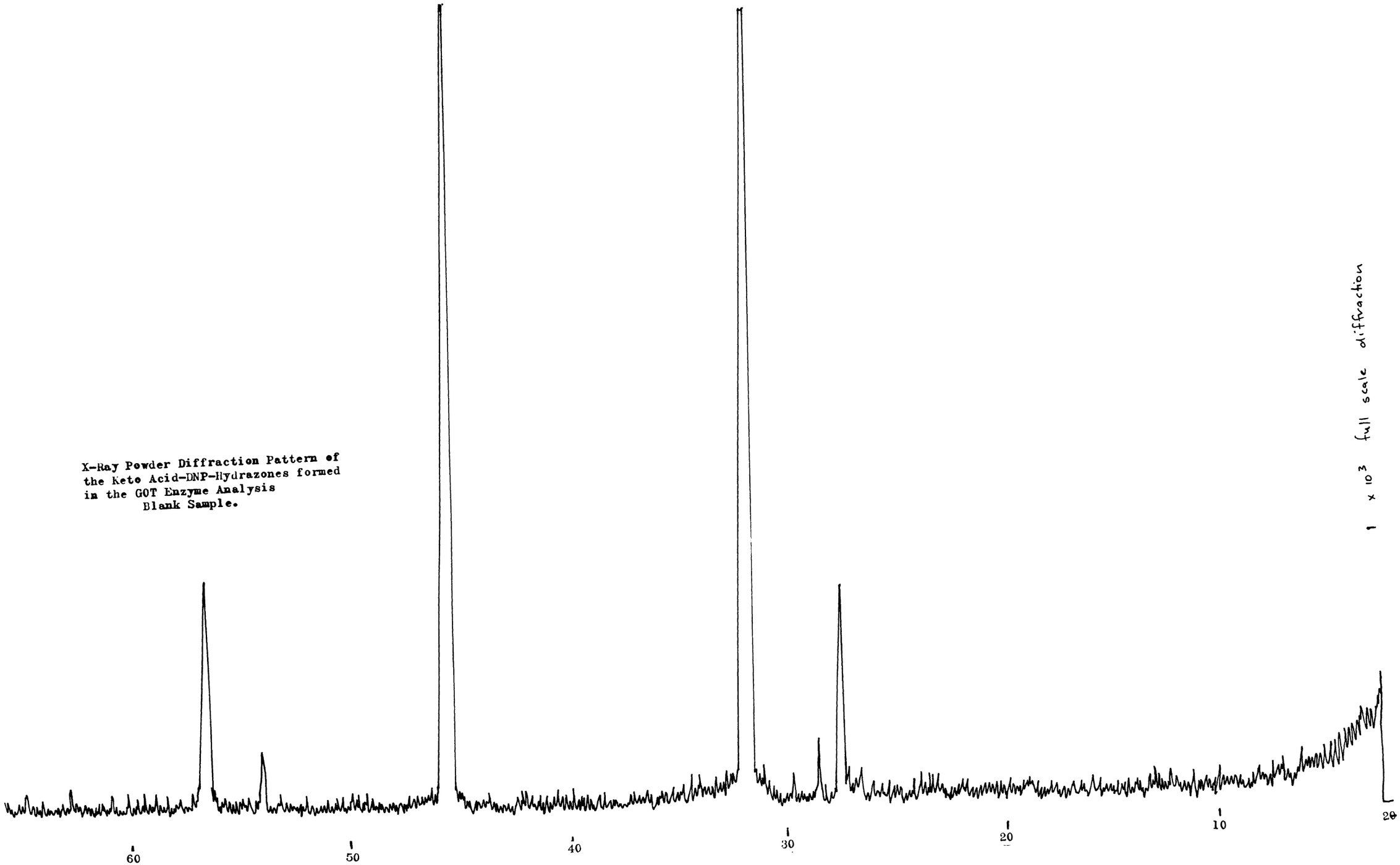
X-Ray Powder Diffraction Pattern
for Pyruvic Acid-DNP-Hydrazone.



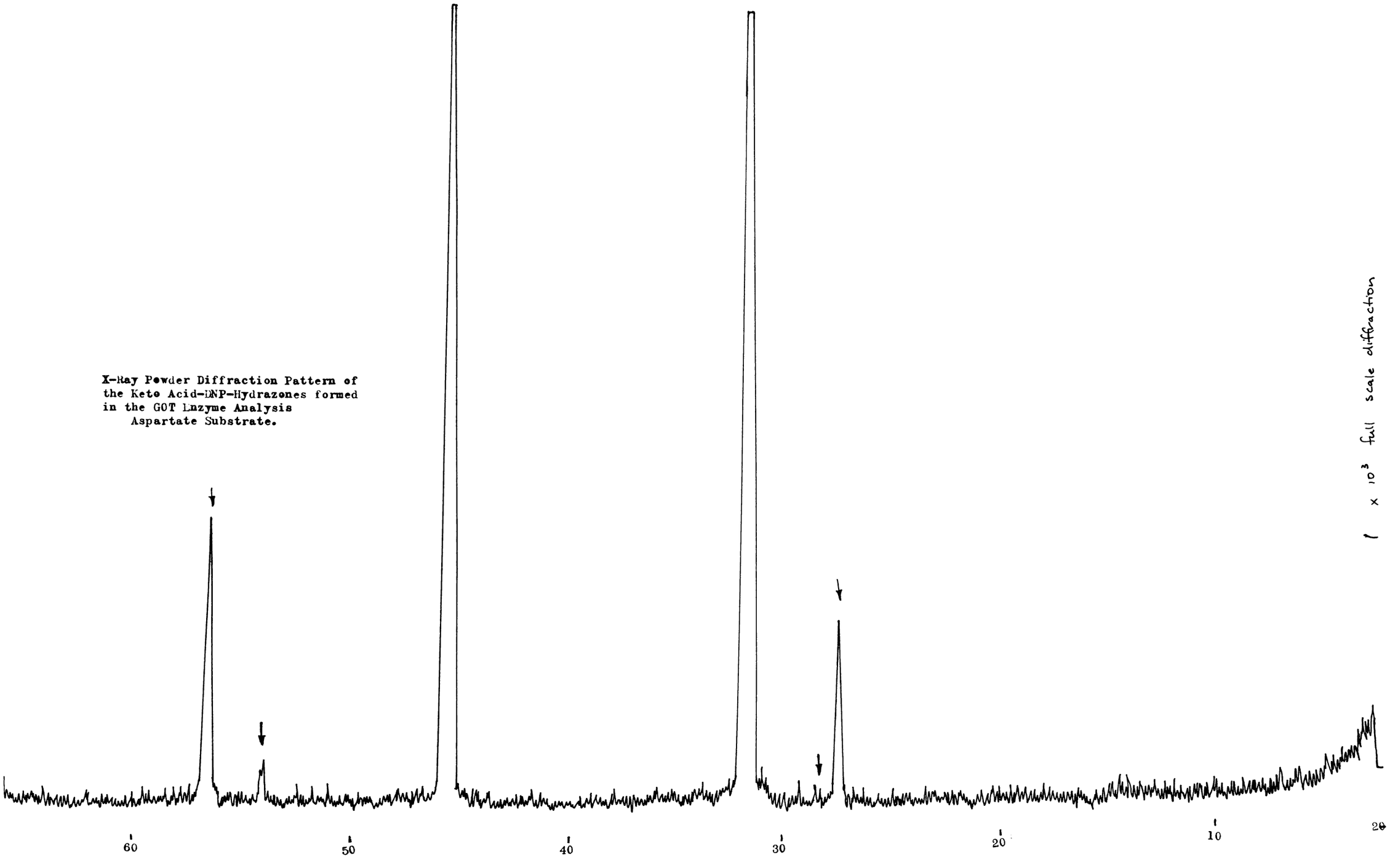
X-Ray Diffraction Pattern for
2,4-Dinitrophenyl Hydrazine.



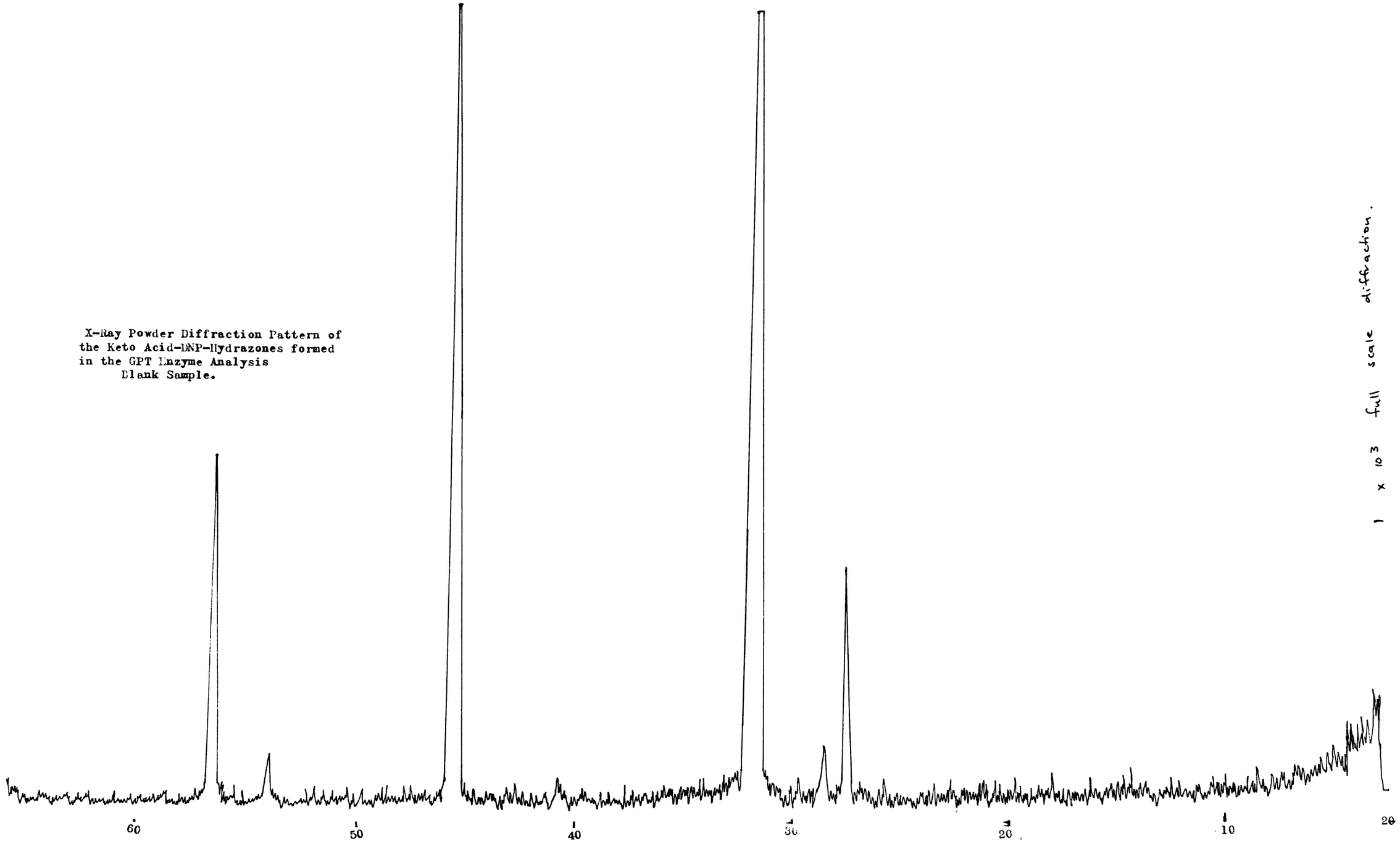
X-Ray Powder Diffraction Pattern of the Keto Acid-DNP-Hydrazones formed in the GOT Enzyme Analysis Blank Sample.



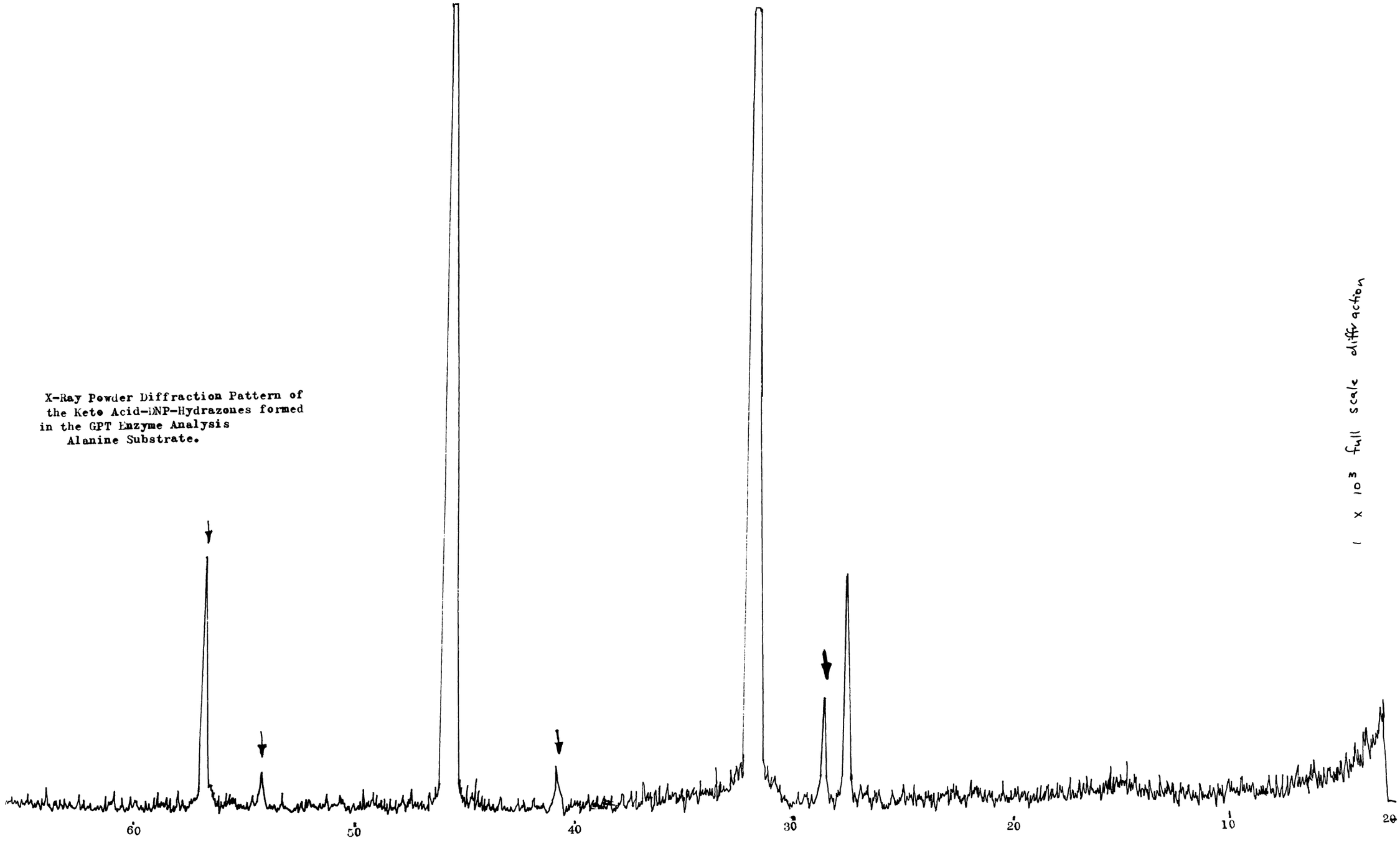
X-Ray Powder Diffraction Pattern of the Keto Acid-DNP-Hydrazones formed in the GOT Enzyme Analysis Aspartate Substrate.



X-Ray Powder Diffraction Pattern of
the Keto Acid-DNP-Hydrazones formed
in the GPT Enzyme Analysis
Blank Sample.



X-Ray Powder Diffraction Pattern of the Keto Acid-DNP-Hydrazones formed in the GPT Enzyme Analysis
Alanine Substrate.



An attempt was made to identify the keto acid DNP hydrazones formed in the plant extracts and to compare these to the standard hydrazones using chromatography. It was found, however, that discrete spots could not be obtained for the unknown samples using any of the solvents recommended by Smith[331] for DNP hydrazones of keto acids. Furthermore it was found that the R_F values for the pure compounds depended to an extremely large extent (20% variation or more) on the actual concentrations of the compounds applied to the paper. The more concentrated samples travelled further and their bands corresponded to the tail of the "streak" for the DNP-hydrazones of the keto acids in the seeds. At lower concentrations, however, the pure standards had R_F's below that of the unknowns.

The keto acids quoted by Smith[331] whose hydrazones have R_F's above that of pyruvic acid are acetoacetate, acetone, ketobutyric, phenyl-pyruvic and hydroxyphenyl-pyruvic acid.

10-6 Discussion of Results

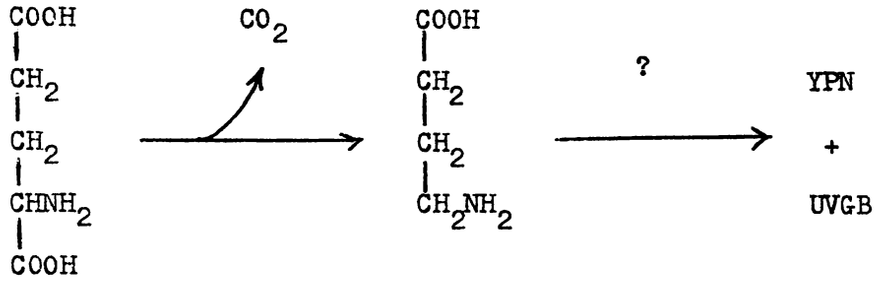
In the paper chromatographic analysis conversion of GABA to two unknowns called UVGB (Green-Blue fluorescence in U.V. light) and YPN occurred. The latter compound YPN was detected by its reaction with ninhydrin, a yellow colour appearing first and this gradually changed to purple in 12-24 hours. Ninhydrin reacts with primary amino groups, normally, to give purple colours but sometimes purple-brown or mauve. It also reacts with most secondary amines and with several alpha-keto acids. The colour tends to be purple with primary amines where the amine group is on an aliphatic carbon and mauve, or rarely, brown or yellow with aromatic primary amines. The spots given by the aromatic keto acids were mauve or pale brown while those with the secondary amines

tended to be some shade of purple.[336] Isatin is more specific for alpha amino acids than is ninhydrin. It rarely gives colours with purines, amines or keto acids.

The colour reaction observed for YPN is extremely unusual, the reverse change being much more common. The same reaction was observed if the chromatogram was not placed in an oven after spraying, conditions under which many non-alpha amino acids do not give colours. The lack of reaction to isatin implies that YPN is probably not an alpha-amino acid. Compounds reported to give a similar colour reaction are 4-aminobenzoic acid (although also often reported to be without reaction), phenyl pyruvic acid, proline (more often reported and detected in this laboratory, as giving a stable yellow colour), and L-(t)-carnosine. The latter compound is more correctly named beta-alanylhistidine and has a very low R_F value for the solvents used so it cannot be YPN. The unknown did not chromatograph with proline in any of several solvent systems. An attempt was made to determine the R_F values for 4-aminobenzoic acid in the solvents used and also to co-chromatograph this compound and YPN but 4-aminobenzoic acid did not, at any of the concentrations studied, react with ninhydrin at all. The R_F values quoted for phenyl pyruvic acid[336] are similar to those found for YPN. The unknown was tested using the ferric chloride reagent but no colour could be detected. It is possible that the reagent was not sufficiently sensitive but the colour produced with ninhydrin implied large quantities of YPN. This compound does seem to be one of the major products of increased quantities of GABA. In the human body, animals and most plants so far studied, glutamine acts as a donor of nitrogen for the formation of many very important compounds, including the nucleotides. In germinating wheat embryos it has been shown that the amide nitrogen of asparagine is more active than that of glutamine for the formation of glycinamide ribonucleotide. This compound

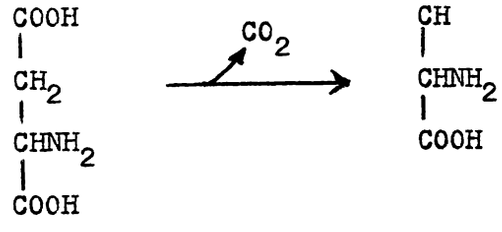
YPN could be associated with GABA, which does appear to be very important during germination, acting in a similar manner as a source of nitrogen.

Glutamic acid was converted to GABA and the two unknowns, UVGB and YPN.



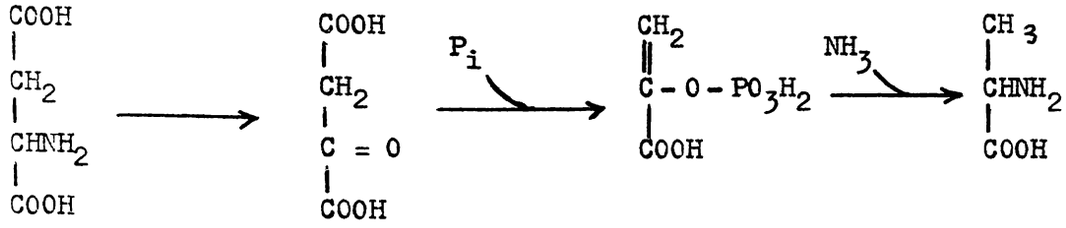
Glutamic Acid GABA

Aspartate appeared to be converted to alanine and GABA. The alanine could be formed by beta-decarboxylation of aspartate.



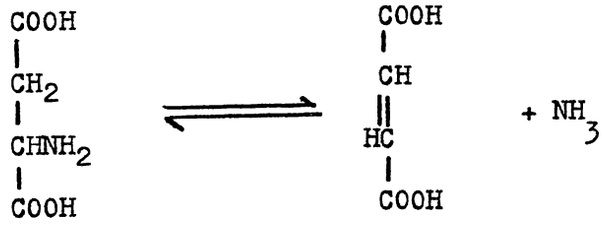
Aspartic Acid Alanine

It is of interest that Vickers[334] when measuring the production of carbon dioxide in the Warburg manometer obtained results which showed increased carbon dioxide production with added aspartate. A second possible pathway exists if transaminases or deaminases are active. These would involve the conversion of aspartate to oxaloacetate and decarboxylation to either phosphoenol pyruvate or pyruvate followed by conversion to alanine. One such possibility is illustrated.



Aspartate | Oxaloacetate | Phospho-enol pyruvate | Alanine

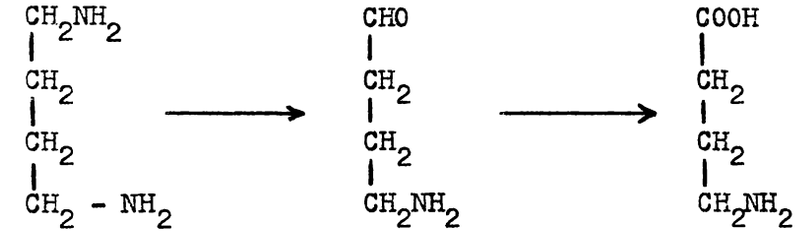
The formation of GABA poses a greater problem as this requires the conversion of the two lower carbon atoms. A possible explanation could be the involvement of a transaminase in the first step above. Most transaminases require alpha-KG or glutamate as one of their substrates and hence the first reaction shown above could convert the endogenous alpha-KG to glutamate which could then decarboxylate to form GABA. It is most unlikely, however, that the supply of alpha-KG is sufficient to cause such an increase in the amount of GABA. Another possible pathway is deamination of aspartate to oxaloacetate which could be metabolised via the TCA cycle to alpha-KG which is then converted to glutamate and hence to GABA. This reaction is most unlikely as the conditions within a seed especially at such an early stage of germination would not be conductive to the operation of the TCA cycle. A third pathway could involve an enzyme similar to the fumarase of microorganisms which converts aspartate to fumarate.



Aspartate | Fumarate

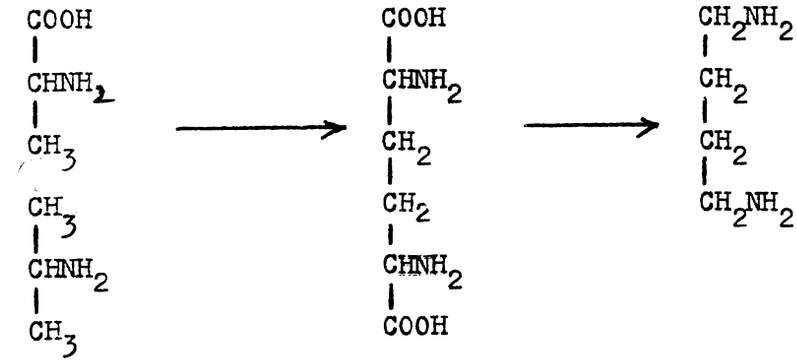
Addition of hydrogen across the double bond would form succinate which could be converted to GABA via succinic semi-aldehyde.

Alanine was also associated with higher concentrations of GABA and possibly transaminated with alpha-KG to form glutamate. These results, with those from added aspartate imply that a direct pathway could exist between alanine and GABA. In the trials with added aspartate, the GABA may have been formed from the alanine. Putrescine can be converted to GABA in some organisms by a pathway involving oxidative deamination to gamma-butyraldehyde. The latter compound is then oxidized to GABA. Conversion of alanine to GABA could initially involve some



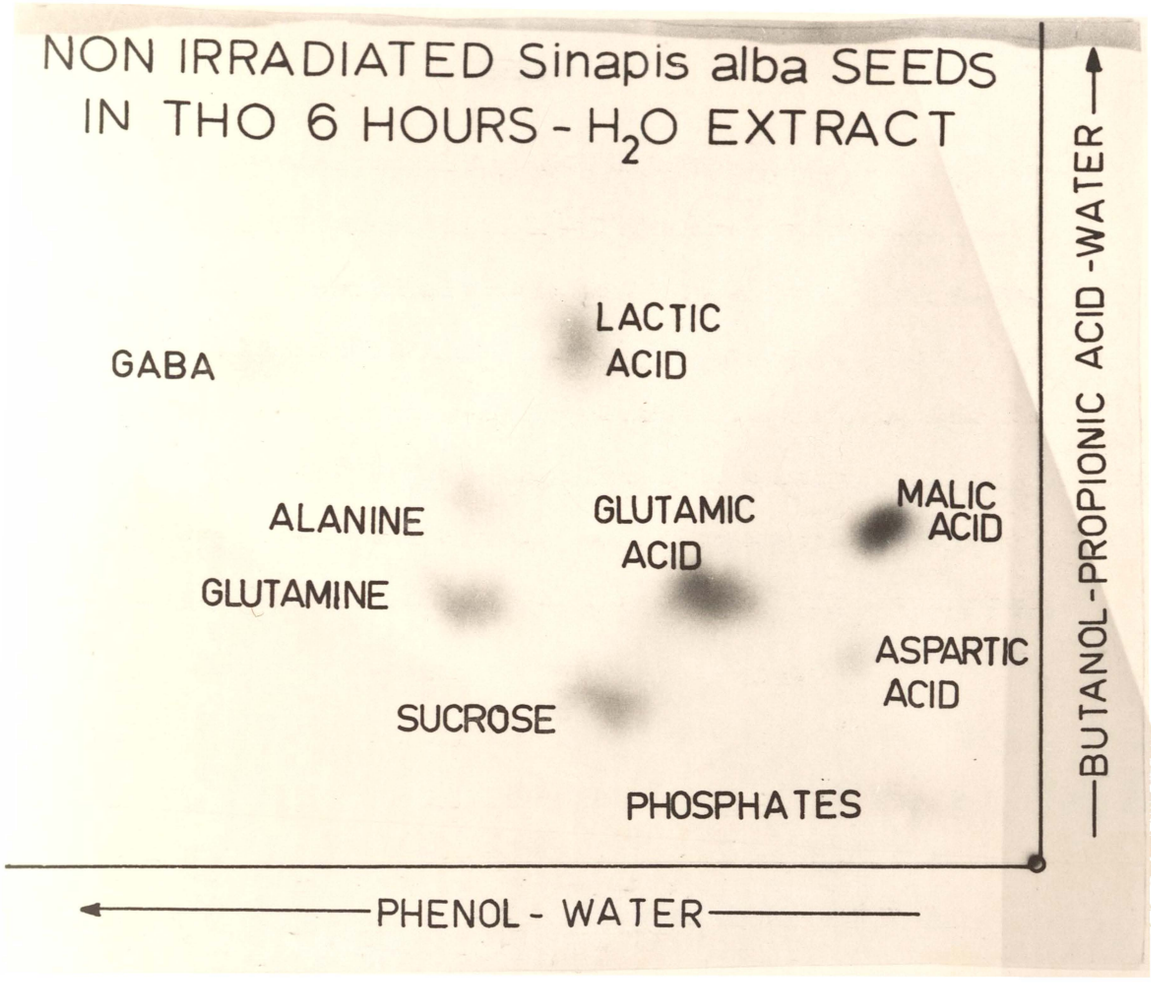
Putrescine — Gamma-amino butyraldehyde — GABA

reaction linking two molecules of alanine followed by double decarboxylation resulting in the formation of putrescine.



Alanine — Putrescine

For the transaminase analyses the increase in colour of the 2,4-dinitrohydrazones was read at 546 mu, at which value the absorption of an alkaline solution of the hydrazone of alpha-KG is small comparable to those of pyruvate or oxaloacetate. If this increase in colour is not due to either of these compounds it must be due to the formation of some


NON IRRADIATED Sinapis alba SEEDS
IN THO 6 HOURS - H_2O EXTRACT
LACTIC ACID
GABA
ALANINE
GLUTAMIC ACID
MALIC ACID
GLUTAMINE
ASPARTIC ACID
SUCROSE
PHOSPHATES
BUTANOL-PROPIONIC ACID-WATER
PHENOL - WATER

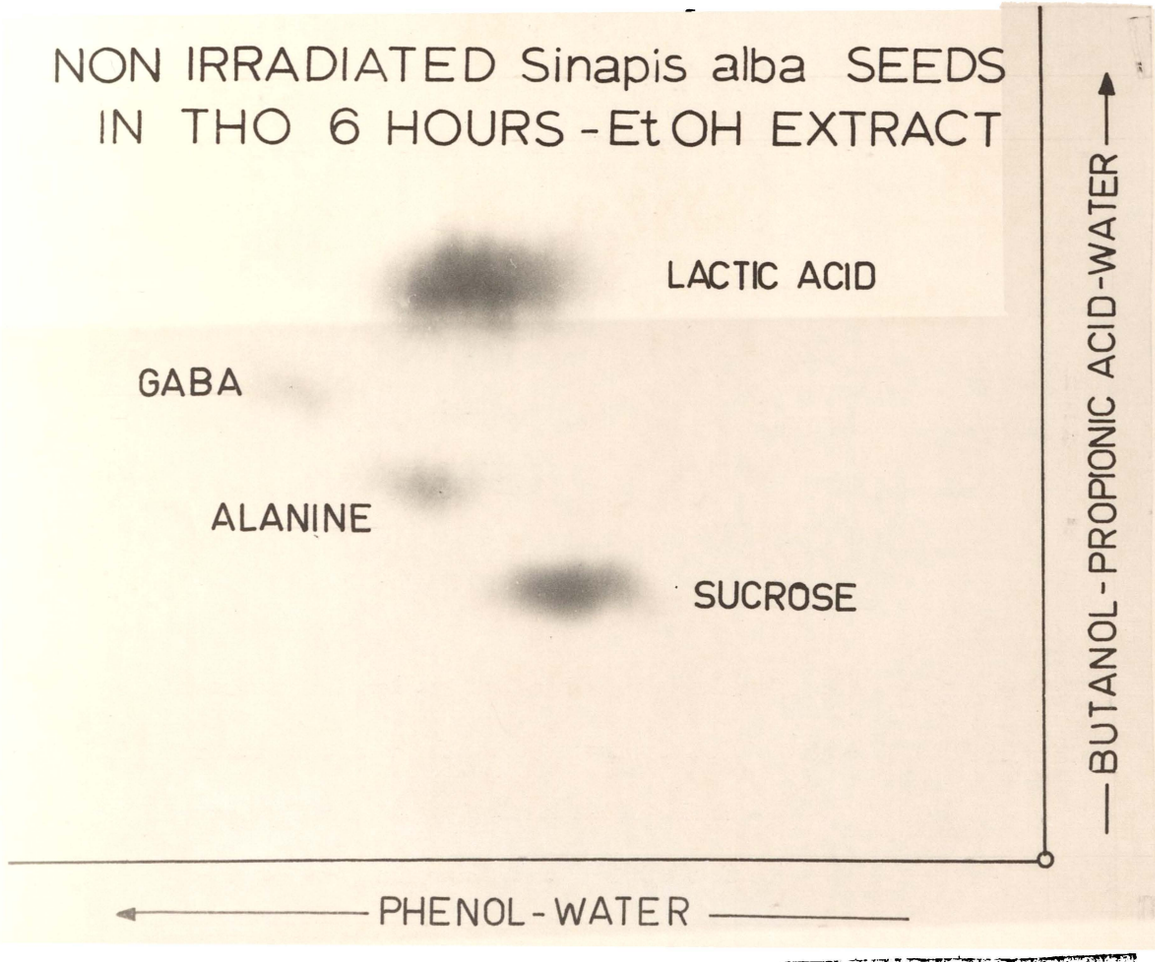

NON IRRADIATED Sinapis alba SEEDS
IN THO 6 HOURS - Et OH EXTRACT
LACTIC ACID
GABA
ALANINE
SUCROSE
BUTANOL-PROPIONIC ACID-WATER
PHENOL-WATER

Plate I.

compound which absorbs at this wavelength. It is possible that this method is in fact more sensitive than the powder diffraction analysis as the latter method related to all keto-acids in the seeds whereas the former method was to a certain degree more specific. Assuming that this widely accepted method was in fact measuring transaminases then they do not appear to be very important during germination, especially after the first hour. The loss of transaminase activity for both GOT and GPT is evident from the graphs 10-4(a) and (b). After only 1 hour imbibition the GPT activity has virtually disappeared.

The effect of excessive heating is also evident from these graphs. After 10 mins at 50°C the major activity for dry seeds is found in the 25-50% saturation separation whereas after 20 mins at 50°C it is in the 50-75% separation. It seems that the extra heat has altered the physical properties of the enzymes in such a way that it is precipitated in a later fractionation.

The X-ray powder diffraction of the keto-acids provides little useful information. The recovery of the hydrazone of alpha-KG using the extraction technique reported is 86% and yet the bands at lower values of 2θ were not observed in the mixtures. It is possible that some form of solid-state reaction has taken place. Slight changes in the intensity of peaks were observed between the blanks and the samples. These results do, however, show that mustard seeds contain some alpha-keto acid in high concentrations which was not one of the three examined.

11. THE INVESTIGATION OF A NOVEL ENZYME ASSAY

11-0. Introduction

During the experiments involving chromatography it was thought that a more rapid method for the analyses could be possible. The substrates could be spotted onto chromatograms and sprayed with a solution of the enzyme, incubated and then allowed to separate out by chromatography in the required solvent. Such a method would only be applicable to 1-D chromatography.

11-1. Method

A piece of cardboard, slightly larger than the intended chromatogram had a 5" x $\frac{1}{4}$" rectangle cut away. The compound(s) whose metabolism was to be examined was streaked along 3 inches of the origin of the chromatogram. The cardboard shield was then placed over the chromatogram in such a way that only the origin was exposed (through the rectangle).

A water extract of the ground-up Sinapis alba seeds was quickly obtained using a Dual Homogenizer and then centrifuging the mixture. The soluble part was placed in a spray bottle and sprayed onto the origin of the chromatogram until the paper was saturated but excess moisture was not visible.

The chromatogram was left in the water saturated atmosphere of a tank overnight and then run in BPW solvent. The lid of this tank was firmly attached by vaseline and the tank contained several beakers of water and toluene.

In the morning the chromatogram was dried and standard samples of the substrate spotted onto areas of the origin which had not been exposed to the spray. The control area for the spray itself consisted

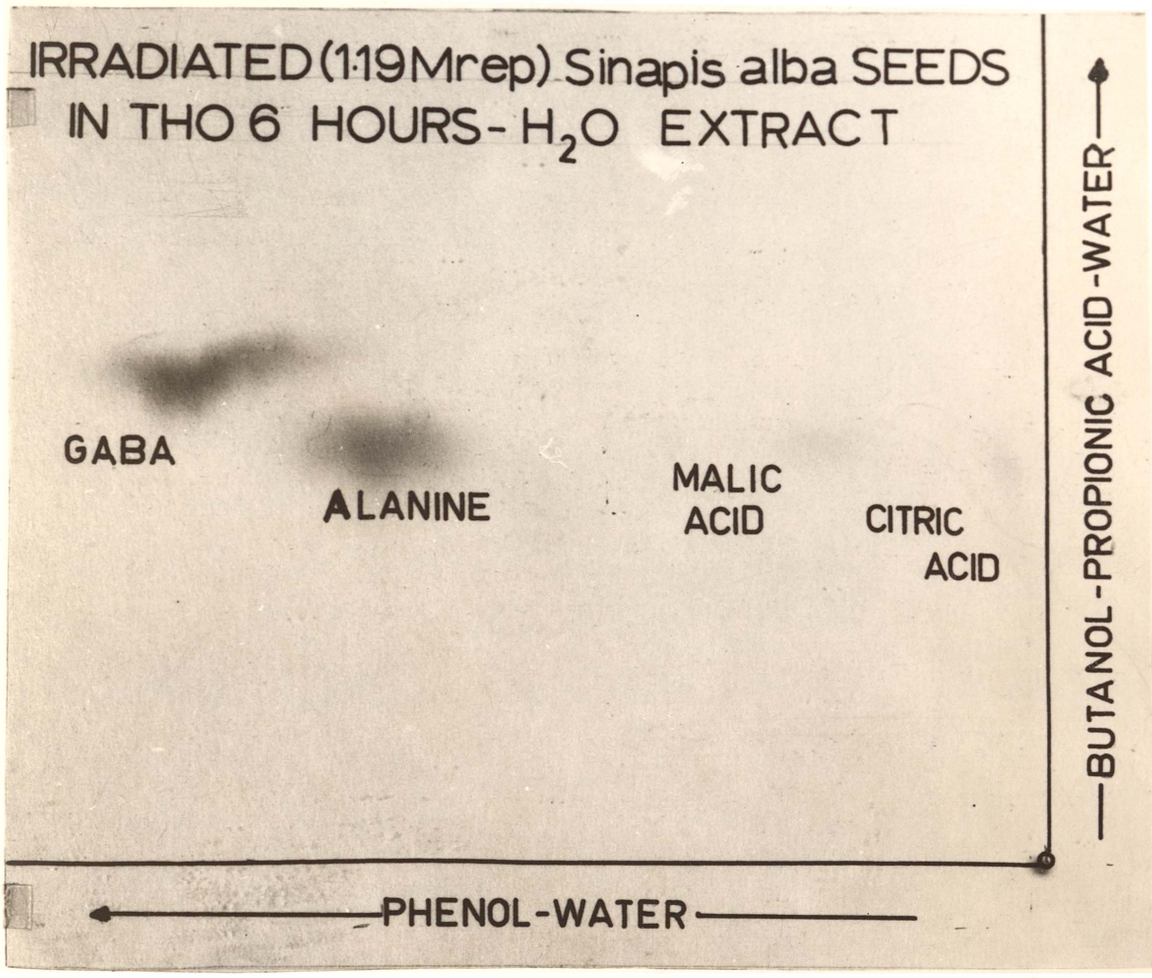
of the two inches of origin which had received the spray but not the substrate solution.

11-2 Results using Seed Extract

At first too much enzyme spray was used and this caused fluctuations in the origins of the chromatograms so that much tailing occurred. After the technique had been mastered runs without substrates gave excellent separation of the compounds contained in the water extract of the seeds. This technique therefore has the added possibility of being a rapid means of applying samples to the origins of many chromatograms. This would be a matter of setting up as many chromatograms as desired, covered by their shields and walking down the row, spraying the solution onto each origin. When substrates were used, however, the endogenous substances in the water extract were present at such high concentrations that few differences could be detected between the areas which had received substrate and spray, and those which had received the spray only.

It was discovered that if substrates were spotted onto the origin in two discrete well separated spots, then the products of the metabolism of the substrates could readily be distinguished from the compounds present in the spray. The latter compounds appeared as relatively faint streaks across the origin but metabolic products even if present in the spray showed discrete well-defined dark spots.

The unknown described in section 10 and called UVGB could barely be detected in the spray but formed intense spots with added GABA or glutamate. The R_F value for this compound was higher using this spray method than had been found previously. The average of 12 analyses gave a value of 0.77 in BPW solvent (as compared to the 0.65 found earlier). In the earlier runs UVGB had had a lower R_F than the other unknown, YPN


IRRADIATED (1·19 Mrep) Sinapis alba SEEDS
IN THO 6 HOURS - H_2O EXTRACT
GABA
ALANINE
MALIC ACID
CITRIC ACID
BUTANOL - PROPIONIC ACID - WATER
PHENOL - WATER

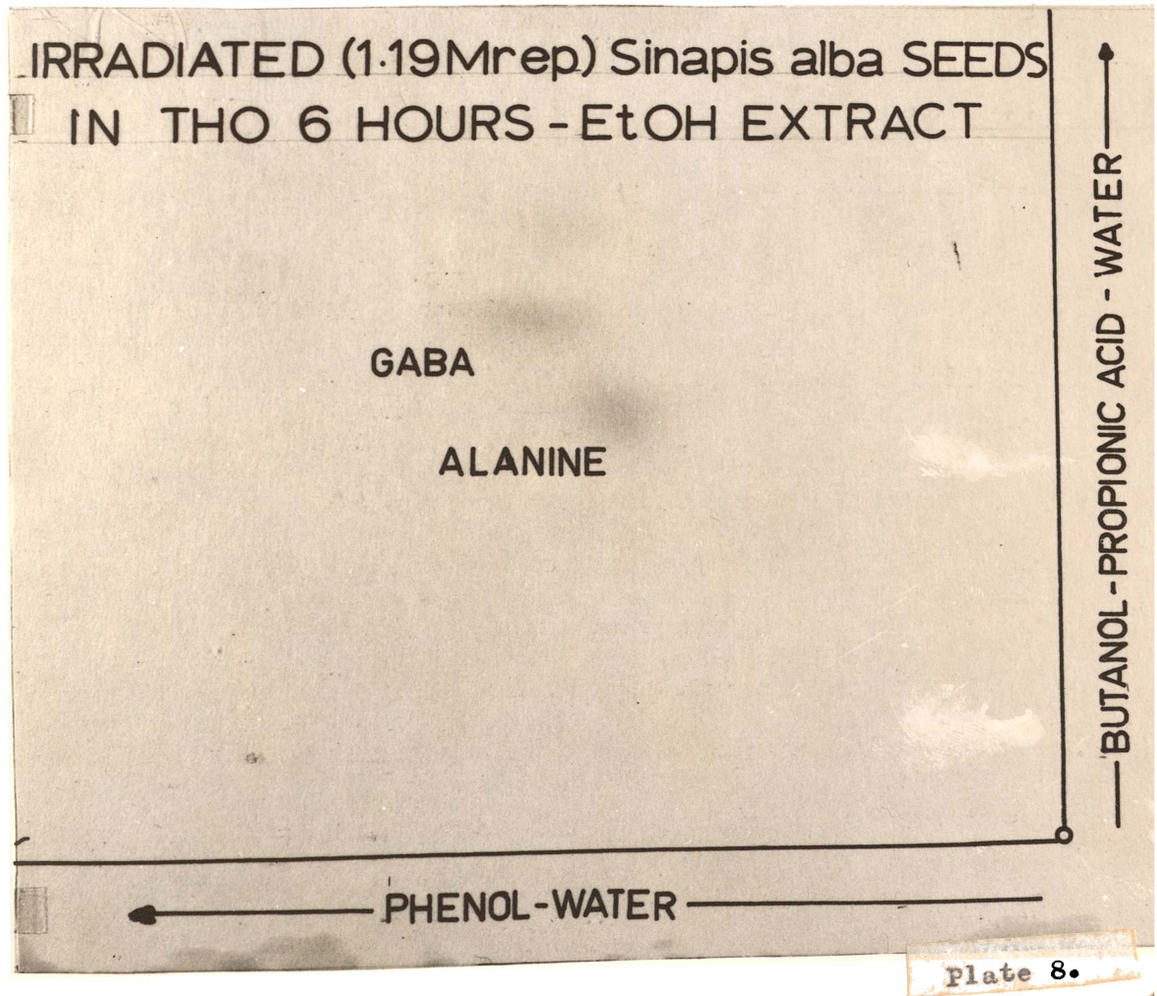

IRRADIATED (1·19 Mrep) Sinapis alba SEEDS
IN THO 6 HOURS - EtOH EXTRACT
GABA
ALANINE
BUTANOL - PROPIONIC ACID - WATER
PHENOL - WATER

Plate 8.

but this was not found to be true in these experiments, in which the two compounds ran together. These results could be due to either solvent effects or to movement of the origin during incubation. No band with similar UV response was detected in either of the standard samples of GABA or glutamate.

The unknown YPN was also found in much higher concentrations above the spots of application of GABA and glutamate. This had an R_F of 0.78 under these conditions and gave its normal ninhydrin reaction of yellow initially turning purple in 12-24 hours. A spot was also detected corresponding to GABA formed from the glutamate substrate. Higher concentrations of alanine were found to be associated with added GABA or glutamate substrate.

Chromatograms sprayed with mercurochrome or the sulphanilimide reagent to detect organic acids showed the presence behind glutamate of an organic acid with an R_F of 0.22 in BPW solvent. From the standard maps it is readily seen that most organic acids, including alpha-KG travel ahead of glutamate in this solvent. Those acids which travel behind glutamate are phosphoenolpyruvate and phosphoglycolic acid.

11-3 Discussion of Results

This method has given more data than the previous chromatographic method. Two additional conversions have been detected, the conversion of GABA and glutamate to alanine, and of glutamate to an organic acid possibly either phosphoenolpyruvate or phosphoglycolic acid.

This method does have great advantages for enzymatic analysis by chromatography, especially if the desired information is qualitative rather than quantitative. No attempt was made to use this method quantitatively, to calculate the amount of conversion of the metaholites

but the only difficulty which could be encountered would be ensuring uniform application of the spray to the origin.

The greatest advantage of the method is the ease of comparison between samples containing substrate and crude enzyme and those containing the crude enzyme preparation only. Once the technique of spraying has been mastered the compounds in the crude extract of seeds appear as faint lines parallel to the origin line. Compounds formed from the substrates appear as much darker and well-defined spots above the point of application of the substrate. In earlier runs a comparison was made between colours developed by spots corresponding to enzyme only and those corresponding to substrate + enzyme. This is much more difficult than comparing a colour to the background line formed behind it.

A second advantage is the small requirements for equipment. The first chromatographic method requires the use of micropipettes, in order that the concentrations of compounds in the sample and in the enzyme solution may be compared. In this novel enzyme assay such control is not required so long as the enzyme solution is applied evenly.

The preferable method of application was found to be sweeping movements in the direction of the long side of the rectangle through which the spray was applied.

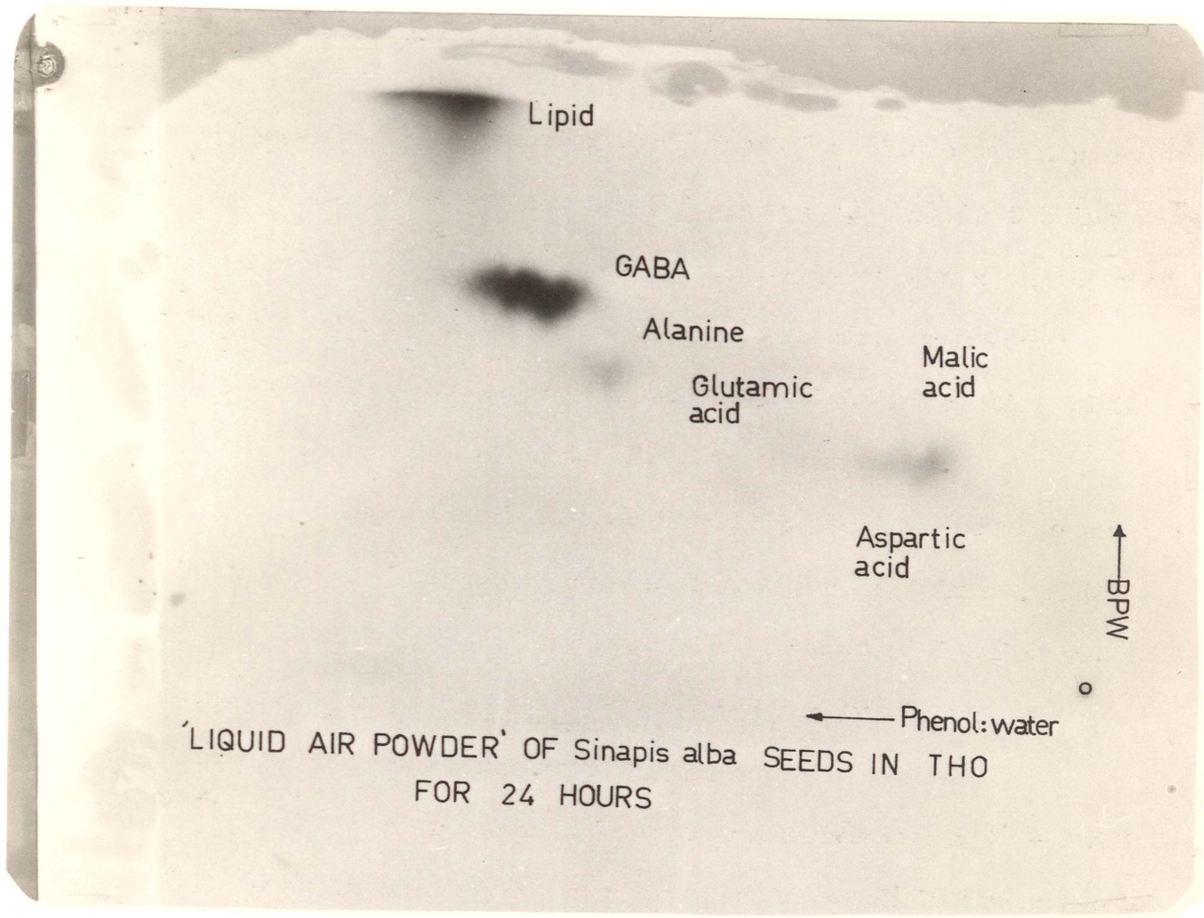
12. STORAGE OF C-14 COMPOUNDS

As mentioned in the discussion earlier radioactive compounds can be contaminated during storage from two main sources, the first being contamination by microbes which metabolise the C-14 compounds and convert them into mixtures of various substances and the second from decay of the material caused by the energy of the radiation interacting with other molecules and breaking bonds to form other compounds.

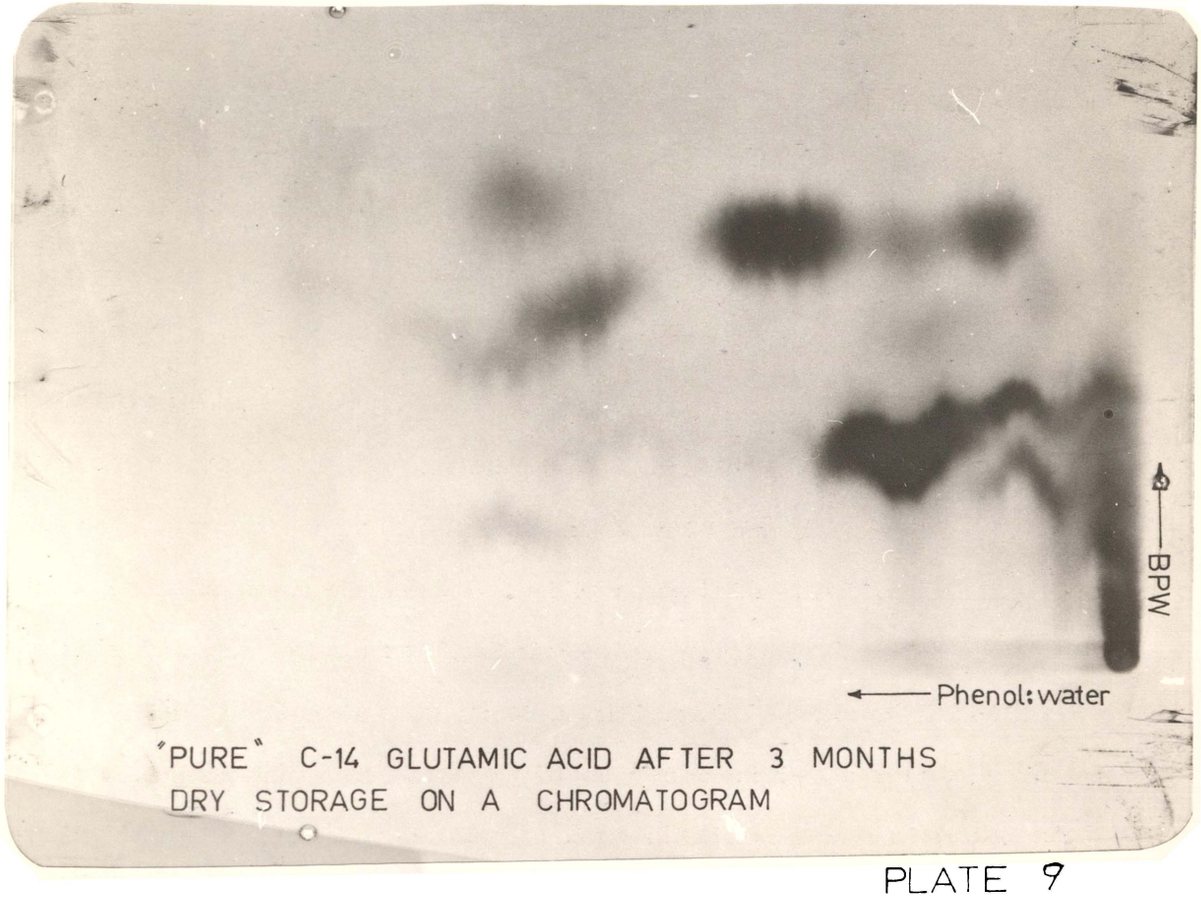
Initially the C-14 materials used for this thesis were made up in 10% isopropanol solution and stored under a layer of toluene. It was found that GABA stored in this manner formed a material which ran close to the solvent front for the chromatography solvents used. Glutamate also gave several bands.

After 12 months' storage in this manner the compounds C^{14}-glutamate, C^{14}-GABA and C^{14}-aspartate were cleaned by spotting each impure compound along the 1-D origin of a whole sheet of Whatman No.4 paper. This was then run in phenol water and exposed to photographic film. For GABA eight discrete bands were obtained, the major activity being in the band corresponding to GABA but a band, which was ninhydrin positive and corresponded closely to the position expected for glutamic acid contained an appreciable portion of the total activity. When this compound was cochromatographed with glutamate, it was found that the two spots did not correspond and hence the compound was not glutamate. With C-14 glutamate chromatographed in phenol:water 10 bands were formed, none of which could be shown to be GABA. The aspartate solution was the less contaminated and only contained three bands.

After the correct band had been identified the radioactive compound was eluted onto the origin of a second 1-D chromatograph and run in BPW solvent. Several bands were obtained for glutamate and GABA but only one for aspartate.



'LIQUID AIR POWDER' OF Sinapis alba SEEDS IN THO FOR 24 HOURS



"PURE" C-14 GLUTAMIC ACID AFTER 3 MONTHS DRY STORAGE ON A CHROMATOGRAM

PLATE 9

After this procedure the compounds were stored as strips on dried chromatograms, as it was thought that microbes would not cause contamination due to the dryness and that self-decomposition would be unlikely due to the large area occupied by each radioactive compound. After three months' storage in this manner a purity check on C^{14}-glutamate was undertaken, using the same solvents as those which had been used for the cleansing process.

The resultant chromatograph contained many spots and amply demonstrated the futility of the method of storage. The next method tried was to prepare solutions of the radioactive compounds, divide each solution into many test tubes, such that the activity in one tube was sufficient for one experiment, and to remove the solvent by freeze drying. This method proved more successful than the two tried earlier but sufficient decay still occurred to make tracer studies ambiguous.

The only way in which tracer studies using C^{14} compounds could give correct and nonambiguous results was found to be by cleansing the compound in the solvents to be used <u>immediately</u> before the desired experiment. This technique was used for the experiments described in the tracer section of this thesis. A 2-D chromatogram of the C^{14}-compound was prepared and run in phenol-water the day before the experiment. First thing the following morning it was run in BPW, exposed to film for 2-3 hours and the spot removed and eluted into the reaction vessel using distilled water. The ground-up seeds were then added and the tracer experiment carried out.

Unless a procedure such as that described is used the results of the tracer experiments using these C^{14}-metabolites just cannot be interpreted. It is also important to remember that contamination will occur after the experiment and so the chromatography, autography and co-chromatography must be carried out as rapidly as possible.

13. TRACER STUDIES

13-0. INTRODUCTION

The essential feature of the method involving tritiated water is that the non-exchangeable incorporation of tritium can occur only as a result of metabolic activity and hence incorporation of tritium may be used as an index of metabolism.

When C-14 compounds are used the label will be observed in those compounds formed which survive the freeze drying procedure and which are soluble in either water or ethanol. A label in the carbon skeleton will not be lost during extraction procedures by exchange with non-labelled carbon atoms.

13-1 Experimental Details

13-1.1 Tritiated Water

The supply of tritiated water was kept in a special fume cupboard. From the bulk source a working supply of about 1 ml was transferred to a small, tightly-stoppered vial. When required tritiated water was taken from this working supply using a Pasteur pipette. All manipulations were carried out on a tray covered in absorbant paper in the fume cupboard, and polythene gloves were always worn.

For the imbibition 5 seeds were placed in a centrifuge tube and one or two drops of tritiated water added. The tube was then tightly stoppered, and shaken until all seeds were wet. They were then left for the specified time and after this they were covered with absolute ethanol, tipped into an all-glass "Dual" tissue homogenizer. The volume of ethanol was made up to about 3 mls and the seeds ground for 4-5 minutes. The extract was then centrifuged and the clear supernatant layer decanted into a small pear-shaped flask with a B14 socket. A

second alcohol extract was obtained, followed by two water extracts in a similar manner. The water extracts were put into a separate pear-shaped flask.

For evaporation, the flasks were attached to one arm of an inverted, Y-shaped yoke; connected to a vacuum line. The other arm held a cylindrical receiver flask, immersed in liquid air. The extract was frozen by liquid air, and the tap leading to the vacuum line opened. The apparatus was evacuated to about 3 microns pressure using a mercury diffusion pump. When the pressure was sufficiently low, the tap was closed and the liquid air surrounding the extract was removed. On warming to room temperature the solvent and any volatile compounds present, passed over into the receiver. This procedure took about 3 hours for the alcohol extract and about 14 hours for the water extract.

A drop of the appropriate solvent was added to the dried extract, and the flask rotated to dissolve any compounds adhering to the walls. This solution was spotted onto the origin of the chromatogram using a fine glass capillary tube. The spot was dried using cool air from a hair dryer. For a particular extract several chromatograms were run, each bearing a different loading. With the lighter loadings resolution was improved but faint spots might not be observed.

The chromatograms were first run in phenol:water and then in Butanol:Propionic Acid:Water, using the technique of descending chromatography. Between developments the chromatograms were left overnight to allow them to dry thoroughly. One dimensional chromatograms were also prepared.

The tritiated spots were detected by scintillation autography. Four chromatograms were stapled onto a used sheet of film with two small pieces of filter paper containing appreciable activity in a form insoluble in toluene stapled in opposite corners to act as "markers".

This film was then placed in a shallow tray of scintillating liquid contained in the dark room. A sheet of X-ray film was placed over the chromatograms, care being taken to ensure that no air bubbles were trapped between the two layers. The lid of the tray was replaced and a heavy lead weight placed on top, to minimise evaporation of the toluene and the entry of stray light.

After 7 days the top film was removed and developed. After drying it was placed over the chromatograms and adjusted until the marker spots coincided with their images. Tritiated compounds were detected as dark spots on the film, the fainter spots being observed by holding the film at an angle and oscillating it slightly, against a white background.

The possible identity of a tritiated compound could be inferred from its position relative to other compounds by the use of standard chromatographic maps, and by its position relative to other compounds. The technique of co-chromatography was used to establish the identity of the compound. The centre of the spot was cut out in the form of an oblong with a point at one end. This minimises the risk of contamination and leaves an area of the spot on the original chromatogram to subject to a spray test. A detectable amount of pure compound was added to the excised strip bearing the spot suspected to be chemically identical to the added carrier.

The elution technique of Calvin and Wilson[333] was used to transfer the compounds onto the origin of a new chromatogram. The compounds were then co-chromatographed in the chosen solvents after which the scintillation autography procedure was repeated. The unlabelled compound was detected by spraying. The size, shape and position of this spot was then compared to that of the tritiated spot on the film. If these coincided in every detail chemical identity was inferred.

13-1.2 C-14 Compounds

The following C-14 compounds were used in this section.

Gamma-Aminobutyric-1-C^{14} Acid

$H_2NCH_2CH_2CH_2\overset{*}{C}OOH$ was purchased from New England Nuclear, Boston, Mass., U.S.A. This sample had a specific activity of 1.64 MCi/mM and was purchased as a crystalline solid. The radiochemical purity was reported as greater than 98%.

L-Glutamic Acid-C^{14}-(U) was purchased from the Radiochemical Centre, Amersham, England. This had a specific activity of 260 mCi/mM and a reported radiochemical purity of 98%. It was supplied in deoxygenated aqueous solution containing 2% ethanol.

L-Aspartic Acid-C^{14} (U) was purchased from the Radiochemical Centre, Amersham, England. This had a specific activity of 208 mCi/mM (1.49 mCi/mg) and a reported radiochemical purity of 99%. It was supplied in deoxygenated aqueous solution containing 2% ethanol.

The procedure used differed only slightly to that described above. For most experiments a "Liquid Air Powder" (LAP) of the seeds was used. This was obtained by freezing the seeds in liquid air and grinding them while they were still frozen. The LAP powder was stored in a desiccator.

The C^{14} compound was obtained in a chromatographically pure state immediately before the experiment by the method described previously. The use of a scintillator is not necessary for C-14 work as the energy of the beta radiation is sufficient to develop the film. Therefore in the autography procedure the film was placed over the chromatograms and stored for several days in a Kodak X-ray holder.

13-2. Tritiated Water Studies on Whole Seeds

Tritiated extracts were prepared and examined as described earlier. By comparing the labelling pattern of non-irradiated seeds with those which had received a dose of 5.08 Mrep of gamma-radiation it was hoped to determine whether or not enzyme systems had been inactivated or activated. After this particular set of seeds had recovered their ability to germinate a further series of tritiated runs were carried out to examine the recovered seeds for unusual metabolism caused by the radiation.

Seeds which had received 1.19 Mrep (one in four could germinate) and 5.08 Mrep of Co^{60} gamma-radiation, when treated with tritiated water for 6 or 24 hours incorporated tritium into fewer compounds than did seeds which had not been irradiated. Five seeds from each dose group and five non-irradiated seeds were killed by autoclaving in a domestic pressure cooker for 1 hour. Extracts of these seeds after exposure to tritiated water did not contain any non-labile tritium atoms. No labelled compounds were detected on the chromatograms showing that dead seeds whose enzyme systems have been inactivated lack the ability to incorporate tritium into metabolites.

The compounds labelled by irradiated seeds were GABA and alanine in the alcohol extract and GABA, alanine, malic acid and citric acid in the water extract. The compounds labelled by non-irradiated seeds in a similar time period were GABA, alanine, glutamine, lactic acid, glutamic acid, sucrose, aspartic acid, malic acid and citric acid. Typical chromatograms are shown in the plates and the results are tabulated in Table 13-2.1.

Once recovery of this particular set of seeds had occurred, tritiated runs of 6, 24 and 30 hours were carried out to compare the metabolism of the irradiated seeds which germinated normally to that of

TABLE 13-2.1

Tritiated Metabolites Occurring in Irradiated and Non-irradiated *Sinapis alba* seeds before Recovery [338]

% Germination	96		96		26		0		0	
Dose (Mrep)	0.00		0.00		1.19		5.08		5.08	
Time in THO (hrs)	6.0		24.0		6.0		6.0		24.0	
Metabolites Labelled	H_2O	EtOH	H_2O	EtOH	H_2O	EtOH	H_2O	EtOH	H_2O	EtOH
GABA	+	+	+	+	++	++	+++	+++	+++	++
Lactic acid	++	+++	+++	+++						
Alanine	+	+	+++	++	++	++	++	+++	++	++
Glutamine	++		+++							
Sucrose	++	++	+++	+++						
Glutamic Acid	+++		+++							
Aspartic Acid	+		++							
Malic Acid	+++		+++		+		++		+	
Sugar Phosphates	+		++							
Citric acid			+		+		++		+	

\+ ++ +++ Increasing relative intensity

non-irradiated seeds. These results are tabulated in Table 13-2.2. The intensity of spots on the chromatograms have been indicated but due to the nature of the method used no attempt has been made to make a quantitative survey and intensity comparisons are only strictly valid for comparison of spots on the same chromatogram. The metabolism of the irradiated seeds after recovery was similar to that of non-irradiated seeds.

The unknown, YPN, already mentioned in sections 10 and 11 was found in extremely high concentrations but unlabelled on the chromatograms of irradiated but not recovered seeds, in which GABA, alanine, citrate and malate were the only labelled compounds. Once again it appears to be associated with the metabolism of GABA. It was also present on

TABLE 13-2.2

Tritiated Metabolites Occurring in Irradiated and Non-Irradiated Sinapis alba Seeds after Recovery [338]

% Germination	94%						98%						97%					
Dose (Mrep)	0.0		0.0		0.0		1.19		1.19		1.19		5.08		5.08		5.08	
Time in THO (hrs)	6.0		24		30		6.0		24		30		6.0		24		30	
Metabolites labelled	H_2O	EtOH	H_2O	EtOH	H_2O	EtOH	H_2O	EtOH	H_2O	EtOH	H_2O	EtOH	H_2O	EtOH	H_2O	EtOH	H_2O	EtOH
GABA	+	+	+	+	++	++	+	++	+	+	+	+	++	++	++	++	+	++
Lactic acid	++	+++	+++	+++	+++	+++		++	+++	+++	+	++	++	+++	+++	+++	+++	+++
Alanine	+	+	+++	++	+++	+++	++	+++	+++	+	+	+	++	++	+++	++	++	+++
Glutamine	++		+++		+++		+++		+++		++	t	+		+++	++	+++	+++
Sucrose	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	++	+++	+++	++	+++
Glutamic acid	+++		+++		+++		+++		+++		+++		++		+++		+++	
Aspartic acid	+		++		+		+		++		++		+		++		++	+
Malic	+++		+++		+++		+++		++		++		++		++		++	+
Sugar phosphates	+		++		++				+		+		+		++		++	
Citric acid			+		++		+		++		+		++		+		+	

+ ++ +++ Increasing relative intensity

t trace

the chromatograms of non-irradiated seeds and recovered seeds but at comparatively low concentrations.

13-3 Tritiated Water Studies on the Liquid Air Powder of Sinapis alba Seeds

Difficulty was experienced in enabling sufficient C^{14}-substrate to enter whole seeds for studying the metabolism of these compounds. It was therefore decided to grind the seeds while in liquid air and to study the metabolism of these amino acids when exposed to this powder in a moist state.

The metabolism in tritiated water of the Liquid Air Powder (LAP) of Sinapis alba seeds was examined, as a means of testing whether or not the grinding procedure and resultant disorganisation had destroyed the enzyme systems of the seed.

The labelling pattern of ground-up seeds was less reproducible for a given time period than that of whole seeds, and this effect was most noticeable for the shorter time periods. The results shown in Table 13-3.1 were those obtained by myself but Vickers[334] has obtained differing results, supposedly for the same time intervals. Within 10 mins of imbibition Lipid, GABA and aspartate were always labelled, and occasionally traces of labelled glutamate were present. This is equivalent to the pattern obtained for the imbibition of whole seeds for only 5 mins. except that labelled lipid is not formed in whole seeds until 180 mins after the beginning of imbibition. Vickers[334] has reported 10 min. runs giving labelled Lipid, GABA, Alanine, Glutamate and Aspartate. This result is equivalent to 10 mins imbibition of whole seeds with the addition of labelled lipid.

After 30 minutes imbibition the labelled metabolites found in the LAP are Lipid, GABA, Aspartate, Alanine, Glutamate, Malate and Citrate.

With whole seeds for the same time interval lactic acid and sucrose are labelled in addition to the compounds mentioned but the lipis is not. After only 15 minutes imbibition whole seeds give an equivalent labelling pattern to the THO at 30 mins but without the labelled lipid.

The labelling pattern of the LAP shows little change between 30 mins and 24 hours imbibition. In the latter case citrate was not detected in any experiments.

TABLE 13-3.1

Labelled Compounds Formed on Imbibition of the "Liquid Air Powder" of Sinapis alba Seeds in Tritiated Water

	LAP			Whole Seed		
Time in THO	10mins	30mins	24hrs	10mins	30mins	24hrs
Labelled Metabolites						
Lipid	+++	+++	+++			+
GABA	++	++	+++	+	+	+
Aspartate	++	+++	++	+	+	++
Alanine		+	+	+	+	+++
Glutamate		+	t	+	+	+++
Malate		t	t		+	+++
Citrate		+			+	+
Lactic Acid					+	+++
Glutamine						+++
Sucrose					+	+++
Sugar phosphates						++

It appeared to have been utilised completely and the metabolic pool depleted.

After 30 mins the unknown compound YPN, was present in an unlabelled form at large concentrations, higher than that found at any stage in whole non-irradiated seeds. Glutamine was not evident in either a labelled or unlabelled form after 30 minutes and 24 hours imbibition in THO. It was, however, present but unlabelled after

10 minutes imbibition and hence, appears to have been completely utilized.

The patterns obtained for the labelling of compounds in the LAP closely resemble those for whole seeds in the early time intervals but does not develop the complex pattern characteristic of whole seeds in longer intervals. Lactic acid, glutamine, sucrose and sugar phosphates do not become labelled. Two metabolites thought to be extremely important for germination, glutamine and citrate are depleted within 30 mins. and 24 hours imbibition.

The labelled compound referred to as "lipid" in this section runs with the solvent front in both BPW and phenol-water solvents, a position characteristic of lipids. This compound must be insoluble in toluene as this was used for the scintillating liquid. For one of the runs of LAP which had imbibed THO for 10 minutes a strip of the chromatogram was sprayed before being exposed to film. The ninhydrin positive compounds were marked and they did not include the lipid. When this chromatogram was withdrawn from the tank a vivid purple on the chromatogram corresponded exactly to the labelled spots of "lipid". It was therefore assumed that this compound contained two parts, one of lipid-like material and the other an amino acid. This "lipid" is soluble in ethanol and is stable in Butanol:Propionic Acid:Water solvent but not in phenol:water. In the latter solvent it decomposes apparently while the chromatogram is being dried and when run in a second direction in Butanol:Propionic Acid:Water one labelled spot does not move while another moves with the solvent front. Similar results are obtained if the second solvent is phenol:water. In butanol:propionic acid:water (BPW) solvent only no decomposition has ever been observed. The lipid was eluted onto the origin of a new 1-D chromatogram and run in phenol:water alongside several standard amino acids. The lipid formed

an active spot travelling with the solvent front and a ninhydrin positive spot whose position closely approximated that of serine.

13-4 Metabolism of C^{14}-Aspartate

The "Liquid Air Powders" of both non-irradiated and irradiated (3.5 Mrep) Sinapis alba seeds were allowed to imbibe in the presence of C^{14} aspartate for two time intervals. The results are shown in Table 13-4.1.

TABLE 13-4.1

The Labelled Compounds Formed when the Liquid Air Powders of Irradiated (3.5 Mrad) and non-irradiated Sinapis alba Seeds were Imbibed with Universally Labelled C^{14} Aspartate

Dose (Mrad)	3.5		0.0	
Time C^{14}-Asp (hrs)	1	3	1	3
Labelled Metabolites				
Aspartate	+++	+++	+++	+++
Succinyl-arginine (?)(U1)				t

No conversion of aspartate has occurred in the irradiated seeds. In 2-D chromatograms for the non-irradiated seeds aspartate was the only spot observed. On 1-D chromatograms, however, an extremely weakly labelled spot was observed. This spot was ninhydrin positive, giving a mauve colour and had an R_F below aspartate in BPW solvent as well as in Butanol:Pyridine:Water (1:1:1) and from R_F values has been tentatively identified as succinyl-arginine.[46,331,335] Attempts to cochromatograph this sample were unsuccessful due to the low activity.

These results show that the LAP of Sinapis alba seeds apparently lacks the ability to metabolise aspartate in short time intervals although this compound was rapidly labelled in all experiments with tritiated water. After 3 hours' imbibition a compound thought to be succinyl

PLATE 10

Plate 10.

arginine was very lightly labelled.

13-5. The Metabolism of Gamma-aminobutyric-1-C^{14} Acid

$H_2NCH_2CH_2CH_2\overset{*}{C}OOH$

Ground-up seeds were allowed to imbibe in the presence of gamma-aminobutyric-1-C^{14}-acid for one and four hours and the metabolism examined by chromatography.

The results are shown in Table 13-5.1.

TABLE 13-5.1

Labelled Compounds Formed When the Liquid Air Powders of Irradiated (3.5 Mrad) and Non-irradiated Sinapis alba Seeds were Imbibed with Gamma-aminobutyric acid-1-C^{14}.

Dose (Mrad)	3.5		0.0	
Time C^{14}-GABA (hrs)	1	4	1	4
Labelled Metabolites				
GABA	+++	+++	+++	+++
Flavonoids	+	+	+	+
U2-unknown		+		+
Alanine		+		t

The label found in GABA remained high. The flavonoids, detected by exposure of the chromatogram to concentrated ammonia, were lightly labelled. A sample of the pure GABA used in this experiment when chromatographed did not give any label in this region. After 4 hours' imbibition a label was detected in a compound with a low R_F of 0.07 in BPW solvent. This compound was ninhydrin positive and was only detected in 1-D chromatograms.

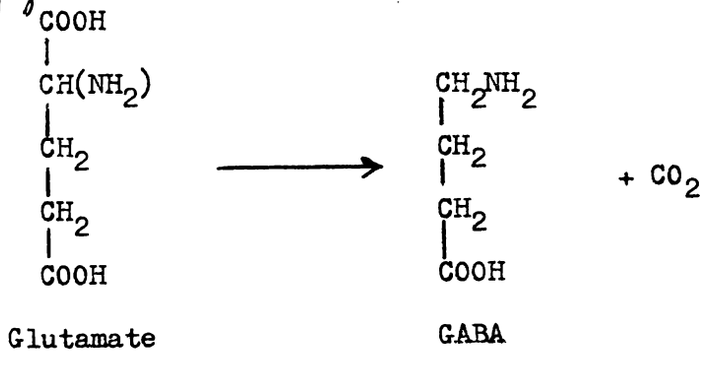
On spraying the chromatograms with ninhydrin large concentrations of lightly labelled alanine were found. The label of GABA must remain

and hence these large amounts of alanine must be formed from C1 of the GABA. The concentration of unlabelled alanine was high after 1 hour and of labelled alanine after 4 hours of imbibition.

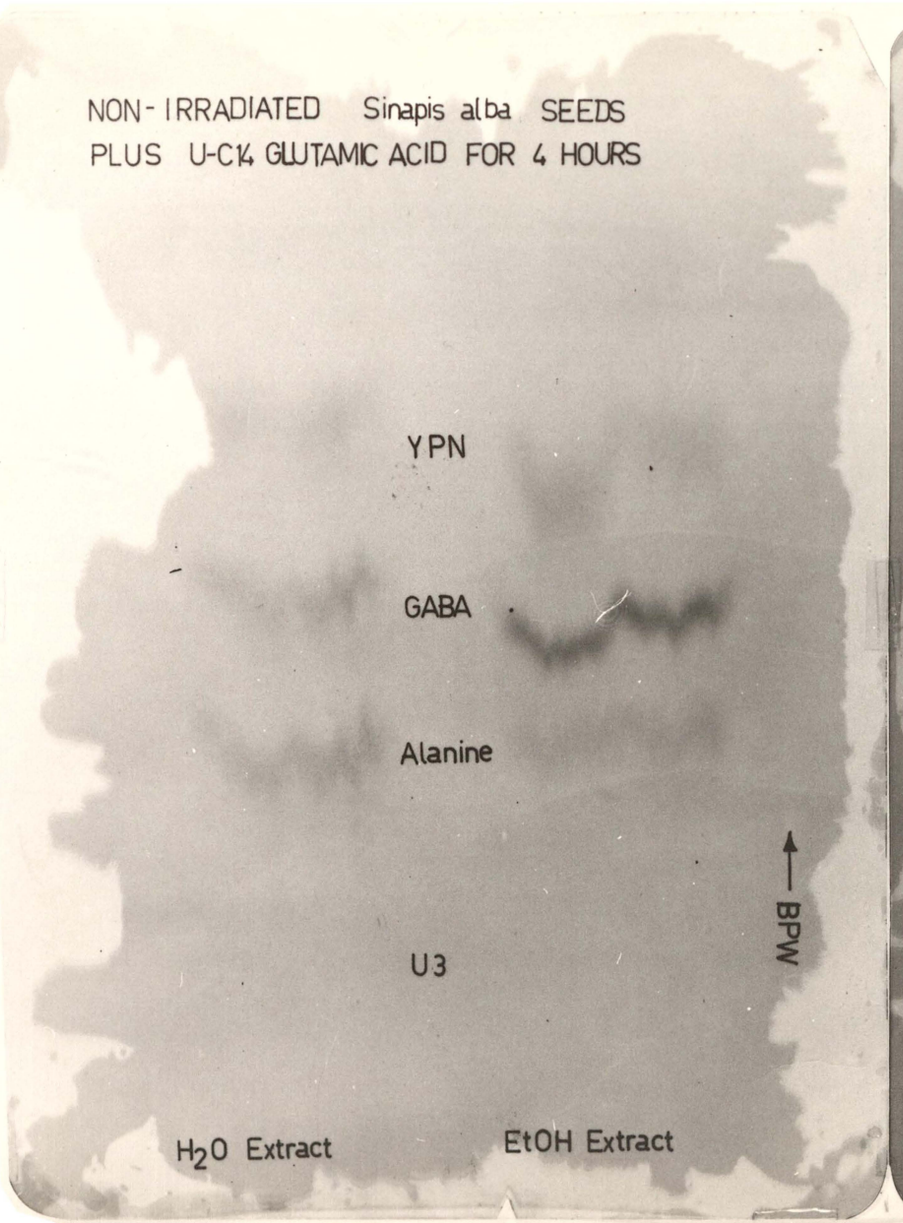
Very few ninhydrin positive compounds have such low values for their R_F in this solvent as that of the labelled unknown. The main group with similar values is that of the dipeptides.

13-6 The Metabolism of C^{14}-Glutamate

When the LAP powder of irradiated and non-irradiated seeds was allowed to imbibe in the presence of universally labelled C^{14}-glutamate for short time intervals the only other labelled compound observed was GABA. Labelled GABA examined in section 13-5 did not give rise to glutamate, and hence this reaction cannot be reversible in seeds. Pyridoxal phosphate is the coenzyme for the reaction, in animals, plants, yeast and bacteria.[332]



After 4 hours' imbibition the extract from the irradiated seeds which lacked the ability to germinate gave more labelled compounds than did the non-irradiated seeds. The results are shown in Table 13-6.1. In the irradiated seeds the lipid was heavily labelled but no label could be detected in that of the non-irradiated seeds. In both cases, however, a slight yellowy colouration of the paper was visible corresponding to the position of the lipid and on spraying with ninhydrin


NON-IRRADIATED Sinapis alba SEEDS
PLUS U-C14 GLUTAMIC ACID FOR 4 HOURS
YPN
GABA
Alanine
U3
BPW
H2O Extract
EtOH Extract

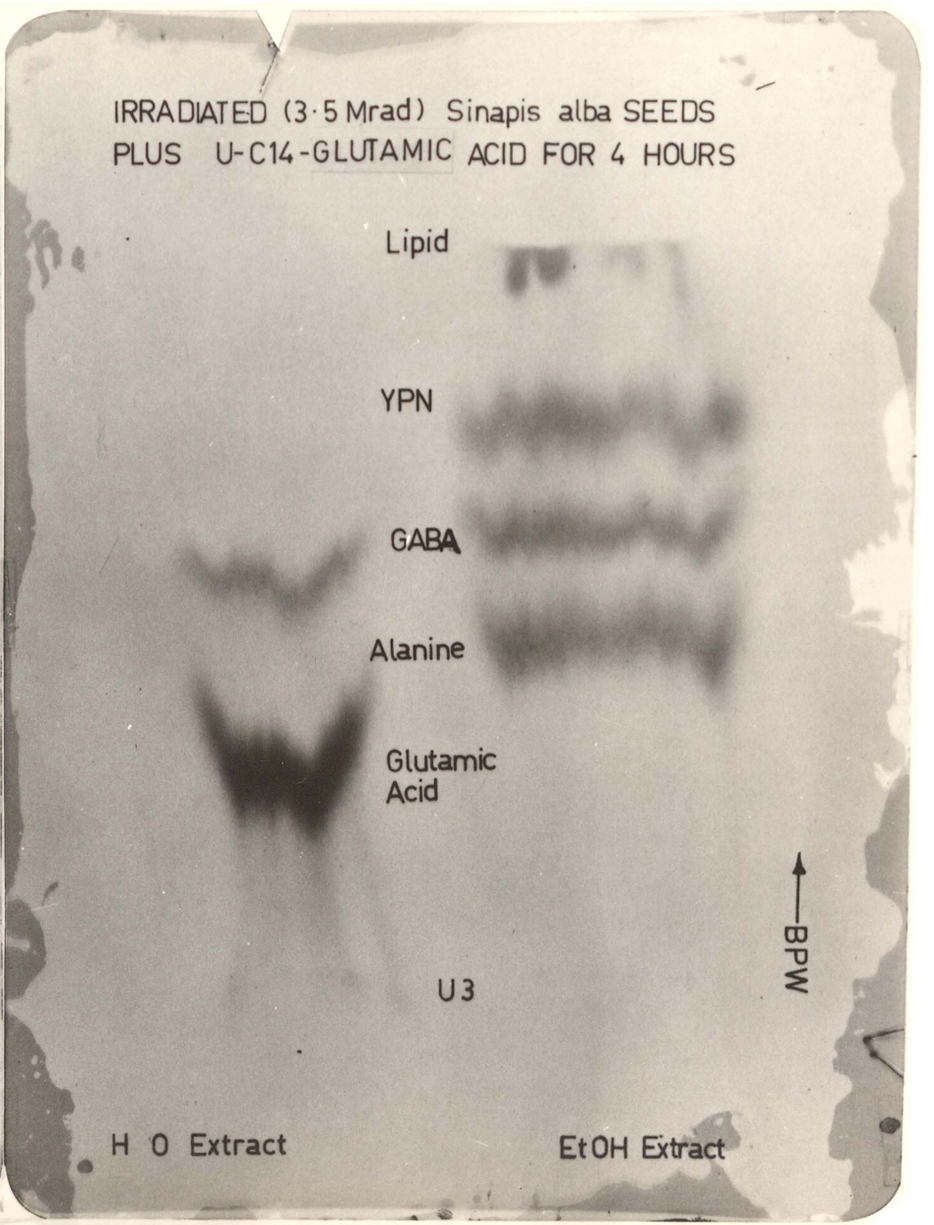

IRRADIATED (3·5 Mrad) Sinapis alba SEEDS
PLUS U-C14-GLUTAMIC ACID FOR 4 HOURS
Lipid
YPN
GABA
Alanine
Glutamic Acid
U3
BPW
H O Extract
EtOH Extract

PLATE 11

TABLE 13-6.1

Labelled Compounds Formed when the Liquid Air Powders of Irradiated and Non-Irradiated Sinapis alba Seeds were Imbibed with Universally Labelled Glutamate.

Dose (Mrad)	3.5			0.0		
Time C^{14}-Glu (hrs)	½	1	4	½	1	4
Labelled Metabolites						
GABA	++	+++	++	++	+++	+++
Glutamate	+++	++	+++	+++	+	
Lipid			++			
"YPN"			++			++
Alanine			++			++
U3-unknown			+			t

the lipid gave a definite darker yellow colouration which turned purple within 24 hours. On the chromatograms for the irradiated seeds this colouration closely corresponded to the shape of the labelling pattern. The unknown YPN and alanine were labelled in all extracts.

For the irradiated seeds the most intense label was contained in glutamic acid and the intensity of the labels in YPN, GABA, alanine and lipid were similar. In the non-irradiated seeds no labelled glutamate could be detected and the most intense label was contained in GABA.

Another unknown was found in this experiment. It was very lightly labelled in the non-irradiated seeds and more heavily in the irradiated seeds. In both cases it corresponded to an intense purple band after spraying with ninhydrin. As mentioned earlier very few compounds which are ninhydrin-positive have such low values for R_F's in acidic solvents. The R_F of this compound which could only be detected after 1-D chromatography in phenol:water was always higher than that

found in the C^{14}-GABA extracts, the value ranging between 0.12 and 0.14. It is likely, however, to be the same compound, because GABA was formed so rapidly from the glutamate any further conversions of GABA which can occur would be expected to be observed in this experiment.

13-7 Discussion of Results

Using tritiated water the metabolism of irradiated seeds did not after 6 or 24 hours show the complex pattern typical of non-irradiated seeds. Only GABA, alanine, malic acid and citric acid were labelled, whereas non-irradiated seeds labelled GABA, alanine, glutamine, lactic acid, glutamic acid, sucrose, aspartic acid, malic acid and citric acid in the same time intervals. Using the C^{14}-substrates aspartate, glutamate and GABA and "Liquid Air Powders" of the irradiated or non-irradiated seeds, however, there was very little observable qualitative difference in the metabolism of these seeds.

The act of grinding the seeds would destroy many membrane systems which in the whole seeds would require active transport for the passage of substances across them. The metabolism of the LAP of non-irradiated seeds as shown using tritiated water as a tracer, differed from that of whole seeds by leading to the formation of labelled lipid in short time intervals and by the non-appearance of labelled sucrose, glutamine or lactic acid. Most cellular membranes would be destroyed in the grinding process and hence cell division would not occur in the LAP. There are, therefore, two possibilities for the differences observed between the metabolism of liquid air powder and whole seeds. The first is that destruction of the organization on grinding has caused the loss of close relationships between enzymes and their substrates and the second is that reactions which lead to the formation of labelled sucrose, glutamine and lactic acid are associated with cell division only.

As discussed earlier it is widely accepted that DNA does act as a primary target for radiation. Severe damage to the DNA would prevent cell division. Irradiation can also damage membrane systems in two possible ways (a) such that active transport is not required and substances can pass through freely, or (b) such that the active transport system is blocked. Hence the effects of irradiation could be similar to the effects of merely grinding seeds. Irradiated seeds did not label glutamate or aspartate as did the LAP of non-irradiated seeds although the lack of labelled sucrose, glutamine and lactic acid was common to both. Using the C^{14} substrates (GABA, glutamic acid and aspartate) no differences could be observed between the metabolism of ground-up irradiated or non-irradiated seeds. It therefore seems likely that the damage on irradiation is similar to that caused by grinding the system. In the whole irradiated seed the tritiated water might be able to pass through certain membranes but not others in such a way that glutamate and aspartate are not labelled and at the same time disruption of organization or lack of cell division prevents the formation of labelled sucrose, glutamine and lactic acid.

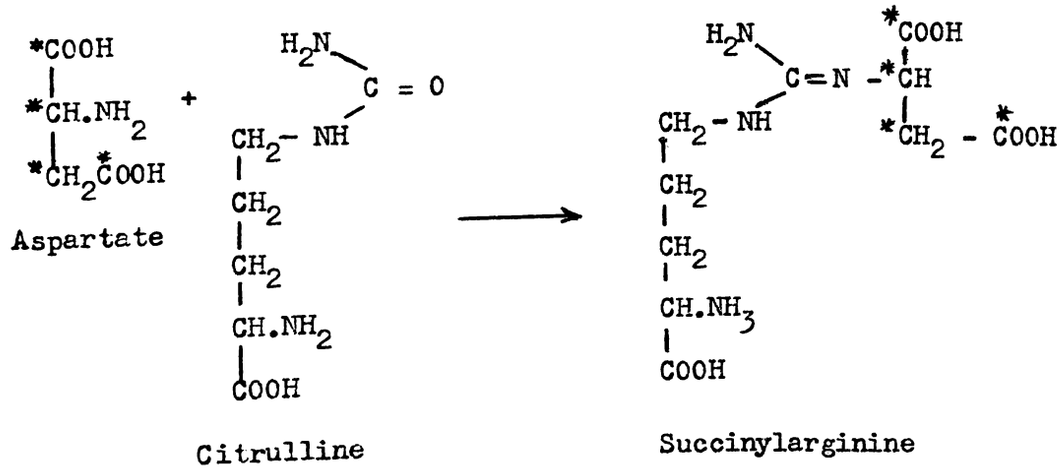
Other possibilities do exist for the effects of both irradiation and grinding on the metabolism of seeds. The formation of sucrose has been postulated to be caused by growth hormones passing from the embryo into the endosperm and activating this synthetic system. In both cases loss of organisation could disrupt these control processes but in irradiated seeds the additional factor, i.e. the radiosensitivity of auxin synthesis is also present.

Destruction of DNA on irradiation would also be expected to prevent the formation of m-RNA and, hence, of new proteins. The metabolism of irradiated seeds could therefore reflect those enzymes which are present in the dry seed in either an active or inactive form and the lack of

labelling in the remaining compounds could be due to the inability for the de nova synthesis of the enzymes required for their formation.

The lipoamino acid seems to be closely connected with both the act of grinding the seeds and with the metabolism of glutamate. It was initially discovered after the imbibition of ground-up seeds in tritiated water and finally found in very large quantities in ground-up seeds which had been incubated with universally labelled C^{14}-glutamate. It is therefore likely that the "amino acid" bond is closely related to glutamate, alanine, GABA and YPN, all ninhydrin positive substances found to be labelled after incubation with C^{14}-glutamate. The lipoamino acid formed during the glutamate experiments gave the ninhydrin reaction of YPN, namely, an initial yellow colour turning purple within 12-24 hours, whereas earlier experiments had shown a delayed reaction leading to purple only or an immediate purple colouration. These results do suggest the possibility of several amino acids being involved.

Metabolism of seeds in the presence of aspartate did not lead to the formation of many labelled compounds. After 3 hours the only labelled compound other than aspartate itself was a ninhydrin-positive compound (U1) with a low R_F in BPW solvent. This compound was tentatively identified as being succinylarginine which could be formed by the reaction:



Earlier experiments (section 10) had suggested that added aspartate was associated with higher concentrations of alanine and GABA and could be involved in transamination during the very early stages of germination. No evidence for these conversions was found in the C-14 experiments. It is possible that aspartate is involved in some control mechanisms involving the metabolism of GABA and alanine. At the relatively high molar quantities of aspartate used in section 10 the enzymes for the further metabolism of these two compounds could be blocked whereas using tracer quantities of the radioactive material they were unaffected. Such control mechanisms could not, however, explain the manometer results of Vickers.

Ground-up seeds incubated with gamma-aminobutyric acid-1-C^{14} rapidly labelled the flavonoids and at longer time intervals alanine and a ninhydrin positive unknown, U2.

The biosynthesis of the flavonoids is intimately connected to the shikimic acid pathway illustrated in Figure 13-7.1. The carbon skeleton of phenylpyruvate is essentially derived from one molecule of erythrose-4-phosphate and two molecules of phosphoenolpyruvate. The phenylpyruvate is converted to cinnamic acid which condenses with two molecules of malonyl CoA and one of acetyl CoA to form the C_{15}-intermediate. Various alterations to the structure of this compound lead to the formation of the flavonoids. The flavonoids have an indirect function as regulators of plant growth. Some, e.g. p-coumaroyl-triglucoside are inhibitors of the enzyme indoleacetic acid oxidase while the majority (e.g. the quercitin glycosides, maringenin and apigenin-7-glucoside) are essential cofactors for this enzyme.

It is also of interest that a branching point in the shikimic acid pathway leads to the formation of indole acetic acid (auxin) which is extremely important during germination; and also to the formation

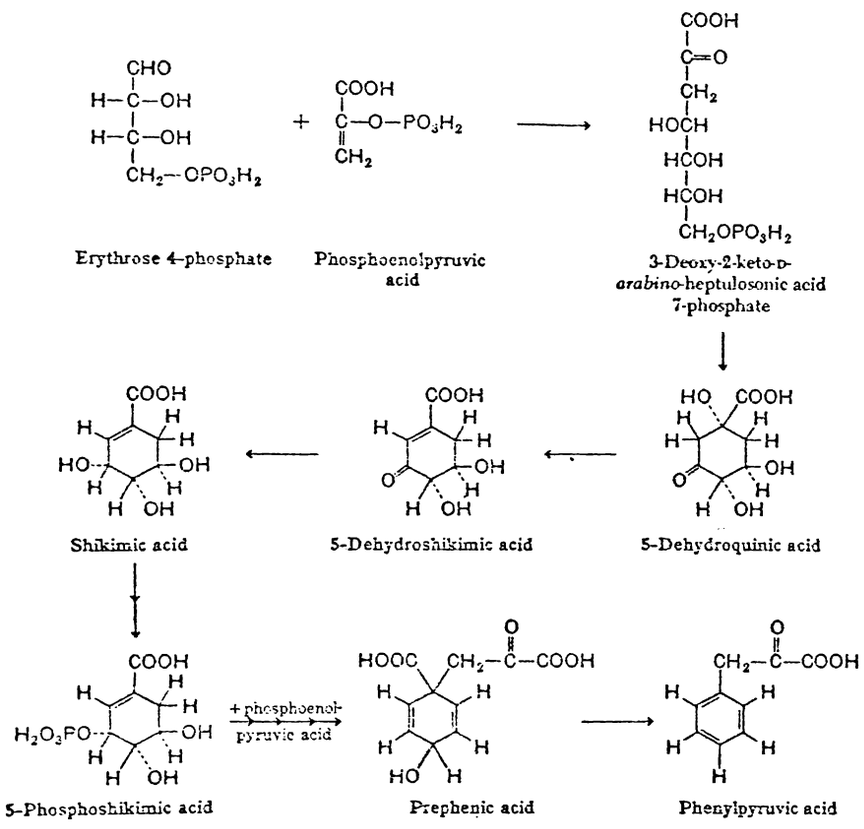


FIG. 13-7.1.

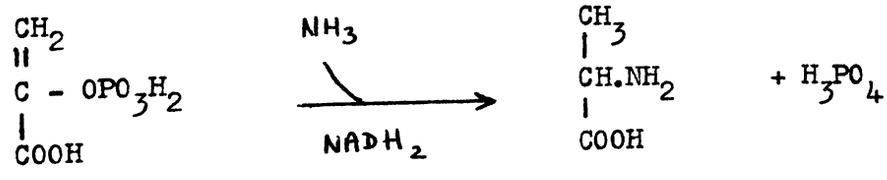
THE BIOSYNTHESIS OF PHENYLPYRUVIC ACID.

of Sinapic acid which exhibits blue-green fluorescence in UV light and has an R_F similar to that of the unknown UVBG shown to be associated with added ^{G}ABA in section 10.

As added GABA-1-C^{14} led to the formation of labelled flavonoids this atom must be involved in the formation of these compounds. It is unlikely to be incorporated via the sugar and hence GABA is probably involved with the formation of phosphoenolpyruvate, malonyl CoA or acetyl CoA. Acetyl CoA carboxylase which catalyses the carboxylation of acetyl CoA to form malonyl CoA is particularly rich in seed embryos. It seems more likely that GABA is a direct source of phosphoenol pyruvate rather than acetyl CoA as alanine has been shown to be closely related to the metabolism of both these compounds.

*COOH | CH_2 | CH_2 | CH_2NH_2	COOH | C = O | CH_3	COOH | $COPO_3H$ || CH_2	COOH | $CH.NH_2$ | CH_3
GABA	Pyruvic acid	Phosphoenol pyruvate	Alanine

Alanine can be formed by transamination from pyruvic acid. However, kinetic studies on photosynthetic tissues have shown the presence of an alternative pathway for the formation of alanine. This involves the reductive amination of phosphoenolpyruvate.



Phosphoenolpyruvate

Alanine

The formation of labelled flavonoids and alanine on incubation with GABA-1-C^{14} shows that GABA is converted to some three-carbon compound, postulated to be phosphopyruvic acid which contains at least the carboxylic acid carbon of the GABA. This compound was not itself detected on the chromatograms but it could be bound to the particulate matter and lost on centrifugation, unstable under the experimental procedures, or very rapidly metabolised on formation.

The unknown detected in these experiments (U2) had a very low R_F and gave an intense purple with ninhydrin spray. Very few ninhydrin positive compounds have been reported to travel in this region. The main group which does possess this property is that of the dipeptides. Gamma-aminobutyrylhistidine (homocarnosine) has been isolated from memmalian brain and human urine. An enzyme from chick muscle catalyzes the synthesis of gamma-aminobutyrylhistidine and gamma-aminobutyryl-arginine.[337] It therefore seems possible that this unknown is in fact a dipeptide of GABA and some other amino acid.

The unknown detected in the experiments involving universally labelled glutamate (U3) is probably identical to that discussed above. Labelled GABA was rapidly formed from the added glutamate and hence the products of its metabolism would be expected after 4 hours' imbibition. The alanine labelled in these experiments would acquire a label in the same manner as discussed earlier. In the experiment with glutamate the unknown YPN was obtained in a labelled form for the first time. If this compound is associated with the metabolism of GABA (as shown earlier) and not directly with glutamate, then YPN must be formed from C-2, 3 or 4 of GABA as it was <u>not</u> labelled using GABA-1-C^{14}

Labelled glutamate was not formed when seeds were incubated with C^{14}-GABA. It is therefore most unlikely that the action of glutamic decarboxylase is reversible.

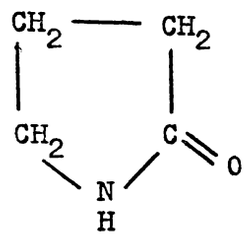
14. DISCUSSION OF THE RESULTS OF THIS THESIS

14-1. The Metabolism of Germination

Little evidence has been found for the operation of the TCA cycle during germination. Labelled ketoacids were not found when seeds were incubated with C^{14} amino acids. GABA did not appear to be converted to succinic acid but rather to be involved in the metabolism of mainly ninhydrin positive or complex aromatic compounds. Transaminases were shown to decrease during imbibition. If a supply of keto acids was required, these could be supplied by transamination or deamination of amino acids formed by the hydrolysis of proteins but this does not seem to be true.

The only three organic acids which do appear to be important during germination are malic acid, citric acid and lactic acid, all of which are involved in glycolysis or the glyoxylate cycle. The initial reactions of germination involve amino acids and in particular the irreversible conversion of glutamic acid, normally considered to be an extremely important metabolite, to GABA.

GABA must play a central role in the metabolism of seeds. The results reported here imply its participation in the pathway leading to the formation of aromatic compounds which could include growth hormones. It could also act as a donor of nitrogen atoms in the synthesis of nucleotides and related compounds. These atoms are normally supplied by glutamine which, although found labelled in both LAP and whole seeds incubated with THO was not formed from the added C^{14}-glutamate during the first 4 hours of imbibition. GABA could also be involved in the synthesis of pyrollidine rings. Formation of an internal amide would form the compound:



which is a pyrollidine. Succinate has been shown to be involved in the synthesis of pyridine rings (e.g. in nicotine) in plants. During germination it is possible that GABA directly supplies these two carbons, not succinate. Some evidence was obtained in this work for the conversion of all of the amino acids studied ultimately to GABA. For aspartate this appeared to be via alanine, probably formed by decarboxylation. The absence of conversion of C^{14}-aspartate to these compounds compared to that of non-labelled aspartate could be due to the larger quantities used in the latter case, or to an isotope effect. The conversion of alanine to GABA was shown to be readily reversible and to involve the carboxylic acid carbon of GABA. A similar reaction was not found in any publications examined and it therefore appears likely that the reactions of seeds differ greatly from those of plants. During germination GABA is an extremely important metabolite.

The unknown "YPN", although it did not react with the ferric chloride reagent, was possibly at insufficient concentrations and hence could be phenylpyruvate or possibly some compound with a similar structure. Phenylpyruvate is involved in the synthesis of growth hormones as well as in the formation of many aromatic compounds. A branch-point of this pathway leads to the formation of sinapic acid which has similar properties to those of the unknown UVGB shown to be associated with GABA.

14-2 The Effects of Gamma-Radiation on Seeds

Using germination as the criterion for radiosensitivity very little variation is shown between the many seed species examined. Only parsnip seeds would not germinate after receiving a dose of 0.5 Mrad. The radiosensitivity of seeds appeared to be closely connected with the germinating ability of the seeds without irradiation. Those seeds which germinated rapidly and which showed a high rate of germination in a short time period (e.g. mustard and lettuce) were not as radiosensitive as those seeds which displayed delayed germination and which showed a low rate of germination extending over many days (e.g. parsnip and spinach).

One group of mustard seeds showed complete biochemical and physiological recovery after storage in a drawer in the laboratory whereas a similar set stored over silica gel did not. The effects of storage at constant relative humidities on the recovery of irradiated seeds was examined. Some recovery was observed, but not to the extent of that discovered earlier. Storage at high relative humidities for prolonged periods led to complete loss of the ability to germinate.

It has been discussed earlier in this thesis (section 1-5.7) that storage at high water contents, above that during the irradiation, can lead to recovery, in terms of seedling growth. The water content used in the work reported in this thesis for the irradiation procedure was 76% R.H. It was shown that prolonged storage above this humidity led to loss of germinating ability even for those seeds which had been able to germinate. It is therefore possible that the seeds stored in the laboratory drawer were exposed to unusual weather conditions involving a short period at very high humidities followed by a much longer period at low humidities. The time of high relative humidities would have to be sufficient to permit the disappearance of any long-lived free radicals in the seeds and to permit repair processes for the

damaged DNA but could not be prolonged as this would lead to deterioration of the seed. At the doses used damage to the DNA must occur and hence it is most likely that seeds contain enzymes capable of such repairs, otherwise stunted plants would be expected. As described in sections 4 and 5 the plants grown from irradiated but recovered seeds were significantly taller and heavier than those from non-irradiated seeds. This difference in growth was shown to be due to increased cell size, possibly caused by interference with the mechanisms controlling the cell size at which division begins. If variable humidity during storage is required then it was unlikely to have been obtained for the other sets of seeds described as stored "in air". These were, in fact, stored in a room which was centrally heated and for which the humidity showed only slight variations.

A second possibility exists which could explain the recovery of this particular group of seeds. At the time of the initial storage a fellow-worker in that laboratory was using quantities of gases; mainly CO_2, H_2 and N_2 which were being expelled into the air of the room. It is possible that the seeds were in fact stored for short periods, at least, in a strongly anaerobic atmosphere and that recovery mechanisms can occur under these conditions but not under storage in air.

The tritiated water patterns of irradiated but not recovered seeds showed that these seeds had a limited metabolism. However, very little difference was found between the metabolism of the LAP of irradiated or non-irradiated seeds when incubated with C^{14}-GABA, aspartate or glutamate. The LAP of non-irradiated seeds, however, did itself show limited metabolism over long time intervals compared to that of whole seeds. The similarity between the tritium patterns of the LAP of non-irradiated seeds and that of whole irradiated seeds has led to the suggestion that disorganization of whole seeds has occured on

irradiation. This could be due to the destruction of the cellular and intracellular membranes.

The patterns could also reflect the enzymes which do not require de nova synthesis during germination. If the DNA was severely damaged, transcription of RNA and hence protein synthesis would not be expected to occur to any great extent. On this basis the close similarity between the patterns obtained could be explained, as these reactions occur early in germination and involves those compounds shown to be labelled extremely early during imbibition in tritiated water. It is most probable that the enzyme systems involved do not require de nova synthesis but are present in the dry seed in an active form or as a zymogen.

APPENDIX

Table 6-2.1

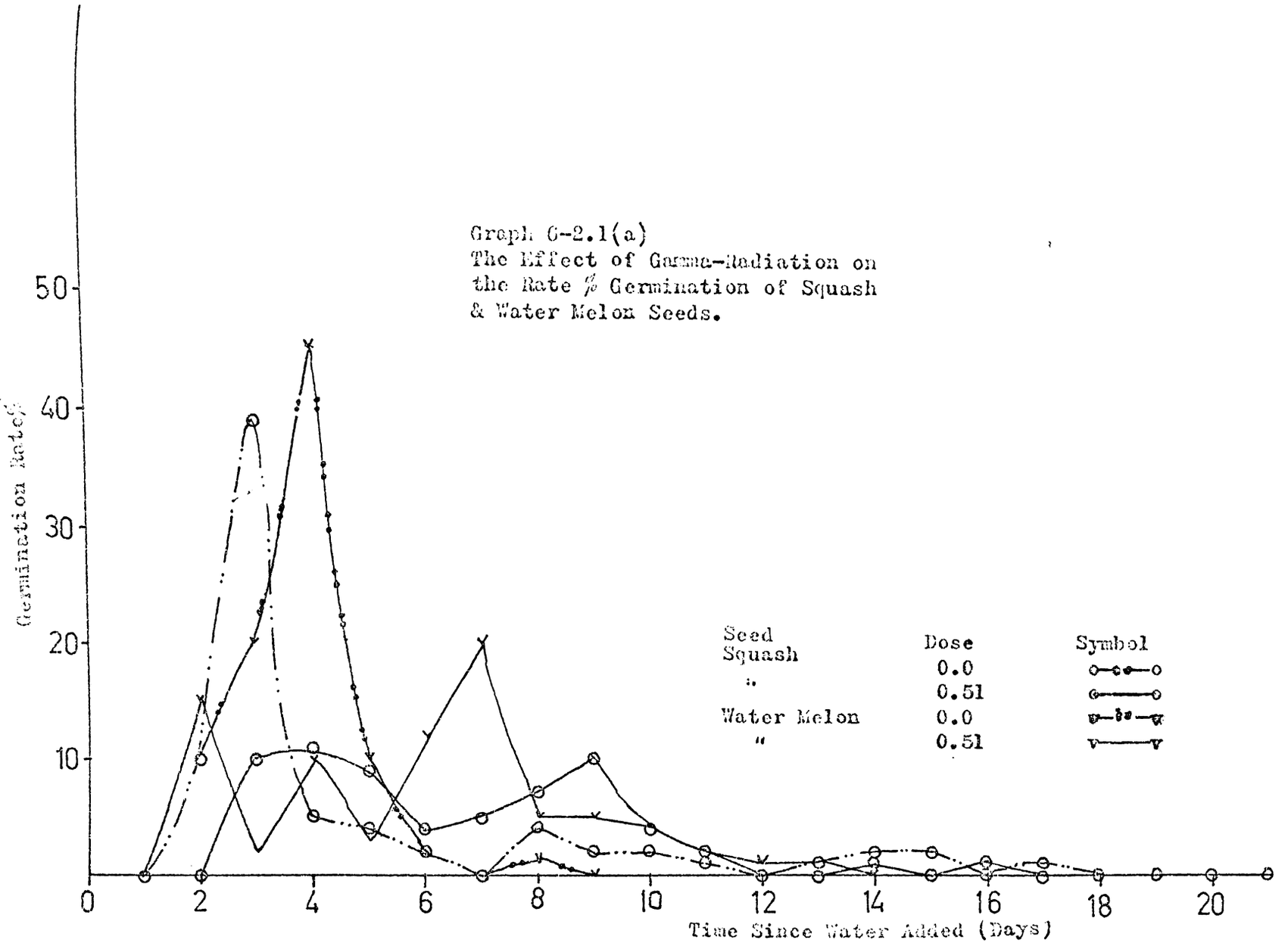
The Effects of Gamma-Radiation on the Germination of Squash (var. "Butternut") and Water Melon (var. Black Diamond Florida Giant).

Seeds	Squash						Water Melon					
Dose (Mrad)	0.0		0.51		5.1		0.0		0.51		5.0	
Dose Rate Mrad/hr	-		0.175		0.175		-		0.175		0.175	
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	10	10	0	0	0	0	10	10	15	15	0	0
3	39	49	10	10	0	0	20	30	2	17	0	0
4	5	54	11	21	0	0	45	75	10	27	0	0
5	4	59	9	30	0	0	10	85	3	30	0	0
6	2	61	4	34	0	0	4	89	12	42	0	0
7	0	61	5	39	0	0	0	89	20	62	0	0
8	4	65	7	46	0	0	1	90	5	67	0	0
9	2	67	10	56	0	0	0	90	5	72	0	0
10	2	69	4	50	0	0	0	90	4	76	0	0
11	1	70	2	62	0	0	0	90	2	78	0	0
12	0	70	0	62	0	0	0	90	1	79	0	0
13	1	71	0	62	0	0	0	90	1	80	0	0
14	2	73	1	63	0	0	0	90	0	80	0	0
15	2	75	0	63	0	0	0	90	0	80	0	0
16	0	75	1	64	0	0	0	90	0	80	0	0
17	1	76	0	64	0	0	0	90	0	80	0	0
18	0	76	0	64	0	0	0	90	0	80	0	0
19	0	76	0	64	0	0	0	90	0	80	0	0
20	0	76	0	64	0	0	0	90	0	80	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph G-2.1(a)
The Effect of Gamma-Radiation on the Rate % Germination of Squash & Water Melon Seeds.



Graph 6-2.1(b)
The Effect of Gamma-Radiation on the Germination % of Squash & Water Melon Seeds.

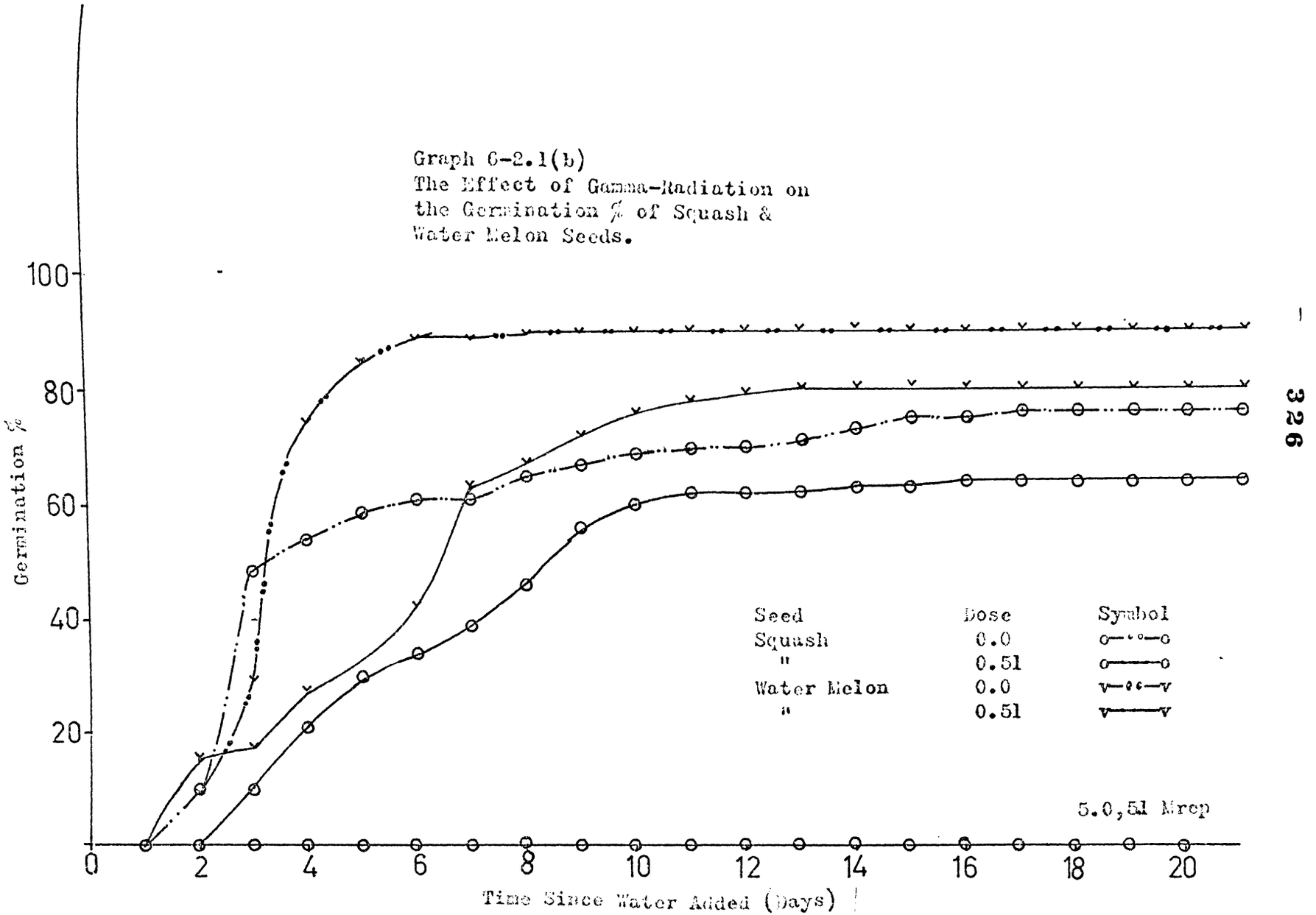


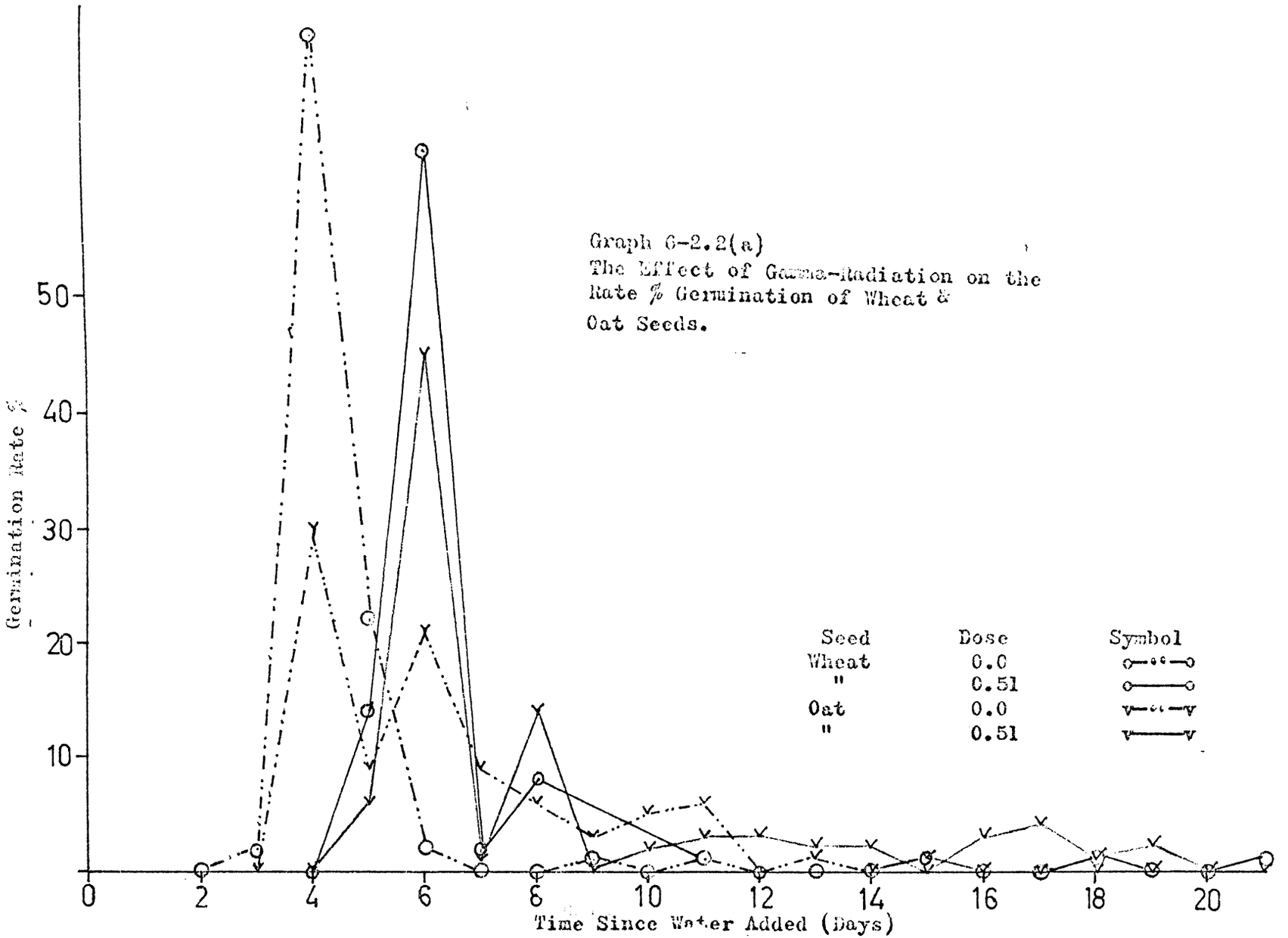
Table 6-2.2

The Effects of Gamma- Radiation on the Germination of Wheat and Oat.

Seeds	Wheat						Oat					
Dose (Mrad)	0.0		0.51		5.14		0.0		0.51		5.10	
Dose Rate Mrad/hr	-		0.175		0.175		-		0.175		0.175	
Time Exposed (hrs)	-						-					
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	2	2	0	0	0	0	0	0	0	0	0	0
4	72	74	0	0	0	0	30	30	0	0	0	0
5	22	96	14	14	0	0	9	39	6	6	0	0
6	2	98	62	76	0	0	21	60	45	51	0	0
7	0	98	2	78	0	0	9	69	1	52	0	0
8	0	98	8	86	0	0	6	75	14	66	0	0
9	1	99	0	86	0	0	3	78	0	66	0	0
10	0	99	0	86	0	0	5	83	2	68	0	0
11	1	100	1	87	0	0	6	89	3	71	0	0
12	-	100	0	87	0	0	1	89	3	74	0	0
13	-	100	0	87	0	0	1	90	2	76	0	0
14	-	100	0	87	0	0	0	90	2	78	0	0
15	-	100	1	88	0	0	0	90	1	79	0	0
16	-	100	0	88	0	0	0	90	3	82	0	0
17	-	100	0	88	0	0	0	90	4	86	0	0
18	-	100	1	89	0	0	0	90	1	87	0	0
19	-	100	0	89	0	0	0	90	2	89	0	0
20	-	100	0	89	0	0	0	90	0	89	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.



Graph C-2.2(a)
The Effect of Gamma-Radiation on the Rate % Germination of Wheat & Oat Seeds.

Graph 6-2.2(b)
The Effect of Gamma-Radiation on the Germination % of Wheat & Oat Seeds.

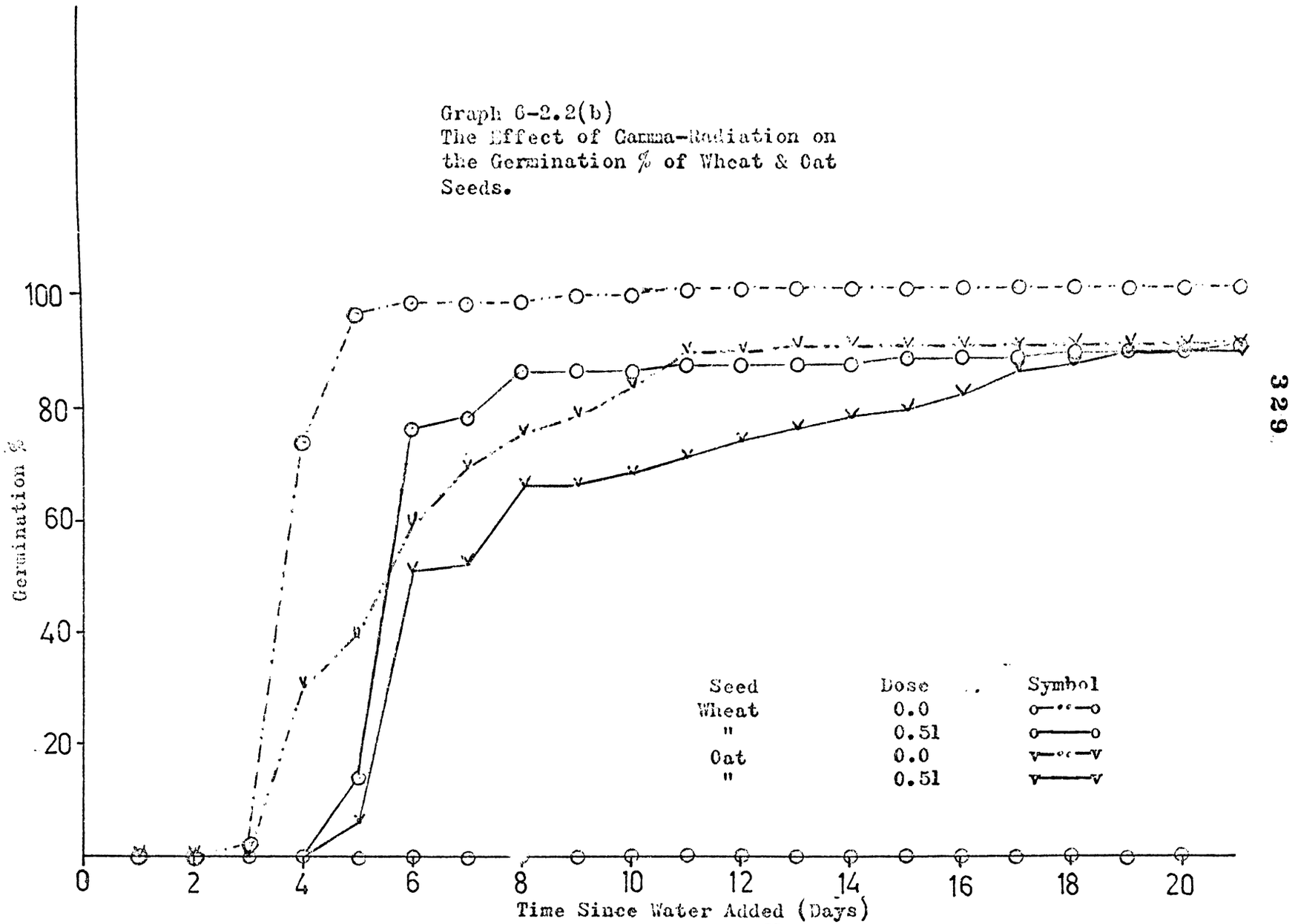


Table 6-2.3

The Effects of Gamma-Radiation on the Germination of Pisum sativum (Pea) Seeds

Dose (Mrad)	0.0		0.1		0.33		0.51		1.0		3.3	
Dose Rate Mrad/hr	-		0.175		0.175		0.175		0.175		0.175	
Time Exposed (hrs)	-											
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	13	13	9	9	1	1	0	0	0	0	0	0
4	38	51	28	37	32	33	7	7	0	0	0	0
5	33	84	37	74	33	66	11	18	5	5	0	0
6	10	94	15	89	16	82	1	19	14	19	0	0
7	5	99	4	93	7	89	1	20	0	19	0	0
8	0	99	0	93	0	89	0	20	0	19	0	0
9	0	99	0	93	0	89	1	21	0	19	0	0
10	0	99	0	93	0	89	0	21	0	19	0	0
11	0	99	0	93	0	89	0	21	0	19	0	0
12	0	99	0	93	0	89	0	21	0	19	0	0
13												
14												
15												
16												
17												
18												
19												
20												

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

Table 6-2.3 continued

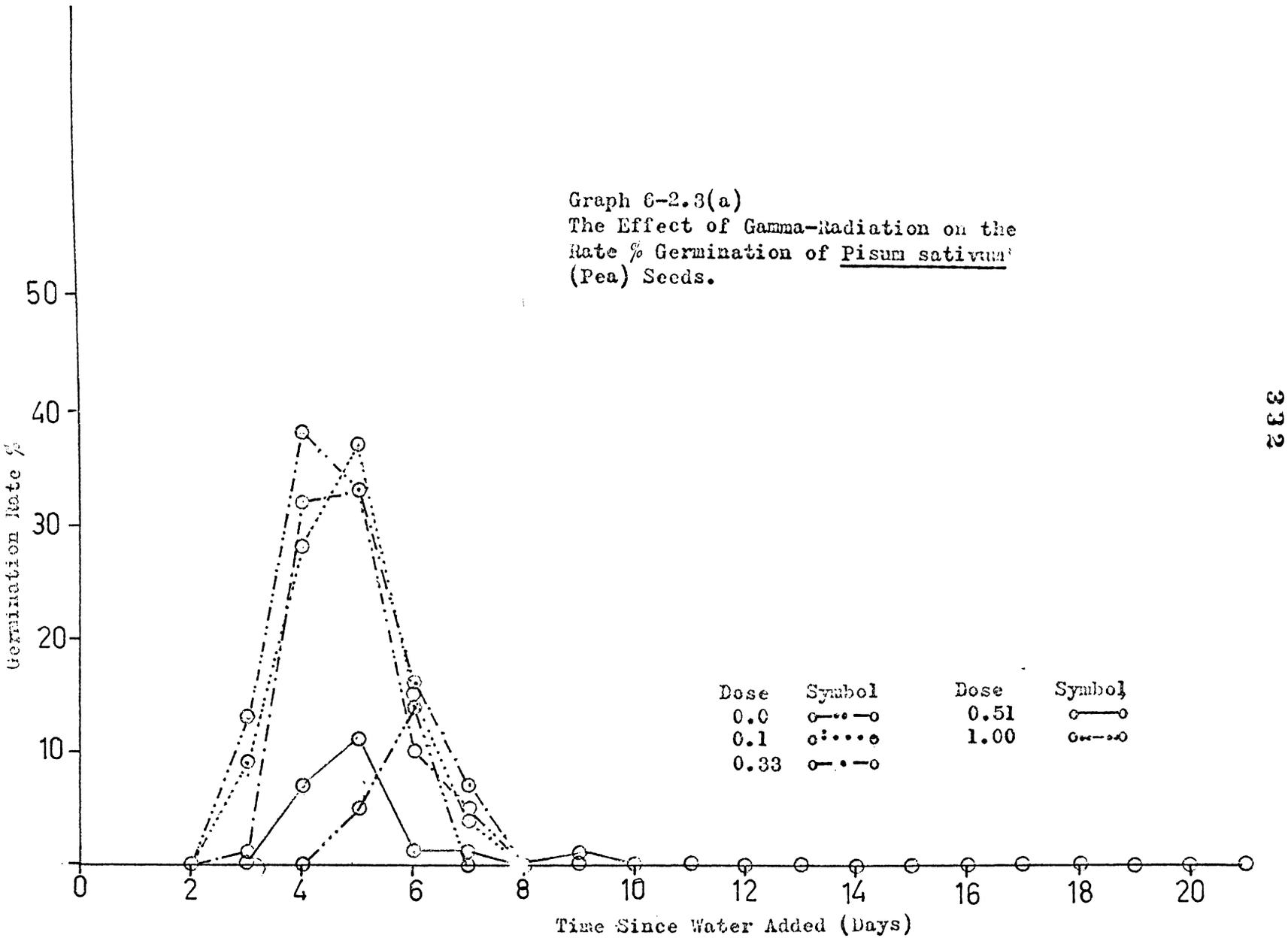
The Effects of Gamma-Radiation on the Germination of Pisum sativum (Pea) Seeds

Dose (Mrad)	5.1											
Dose Rate Mrad/hr	0.175											
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0										
2	0	0										
3	0	0										
4	0	0										
5	0	0										
6	0	0										
7	0	0										
8	0	0										
9	0	0										
10	0	0										
11	0	0										
12	0	0										
13												
14												
15												
16												
17												
18												
19												
20												

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 6-2.3(a)
The Effect of Gamma-Radiation on the Rate % Germination of Pisum sativum (Pea) Seeds.



Graph 6-2.3(b)
The Effect of Gamma-Radiation on the
Germination % of Pisum sativum Seeds

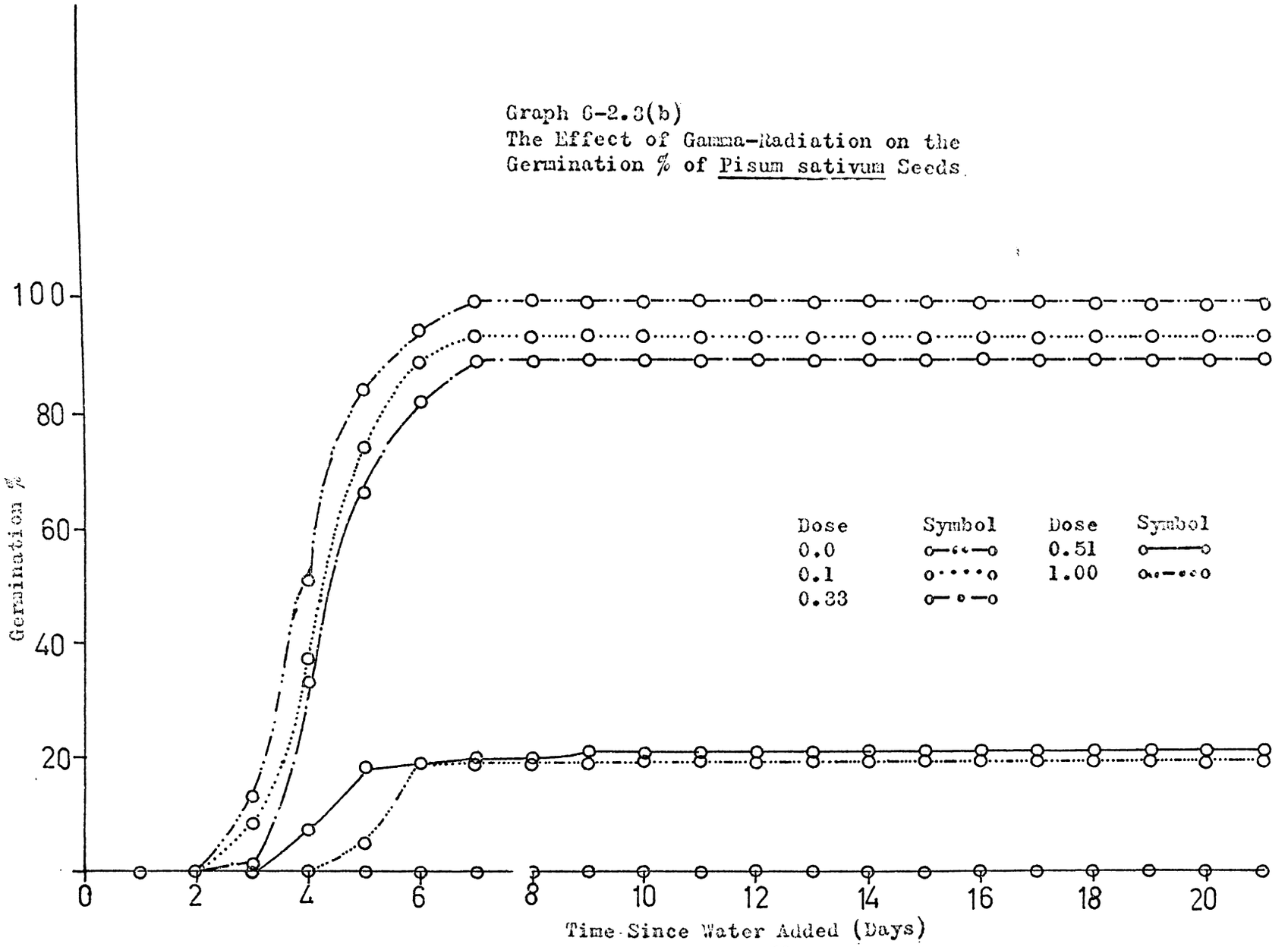


Table 6-2.4

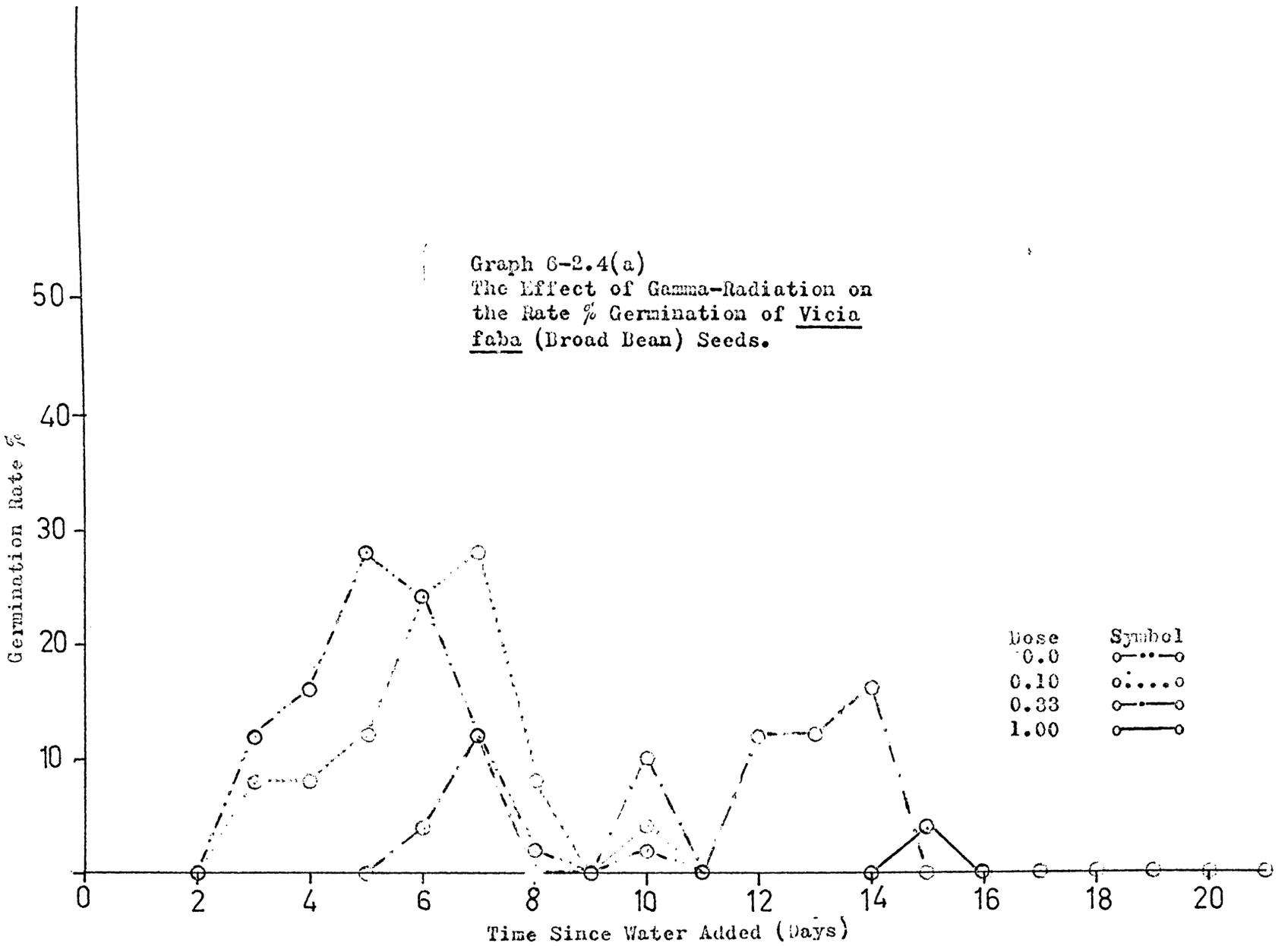
The Effects of Gamma-Radiation on the Germination of Broad Bean (Vicia faba) Seeds

Dose (Mrad)	0.0		0.10		0.33		1.00		3.30		5.10	
Dose Rate Mrad/hr	-		0.175		0.175		0.175		0.175		0.175	
Time Exposed (hrs)	-											
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	12	12	8	8	0	0	0	0	0	0	0	0
4	16	28	8	16	0	0	0	0	0	0	0	0
5	28	56	12	28	0	0	0	0	0	0	0	0
6	24	80	24	52	4	4	0	0	0	0	0	0
7	12	92	28	80	12	16	0	0	0	0	0	0
8	2	94	8	88	0	16	0	0	0	0	0	0
9	0	94	0	88	0	16	0	0	0	0	0	0
10	2	96	4	92	10	26	0	0	0	0	0	0
11	0	96	0	92	0	26	0	0	0	0	0	0
12	0	96	0	92	12	38	0	0	0	0	0	0
13	0	96	0	92	12	50	0	0	0	0	0	0
14	0	96	0	92	16	76	0	0	0	0	0	0
15	0	96	0	92	0	76	4	4	0	0	0	0
16	0	96	0	92	0	76	0	4	0	0	0	0
17	0	96	0	92	0	76	0	4	0	0	0	0
18	0	96	0	92	0	76	0	4	0	0	0	0
19	0	96	0	92	0	76	0	4	0	0	0	0
20	0	96	0	92	0	76	0	4	0	0	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 6-2.4(a)
The Effect of Gamma-Radiation on the Rate % Germination of Vicia faba (Broad Bean) Seeds.



Graph 6-2.4(b)
The Effect of Gamma-Radiation on the
Germination of Vicia faba Seeds.

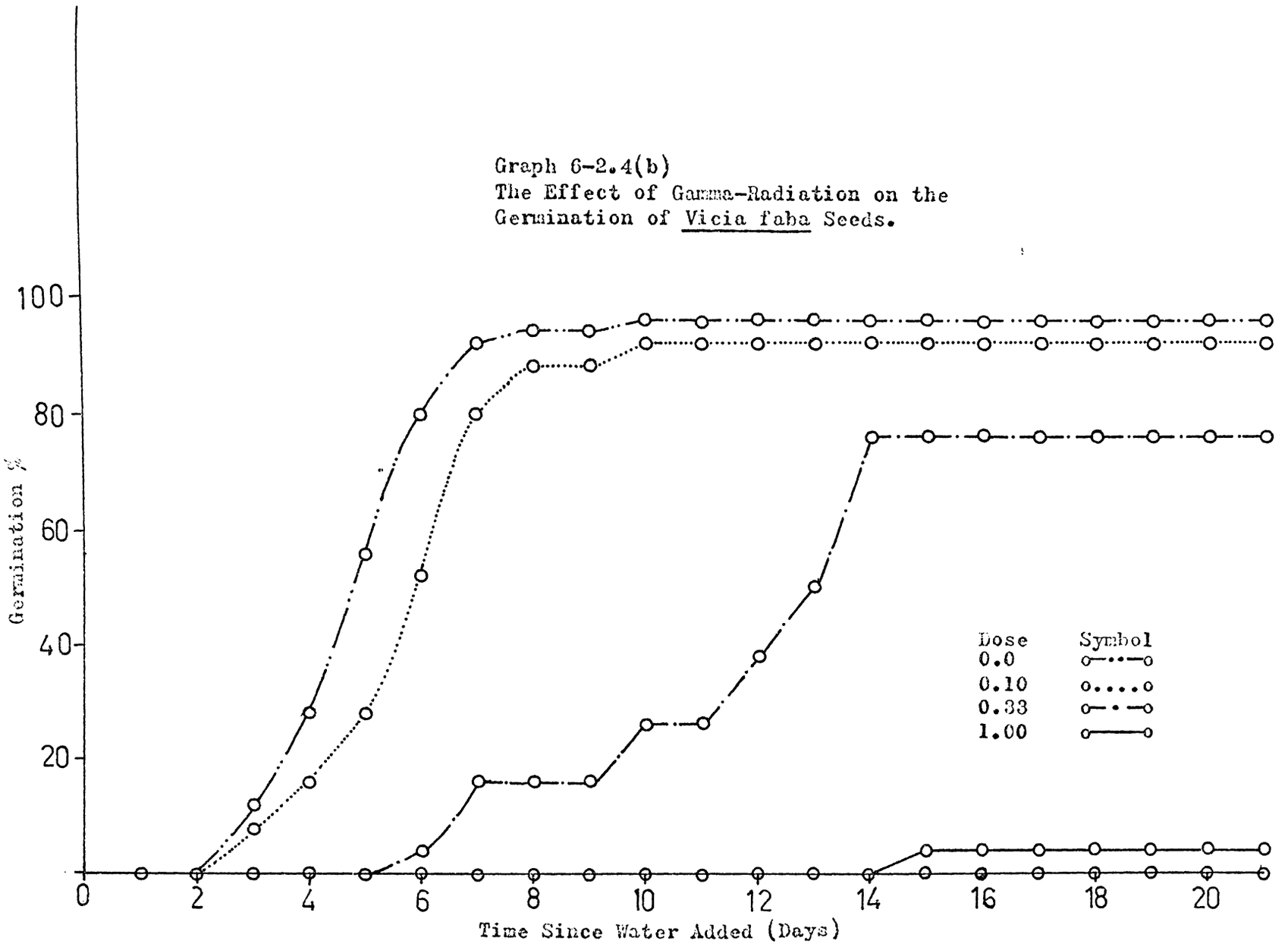


Table 6-2.5

The Effects of Gamma-Radiation on the Germination of Lettuce Seeds (Lactuca sativa) Variety, "Webbs Wonderful".

Dose (Mrad)	0.0		0.168		0.336		0.51		0.672		0.980	
Dose Rate Mrad/hr	-		0.175		0.175		0.175		0.175		0.175	
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	100	100	11	11	0	0	0	0	0	0	0	0
2	-	100	86	97	55	55	5	5	0	0	0	0
3	-	100	2	99	33	88	57	62	1	1	0	0
4	-	100	1	100	8	96	24	86	4	5	0	0
5	-	100	-	100	2	98	0	6	9	14	2	2
6	-	100	-	100	0	98	1	87	12	26	8	10
7	-	100	-	100	0	98	0	87	7	33	5	15
8	-	100	-	100	0	98	0	87	20	53	4	19
9	-	100	-	100	0	98	2	89	20	73	4	23
10	-	100	-	100	0	98	0	89	8	81	6	29
11	-	100	-	100	0	98	1	90	7	88	7	36
12	-	100	-	100	0	98	2	92	3	91	8	44
13	-	100	-	100	0	98	3	95	0	91	8	52
14	-	100	-	100	0	98	0	95	5	96	10	62
15	-	100	-	100	0	98	0	95	0	96	7	69
16	-	100	-	100	0	98	0	95	0	96	5	74
17	-	100	-	100	0	98	0	95	0	96	4	78
18	-	100	-	100	0	98	0	95	0	96	2	80
19	-	100	-	100	0	98	0	95	0	96	2	82
20	-	100	-	100	0	98	0	95	0	96	1	83
21											3	86

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

Table 6-2.5 continued

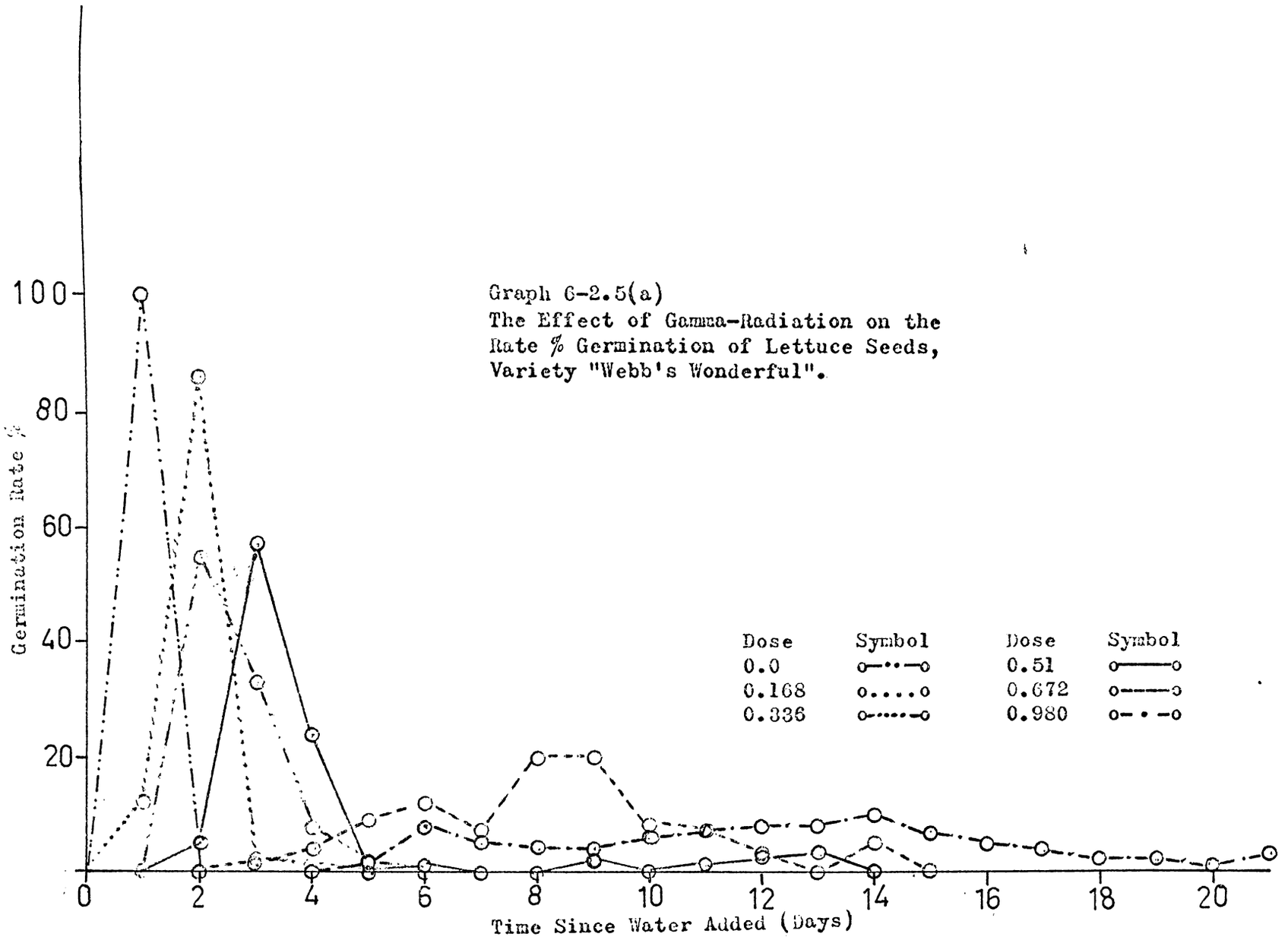
The Effects of Gamma-Radiation on the Germination of Lettuce Seeds (Lactuca sativa) Variety, "Webbs Wonderful".

Dose (Mrad)	2.0		5.0		32.3							
Dose Rate Mrad/hr	0.175		0.175		0.175							
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0						
2	0	0	0	0	0	0						
3	0	0	0	0	0	0						
4	0	0	0	0	0	0						
5	0	0	0	0	0	0						
6	0	0	0	0	0	0						
7	0	0	0	0	0	0						
8	0	0	0	0	0	0						
9	0	0	0	0	0	0						
10	0	0	0	0	0	0						
11	0	0	0	0	0	0						
12	0	0	0	0	0	0						
13	0	0	0	0	0	0						
14	0	0	0	0	0	0						
15	0	0	0	0	0	0						
16	0	0	0	0	0	0						
17	0	0	0	0	0	0						
18	0	0	0	0	0	0						
19	0	0	0	0	0	0						
20	0	0	0	0	0	0						

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 6-2.5(a)
The Effect of Gamma-Radiation on the Rate % Germination of Lettuce Seeds, Variety "Webb's Wonderful".



Graph 6-2.5(b)
The Effect of Gamma-Radiation on the
Germination % of Lettuce Seeds,
Variety "Webb's Wonderful".

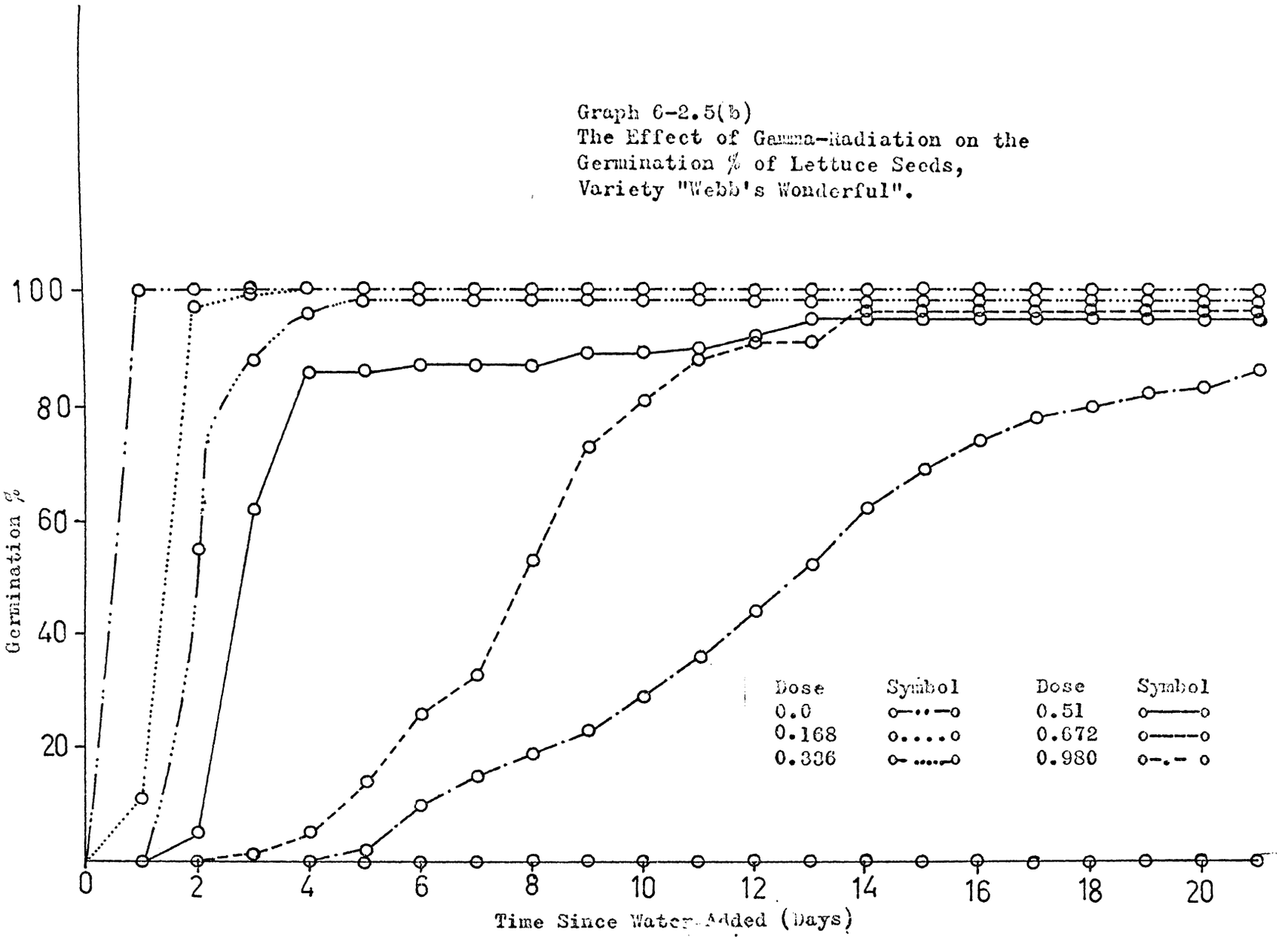


Table 6-2.6

The Effects of Gamma-Radiation on the Germination of Onion (Allium cepa) Seeds Variety "White Silverskin".

Dose (Mrad)	0.0		0.49		0.672		0.98		2.0		5.0	
Dose Rate Mrad/hr	-		0.175		0.175		0.175		0.175		0.175	
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	1	1	2	2	2	2	0	0	0	0	0	0
5	2	3	1	3	2	4	0	0	0	0	0	0
6	75	78	3	6	2	6	1	1	0	0	0	0
7	1	79	4	10	3	9	1	2	0	0	0	0
8	4	83	8	18	7	16	9	11	0	0	0	0
9	3	86	6	24	8	24	10	21	0	0	0	0
10	0	86	4	28	3	27	25	46	0	0	0	0
11	2	88	11	39	10	37	23	69	0	0	0	0
12	1	89	12	51	14	51	7	76	0	0	0	0
13	1	90	12	63	13	64	3	79	0	0	0	0
14	2	92	4	67	4	68	1	80	0	0	0	0
15	0	92	3	70	4	72	0	80	0	0	0	0
16	1	93	3	73	2	74	0	80	0	0	0	0
17	1	94	4	77	3	77	1	81	0	0	0	0
18	0	94	3	80	4	81	0	81	0	0	0	0
19	0	94	2	82	1	82	1	82	0	0	0	0
20	0	94	1	83	1	83	0	82	0	0	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

/continued....

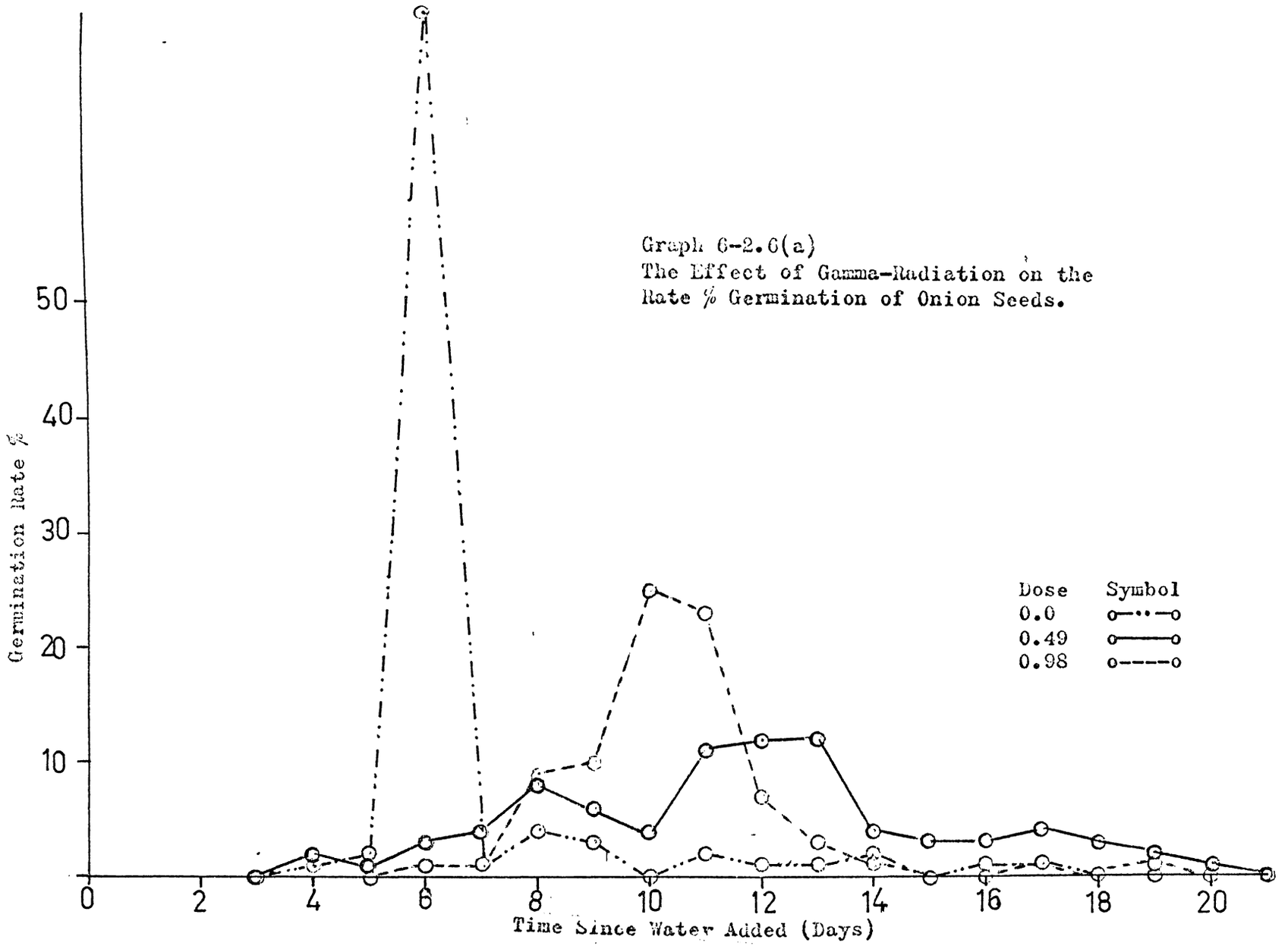
Table 6-2.6 continued

The Effects of Gamma-Radiation on the Germination of Onion (Allium capa) Seeds Variety "White Silverskin".

Dose (Mrad)	32.3											
Dose Rate Mrad/hr	0.175											
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0										
2	0	0										
3	0	0										
4	0	0										
5	0	0										
6	0	0										
7	0	0										
8	0	0										
9	0	0										
10	0	0										
11	0	0										
12	0	0										
13	0	0										
14	0	0										
15	0	0										
16	0	0										
17	0	0										
18	0	0										
19	0	0										
20	0	0										

* (a) % Germinated previous 24 hours

* (b) Total % germination.


Graph 6-2.6(a)
The Effect of Gamma-Radiation on the
Rate % Germination of Onion Seeds.
Germination Rate %
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
Time Since Water Added (Days)
Dose
Symbol
0.0
0.49
0.98

Graph 6-2.6(b)
The Effect of Gamma-Radiation on the
Germination % of Onion Seeds.

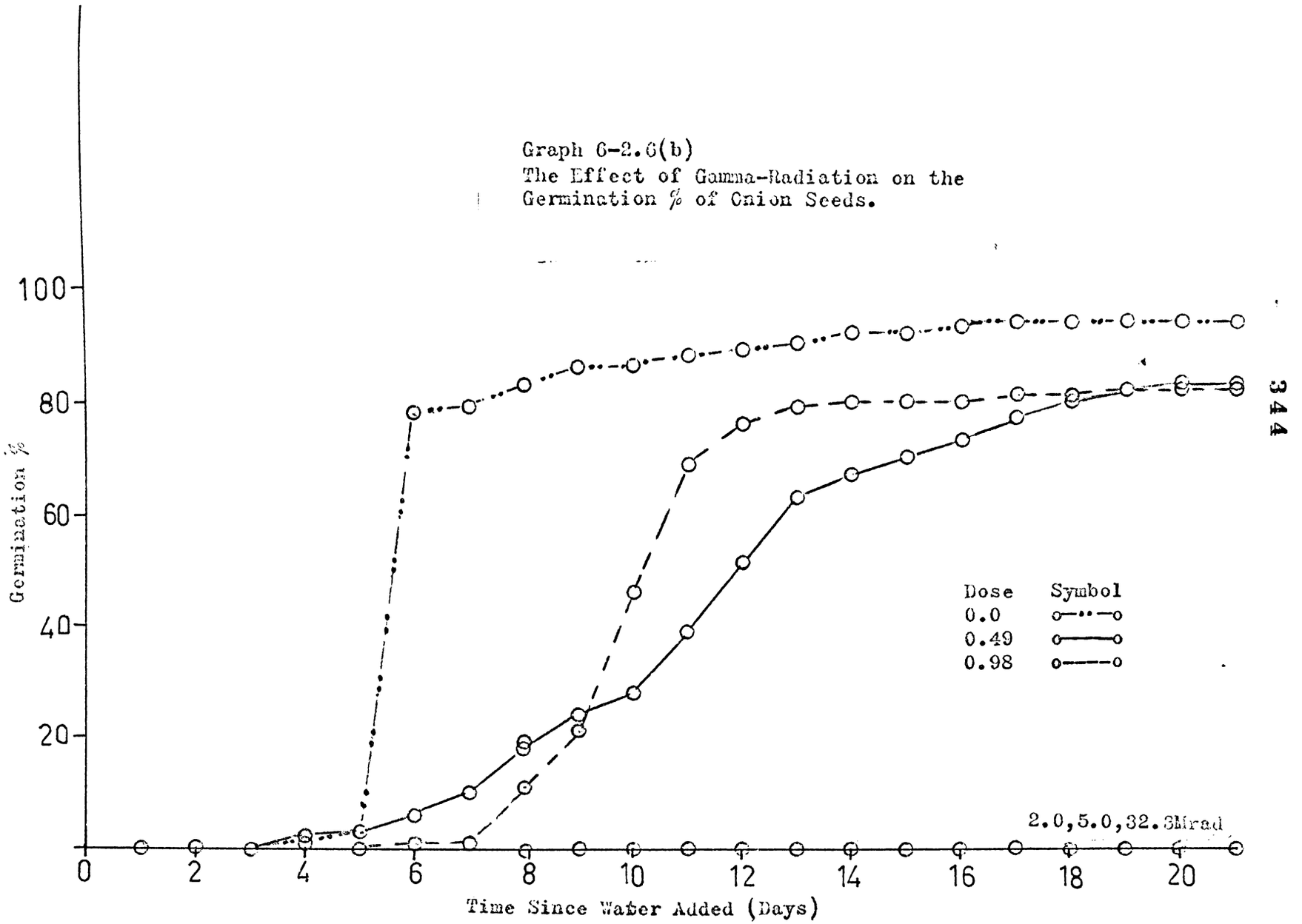


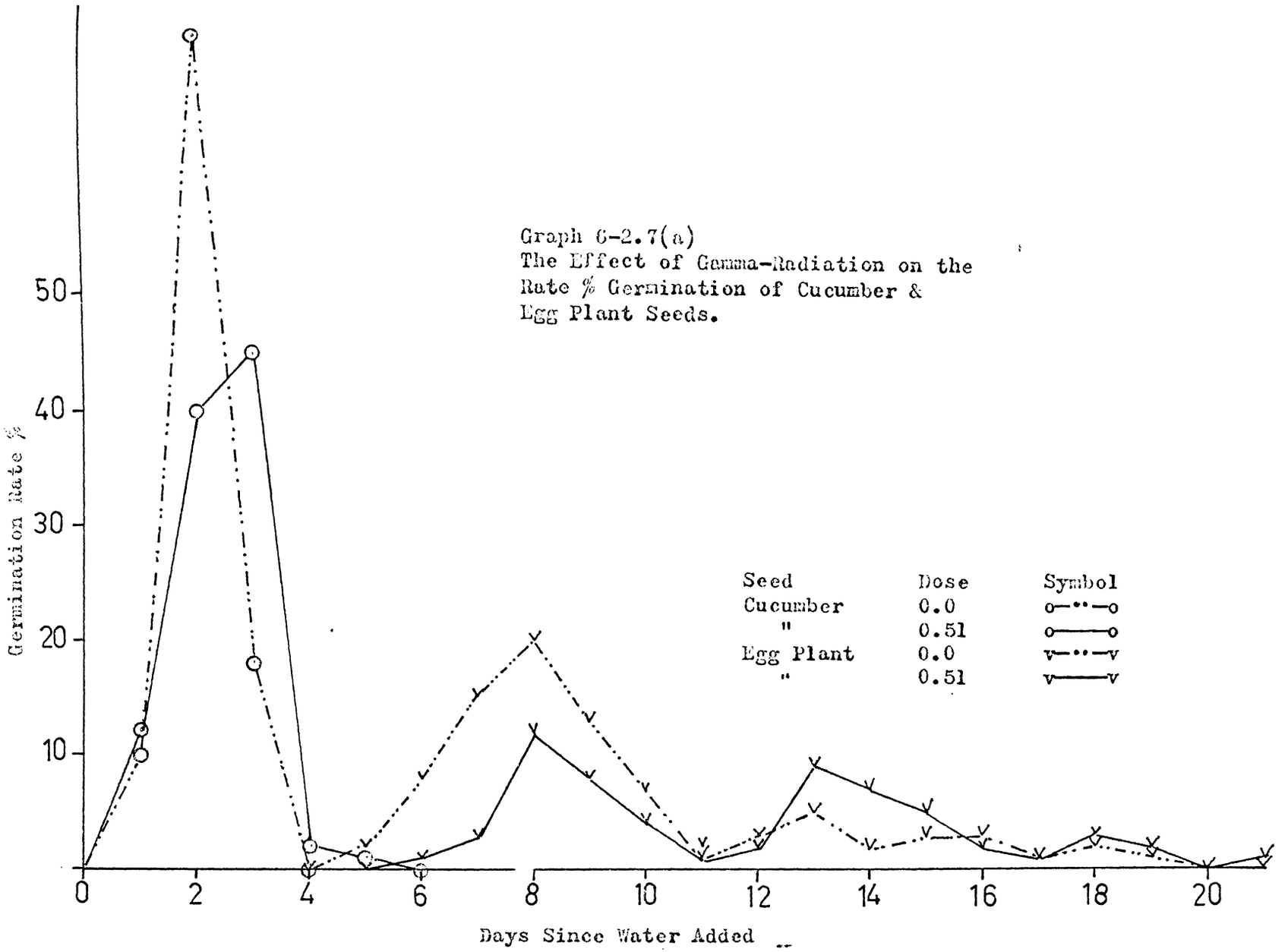
Table 6-2.7

The Effects of Gamma-Radiation on the Germination of Cucumber (var. Heinz Gherkin) and Egg Plant (var. New York Purple) Seeds.

Seeds	Cucumber						Egg Plant					
Dose (Mrad)	0.0		0.51		5.1		0.0		0.51		5.0	
Dose Rate Mrad/hr	-		0.175		0.175		-		0.175		0.175	
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	10	10	12	12	0	0	0	0	0	0	0	0
2	72	82	40	52	0	0	0	0	0	0	0	0
3	18	100	45	97	0	0	0	0	0	0	0	0
4	-	100	2	99	0	0	0	0	0	0	0	0
5	-	100	1	100	0	0	2	2	0	0	0	0
6	-	100	-	100	0	0	8	10	1	1	0	0
7	-	100	-	100	0	0	15	25	3	4	0	0
8	-	100	-	100	0	0	20	45	12	16	0	0
9	-	100	-	100	0	0	13	58	8	24	0	0
10	-	100	-	100	0	0	7	65	4	28	0	0
11	-	100	-	100	0	0	1	66	2	30	0	0
12	-	100	-	100	0	0	3	69	2	32	0	0
13	-	100	-	100	0	0	5	74	9	41	0	0
14	-	100	-	100	0	0	2	76	7	48	0	0
15	-	100	-	100	0	0	3	79	5	53	0	0
16	-	100	-	100	0	0	3	82	2	55	0	0
17	-	100	-	100	0	0	1	83	1	56	0	0
18	-	100	-	100	0	0	2	85	3	59	0	0
19	-	100	-	100	0	0	1	86	2	61	0	0
20	-	100	-	100	0	0	0	86	0	61	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.



Graph 6-2.7(a)
The Effect of Gamma-Radiation on the Rate % Germination of Cucumber & Egg Plant Seeds.

Graph 6-2.7(b)
The Effect of Gamma-Radiation on the Germination % of Cucumber & Egg Plant Seeds.

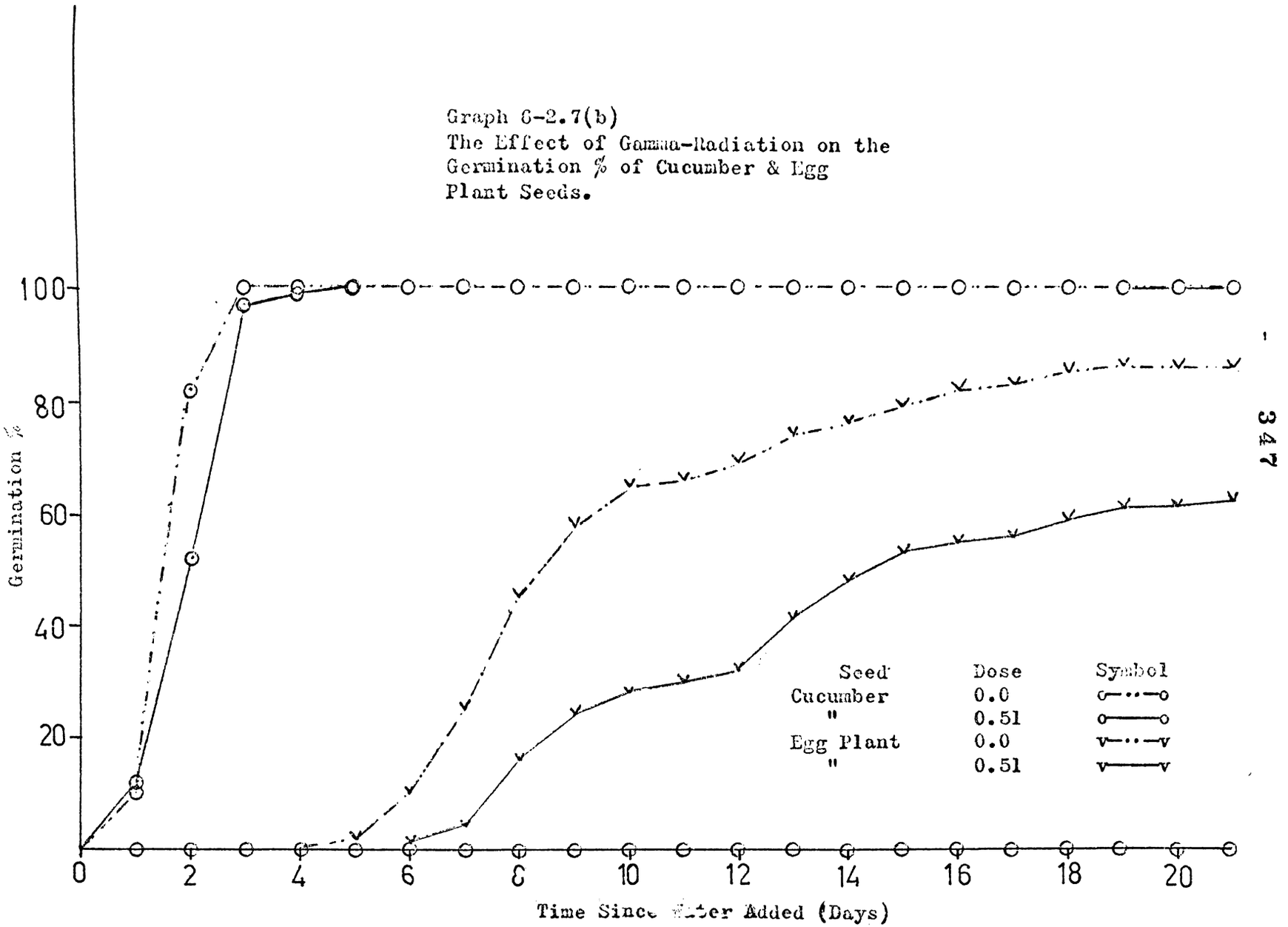


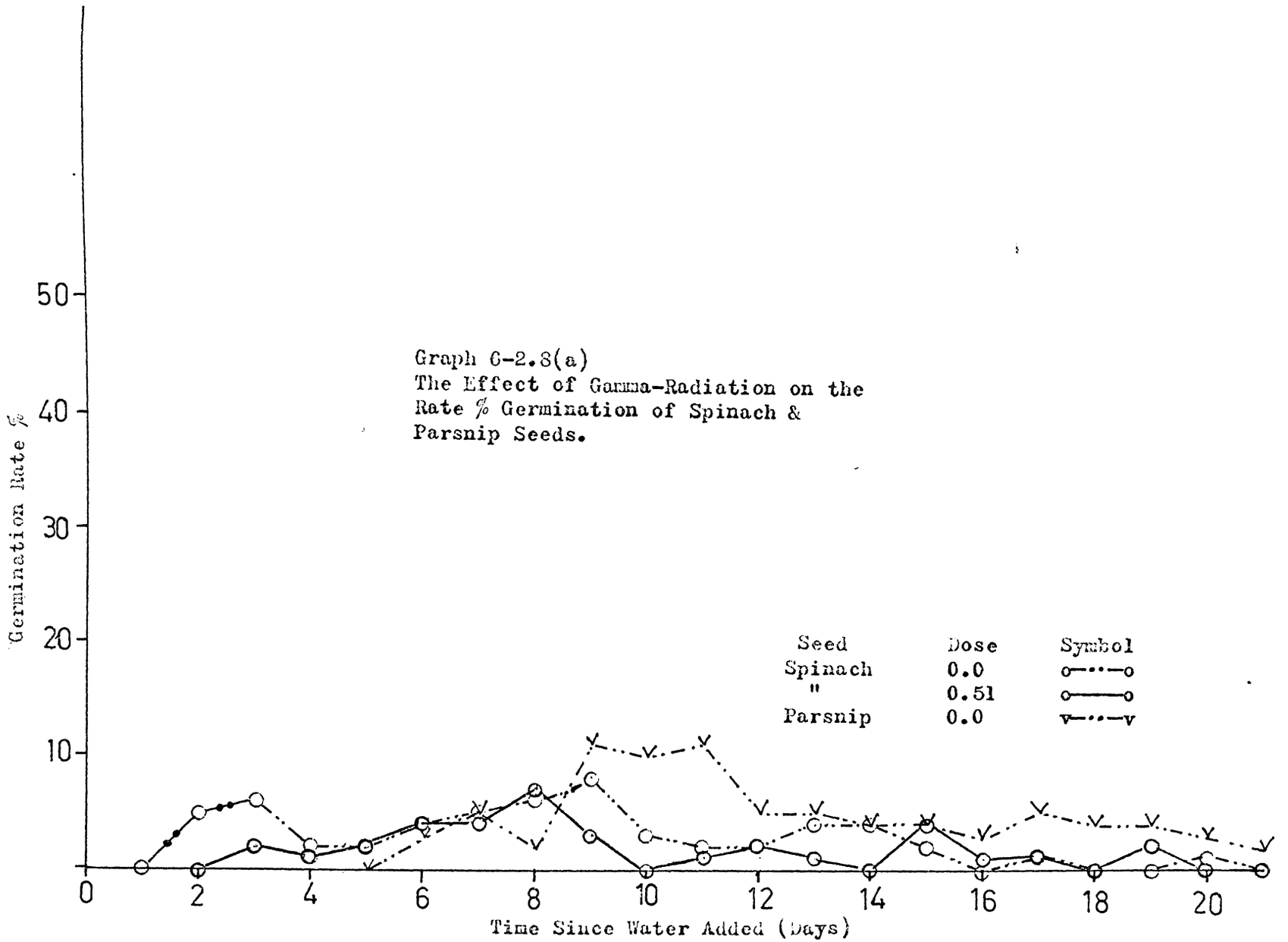
Table 6-2.8

The Effect of Gamma-Radiation on the Germination of Spinach (var. Long Round Standing Viking) and Parsnip (var. Hollow Crown Select).

Seed	Spinach						Parsnip					
Dose (Mrad)	0.0		0.51		5.0		0.0		0.51		5.1	
Dose Rate Mrad/hr	-		0.175		0.175		-		0.175		0.175	
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	5	5	0	0	0	0	0	0	0	0	0	0
3	6	11	2	2	0	0	0	0	0	0	0	0
4	2	13	1	3	0	0	0	0	0	0	0	0
5	2	15	2	5	0	0	0	0	0	0	0	0
6	4	19	4	9	0	0	3	3	0	0	0	0
7	5	24	4	13	0	0	5	8	0	0	0	0
8	6	30	7	20	0	0	2	10	0	0	0	0
9	8	38	3	23	0	0	11	21	0	0	0	0
10	3	41	0	23	0	0	10	31	0	0	0	0
11	2	43	1	24	0	0	11	42	0	0	0	0
12	2	45	2	26	0	0	5	47	0	0	0	0
13	4	49	1	27	0	0	5	52	0	0	0	0
14	4	53	0	27	0	0	4	56	0	0	0	0
15	2	55	4	31	0	0	4	60	0	0	0	0
16	0	55	1	32	0	0	3	63	0	0	0	0
17	1	56	1	33	0	0	5	68	0	0	0	0
18	0	56	0	33	0	0	4	72	0	0	0	0
19	0	56	2	35	0	0	4	76	0	0	0	0
20 21	1	57	0	35	0	0	3 2	79 81	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Graph 6-2.8(a)
The Effect of Gamma-Radiation on the Rate % Germination of Spinach & Parsnip Seeds.



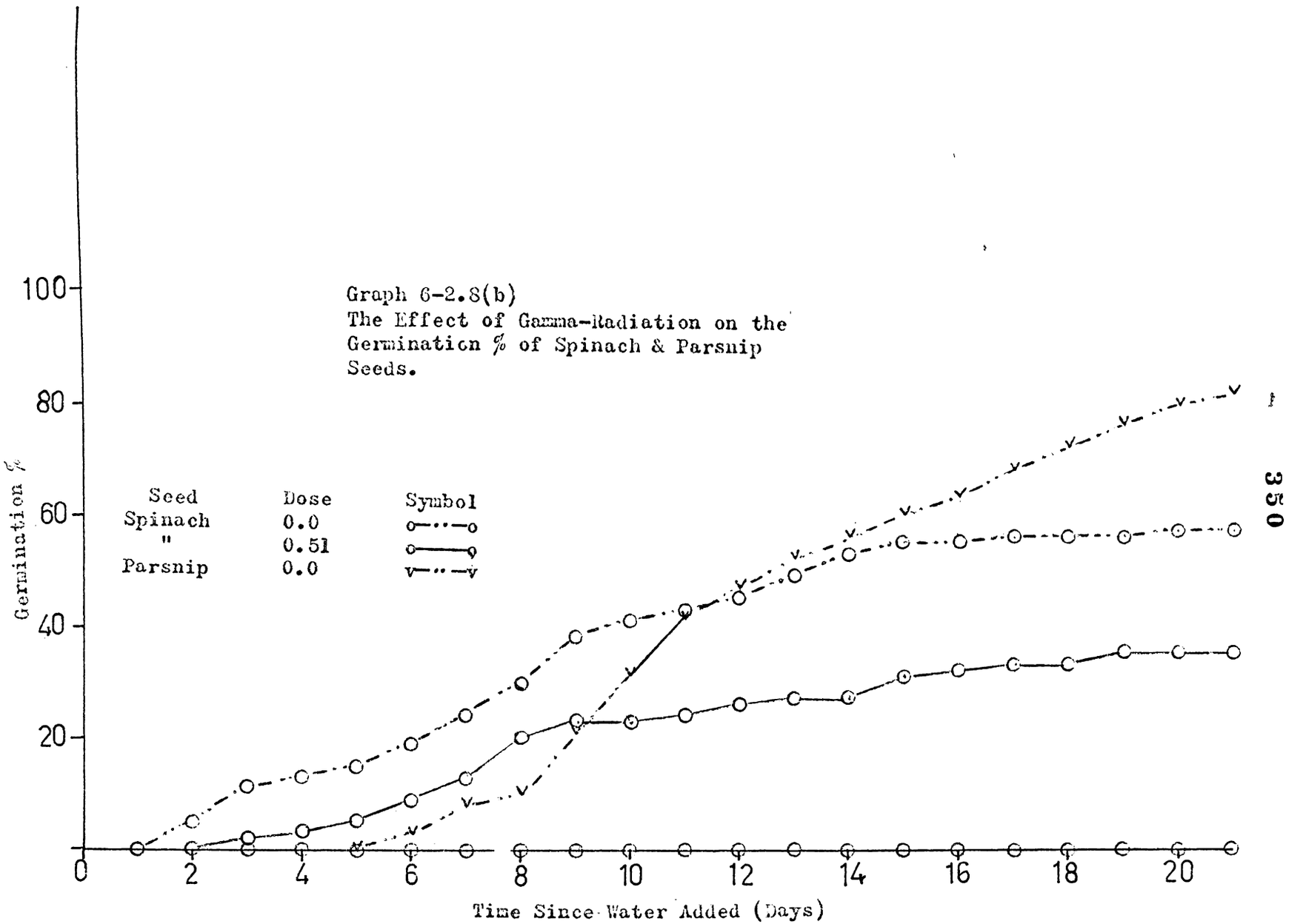
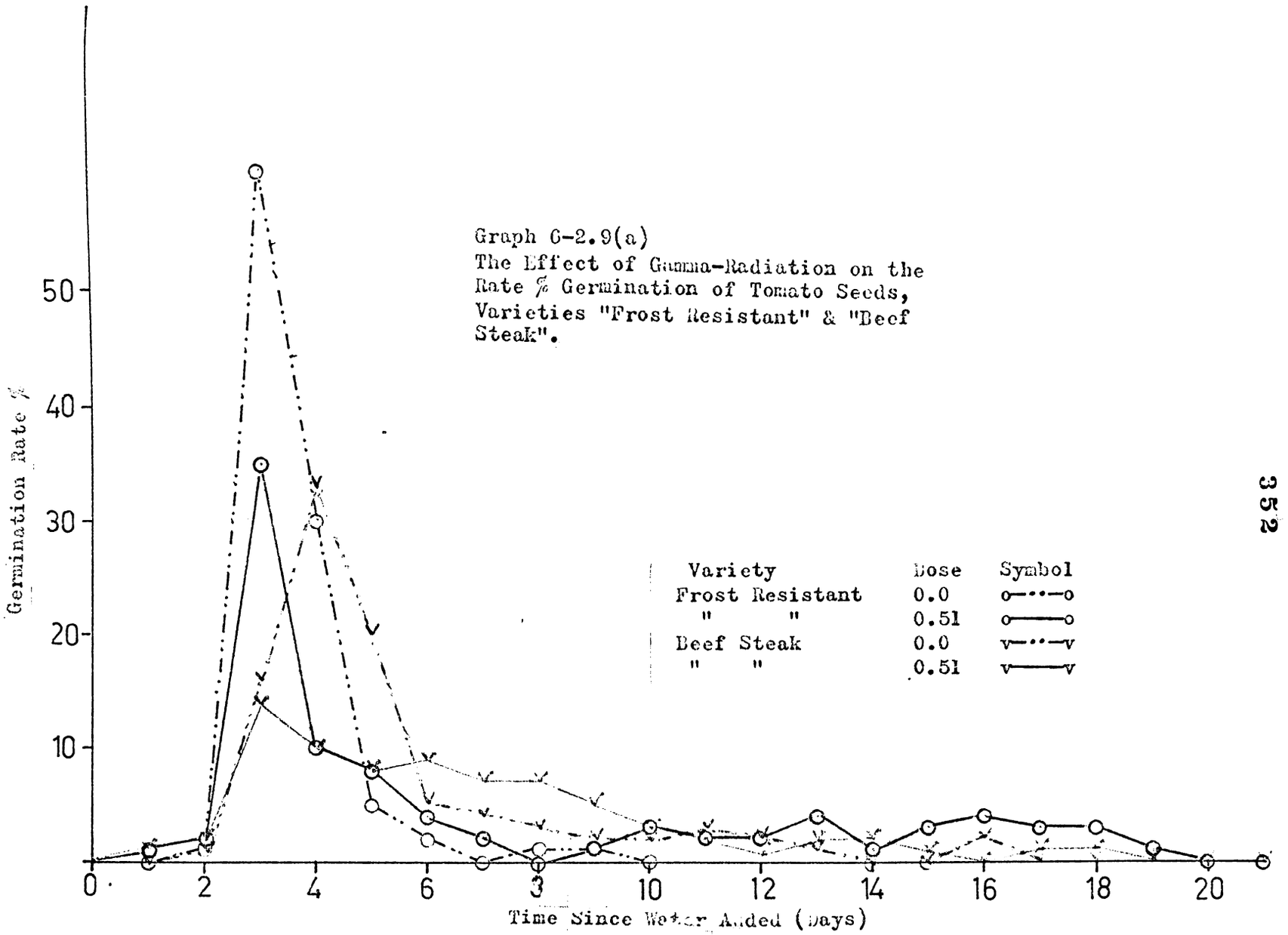

Graph 6-2.8(b)
The Effect of Gamma-Radiation on the Germination % of Spinach & Parsnip Seeds.
Seed
Dose
Symbol
Spinach
0.0
"
0.5l
Parsnip
0.0
Germination %
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
Time Since Water Added (Days)

Table 6-2.9

The Effect of Gamma-Radiation on the Germination of Tomato Seeds (varieties "Frost-Resistant" and "Beefsteak")

Variety	Frost Resistant						Beefsteak					
Dose (Mrad)	0.0		0.51		5.0		0.0		0.51		5.0	
Dose Rate Mrad/hr	-		0.175		0.175		-		0.175		0.175	
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	1	1	0	0	0	0	1	1	0	0
2	1	1	2	3	0	0	1	1	2	3	0	0
3	60	61	35	38	0	0	16	17	14	17	0	0
4	30	91	10	48	0	0	33	50	10	27	0	0
5	5	96	8	56	0	0	20	70	8	35	0	0
6	2	98	4	60	0	0	5	75	9	44	0	0
7	0	98	2	62	0	0	4	79	7	51	0	0
8	1	99	0	62	0	0	3	82	7	58	0	0
9	1	100	1	63	0	0	2	84	5	63	0	0
10	-	100	3	66	0	0	2	86	3	66	0	0
11	-	100	2	68	0	0	2	89	2	68	0	0
12	-	100	2	70	0	0	2	91	1	69	0	0
13	-	100	4	74	0	0	1	92	2	71	0	0
14	-	100	1	75	0	0	0	92	2	73	0	0
15	-	100	3	78	0	0	0	92	1	74	0	0
16	-	100	4	82	0	0	2	94	0	74	0	0
17	-	100	3	85	0	0	0	94	1	75	0	0
18	-	100	3	88	0	0	0	94	1	76	0	0
19	-	100	1	89	0	0	0	94	0	76	0	0
20	-	100	0	89	0	0	0	94	0	76	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.


Graph 6-2.9(a)
The Effect of Gamma-Radiation on the Rate % Germination of Tomato Seeds, Varieties "Frost Resistant" & "Beef Steak".
Germination Rate %
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
Time Since Water Added (Days)
Variety
Dose
Symbol
Frost Resistant
0.0
" "
0.5l
Beef Steak
0.0
" "
0.5l

Graph C-2.9(b)
The Effect of Gamma-Radiation on the Germination % of Tomato Seeds, Varieties "Frost Resistant" & "Beef Steak".

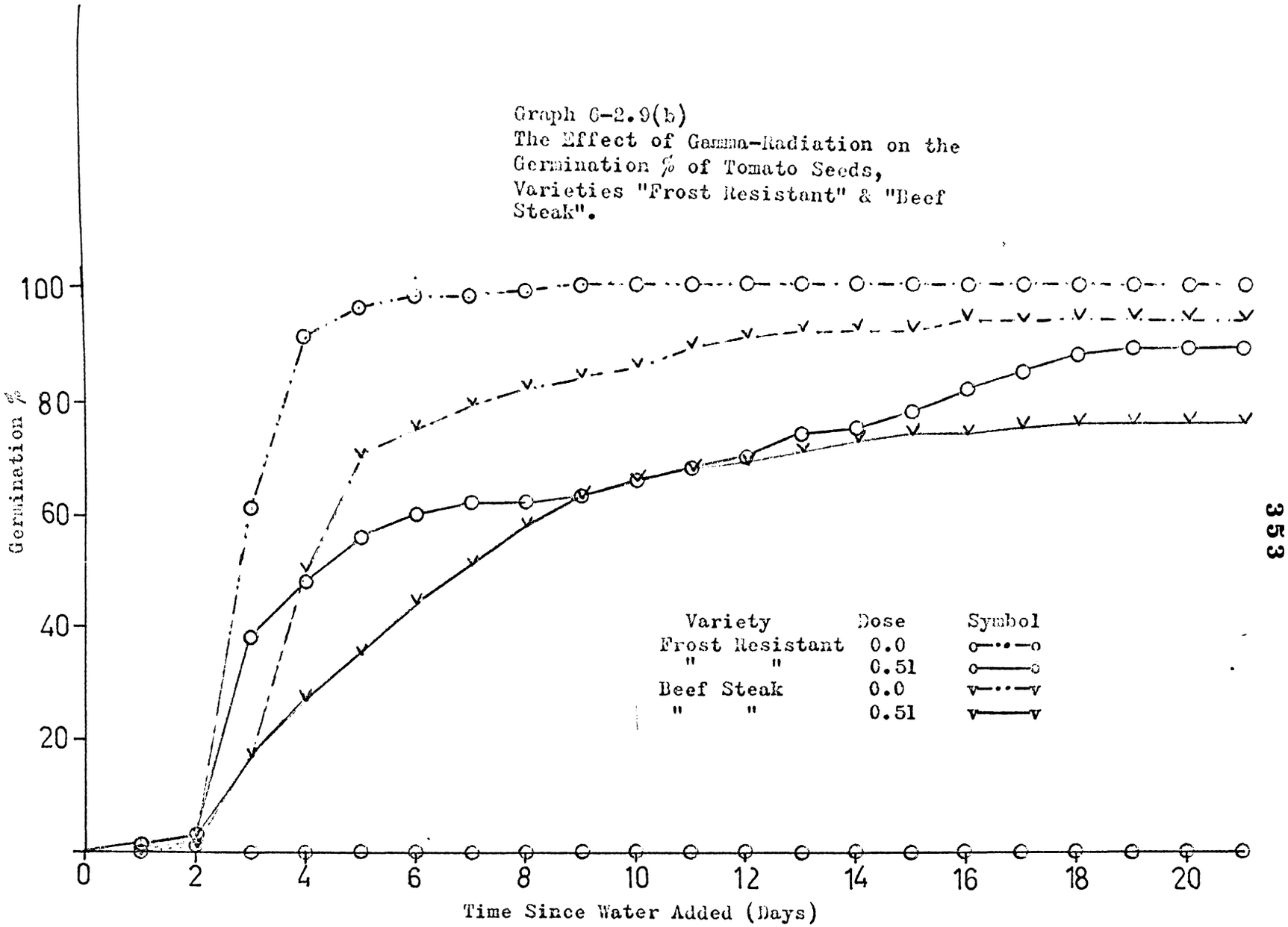


Table 6-2.10

The Effects of Gamma-Radiation on the Germination of Sinapis alba Seeds

Dose (Mrad)	0.0		0.50		2.0		4.38		4.5		5.0	
Dose Rate Mrad/hr	-		0.175		0.175		0.175		0.175		0.175	
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	1	1	0	0	0	0	0	0	0	0	0	0
2	68	69	1	1	0	0	0	0	0	0	0	0
3	12	81	4	5	0	0	0	0	0	0	0	0
4	3	84	41	46	0	0	0	0	0	0	0	0
5	4	88	31	77	0	0	0	0	0	0	0	0
6	4	92	8	85	0	0	0	0	0	0	0	0
7	0	92	2	87	0	0	0	0	0	0	0	0
8	0	92	0	87	0	0	0	0	0	0	0	0
9	1	93	2	89	0	0	0	0	0	0	0	0
10	0	93	0	89	0	0	0	0	0	0	0	0
11	1	94	2	91	0	0	0	0	0	0	0	0
12	0	94	1	92	0	0	0	0	0	0	0	0
13	0	94	1	93	0	0	0	0	0	0	0	b0
14	0	94	0	93	0	0	0	0	0	0	0	0
15	0	94	1	94	0	0	0	0	0	0	0	0
16	1	95	1	95	0	0	0	0	0	0	0	0
17	0	95	0	95	0	0	0	0	0	0	0	0
18	0	95	0	95	0	0	0	0	0	0	0	0
19	0	95	0	95	0	0	0	0	0	0	0	0
20	0	95	0	95	0	0	0	0	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

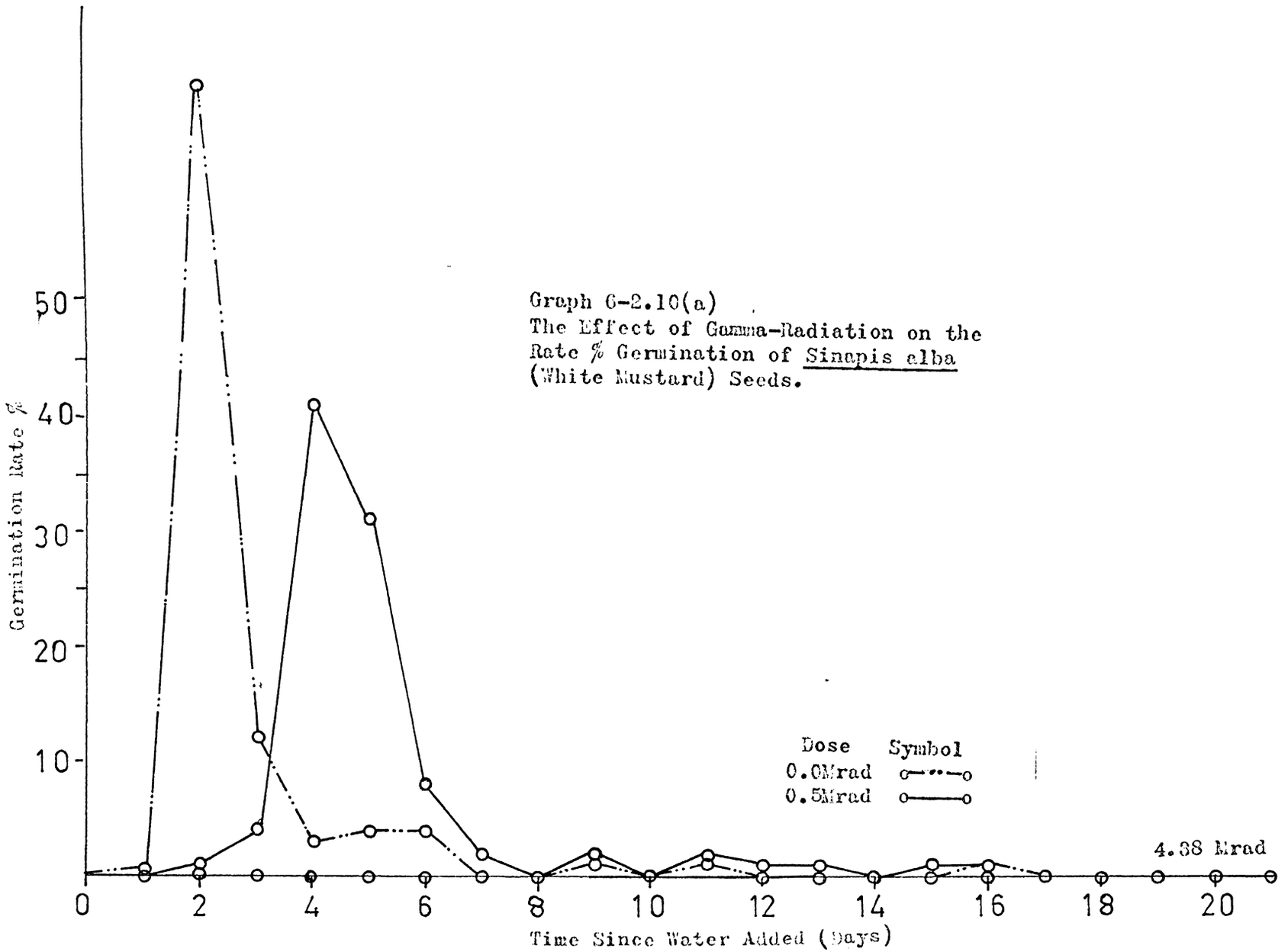
Table 6-2.10 continued

The Effects of Gamma-Radiation on the Germination of Sinapis alba Seeds

Dose (Mrad)	9.0		32.3									
Dose Rate Mrad/hr	0.175		0.175									
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0								
2	0	0	0	0								
3	0	0	0	0								
4	0	0	0	0								
5	0	0	0	0								
6	0	0	0	0								
7	0	0	0	0								
8	0	0	0	0								
9	0	0	0	0								
10	0	0	0	0								
11	0	0	0	0								
12	0	0	0	0								
13	0	0	0	0								
14	0	0	0	0								
15	0	0	0	0								
16	0	0	0	0								
17	0	0	0	0								
18	0	0	0	0								
19	0	0	0	0								
20	0	0	0	0								

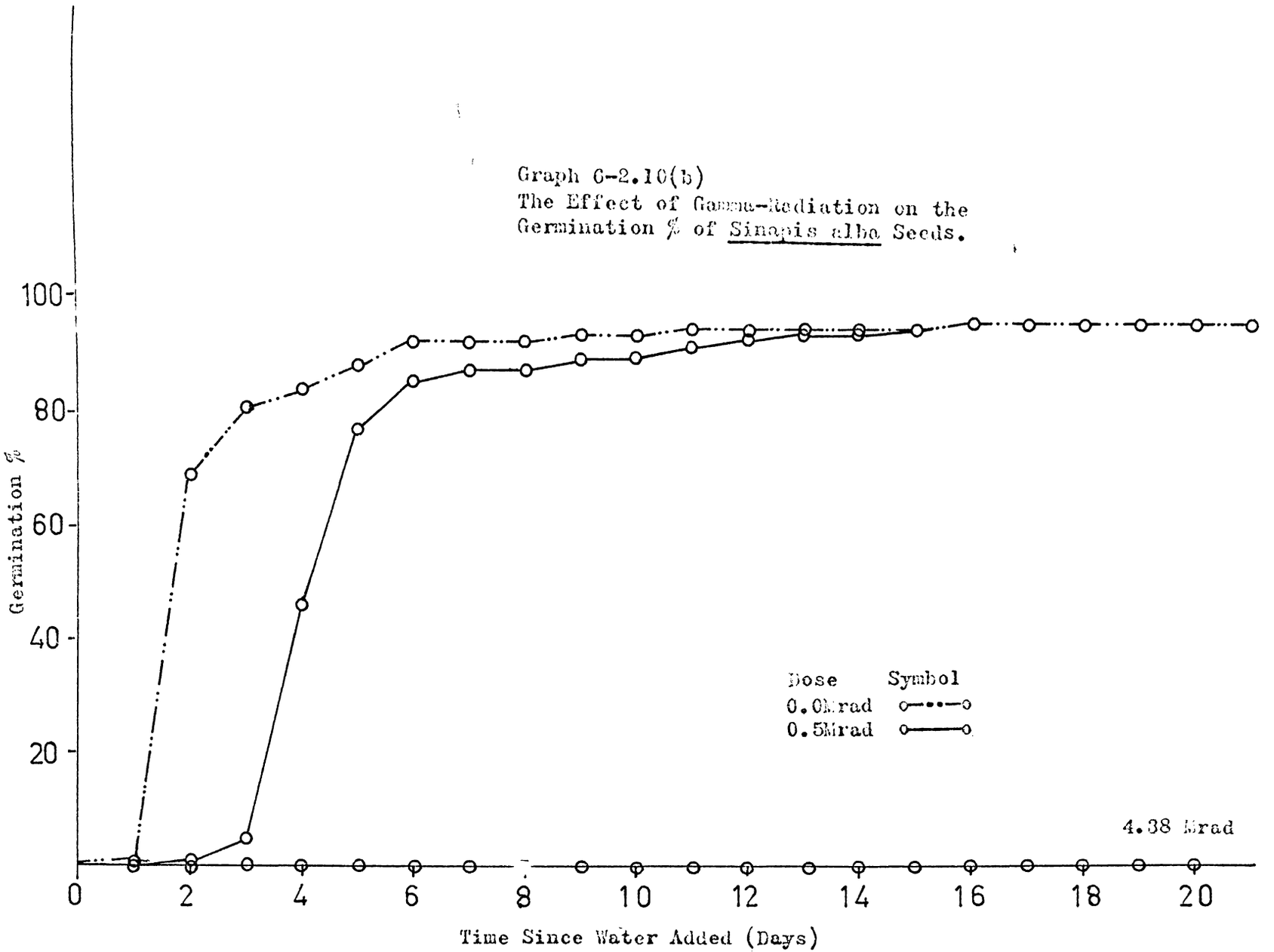
* (a) % Germinated previous 24 hours

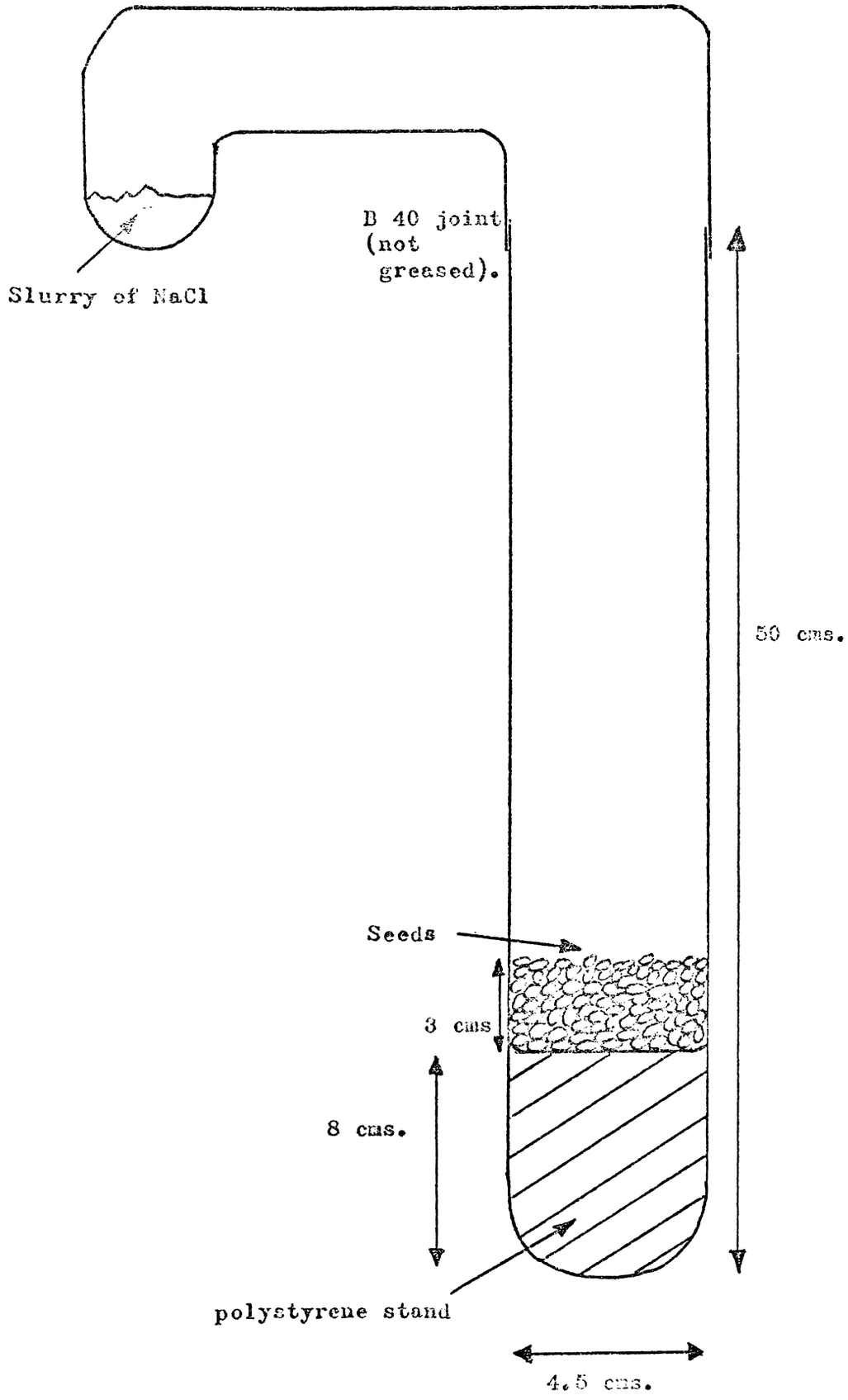
* (b) Total % germination.



Graph G-2.10(a)
The Effect of Gamma-Radiation on the Rate % Germination of Sinapis alba (White Mustard) Seeds.

Graph C-2.10(b)
The Effect of Gamma-Radiation on the Germination % of Sinapis alba Seeds.





THE APPARATUS USED TO KEEP THE R.H. CONSTANT DURING THE IRRADIATION PROCEDURES.

FIG 6-2.1

Table 6-2.11

The Effects of Gamma-Radiation on the Germination of Sinapis alba (White Mustard) Seeds.

Dose (Mrad)	0.0		0.118		0.265		1.14		3.5		10.4	
Dose Rate Mrad/hr	-		0.143		0.143		0.143		0.143		0.143	
Time Exposed (hrs)	-		0.83		2.57		8.0		24.30		70.72	
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	71	71	69	69	40	40	0	0	0	0	0	0
2	15	86	14	83	22	62	2	2	0	0	0	0
3	5	91	6	89	12	74	0	2	0	0	0	0
4	2	93	3	92	10	84	0	2	0	0	0	0
5	3	96	0	92	4	88	0	2	0	0	0	0
6	2	98	2	94	2	90	4	6	0	0	0	0
7	1	99	3	97	2	92	6	12	0	0	0	0
8	0	99	1	98	2	94	7	19	0	0	0	0
9	0	99	1	99	1	95	4	23	0	0	0	0
10	0	99	0	99	2	97	2	25	0	0	0	0
11	0	99	0	99	2	99	2	27	0	0	0	0
12	1	100	0	99	0	99	1	28	0	0	0	0
13	-	100	0	99	0	99	1	29	0	0	0	0
14	-	100	1	100	0	99	0	29	0	0	0	0
15	-	100	-	100	0	99	0	29	0	0	0	0
16	-	100	-	100	0	99	0	29	0	0	0	0
17	-	100	-	100	0	99	0	29	0	0	0	0
18	-	100	-	100	0	99	0	29	0	0	0	0
19	-	100	-	100	0	99	0	30	0	0	0	0
20	-	100	-	100	0	99	0	30	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

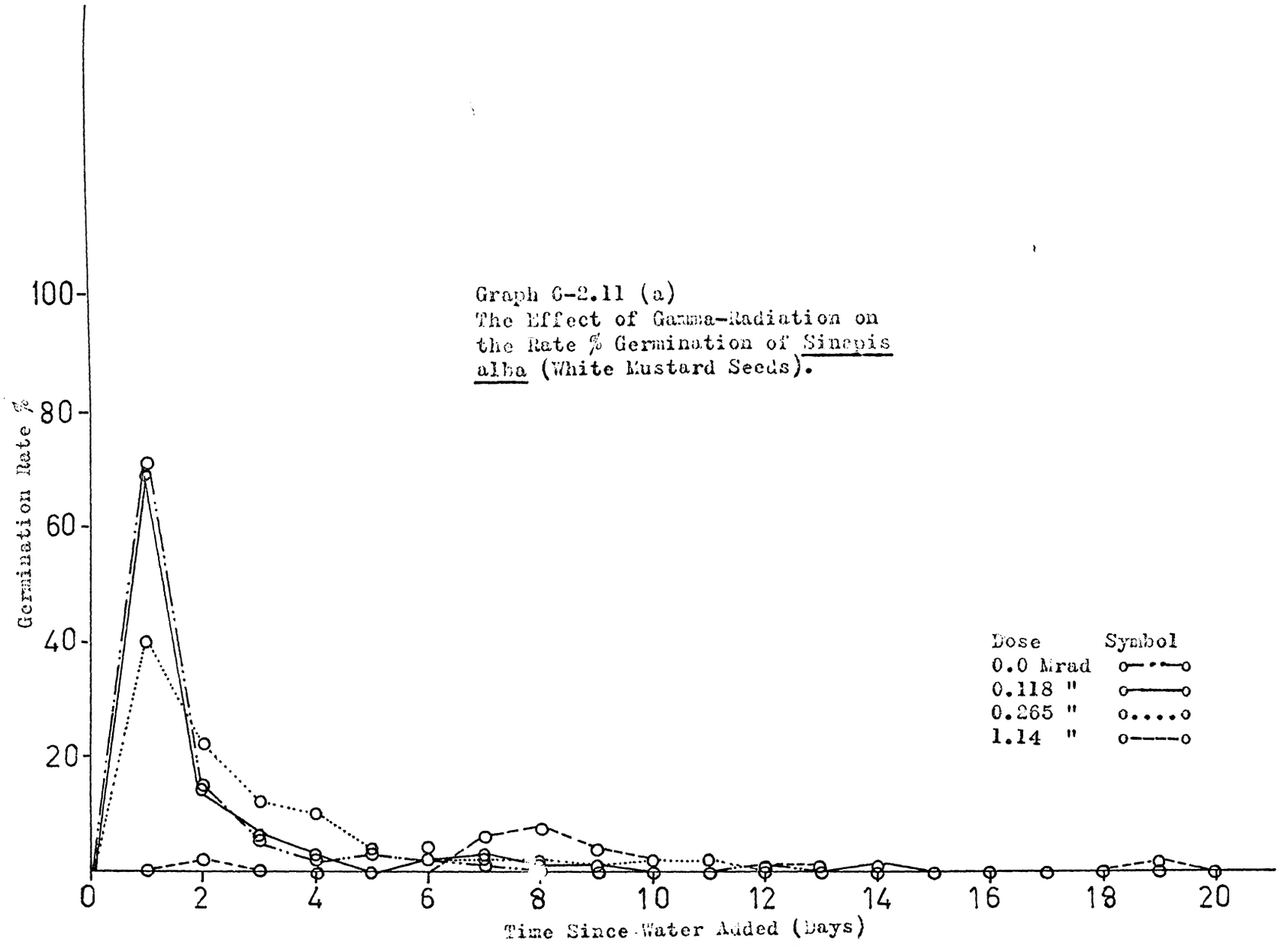
Table 6-2.11 continued

The Effects of Gamma-Radiation on the Germination of Sinapis alba (White Mustard) Seeds.

Dose (Mrad)	10.8		30.7									
Dose Rate Mrad/hr	0.143		0.143									
Time Exposed (hrs)	75.0		214.6									
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0								
2	0	0	0	0								
3	0	0	0	0								
4	0	0	0	0								
5	0	0	0	0								
6	0	0	0	0								
7	0	0	0	0								
8	0	0	0	0								
9	0	0	0	0								
10	0	0	0	0								
11	0	0	0	0								
12	0	0	0	0								
13	0	0	0	0								
14	0	0	0	0								
15	0	0	0	0								
16	0	0	0	0								
17	0	0	0	0								
18	0	0	0	0								
19	0	0	0	0								
20	0	0	0	0								

* (a) % Germinated previous 24 hours

* (b) Total % germination.



Graph G-2.11 (a)
The Effect of Gamma-Radiation on the Rate % Germination of Sinapis alba (White Mustard Seeds).

Graph 6-2.11 (b)
The Effect of Gamma-Radiation on
The Germination % of Sinapis alba
Seeds.

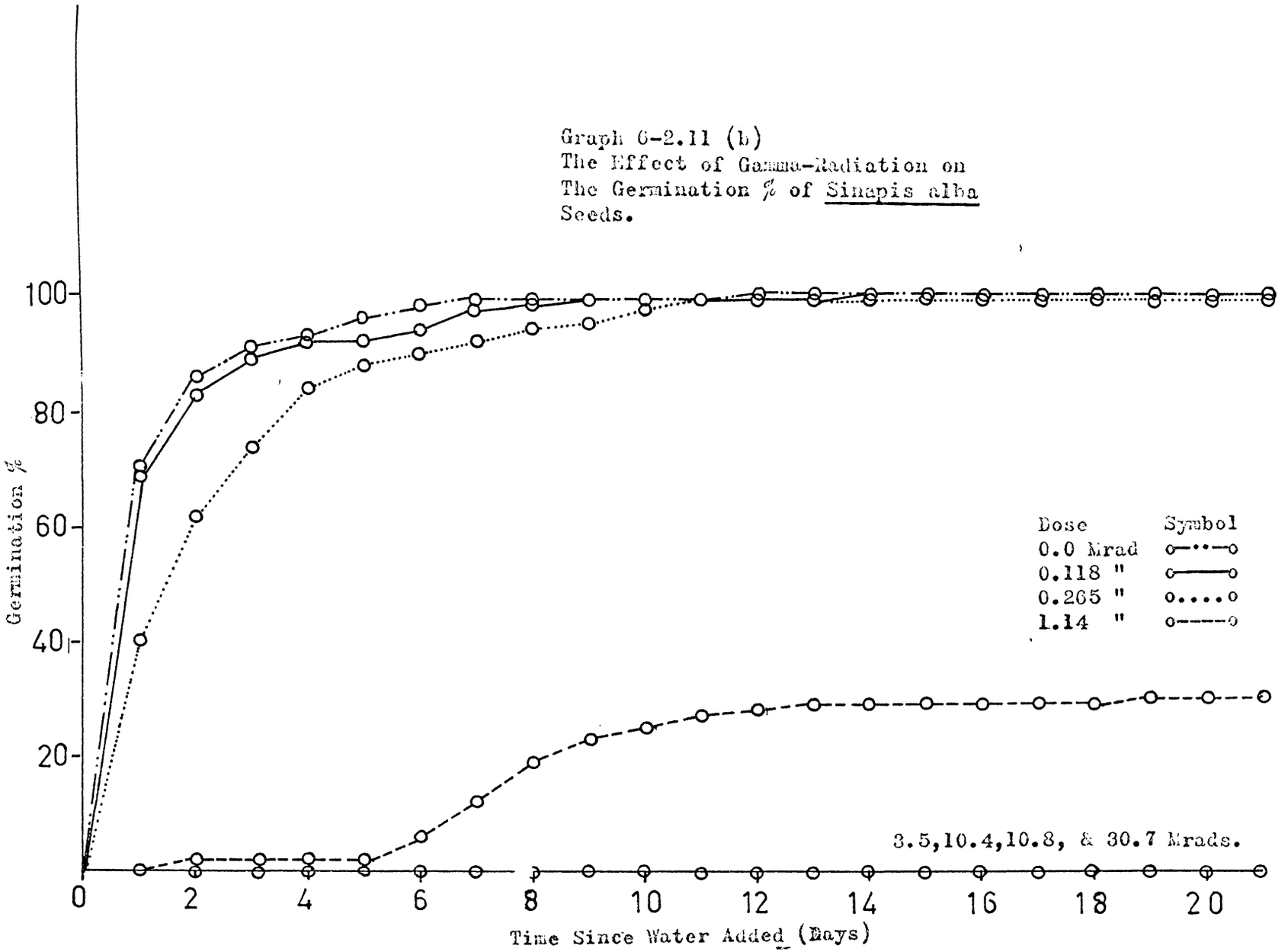


Table 6-2.12

The Effects of Gamma-radiation Without Storage on the Germination of Lettuce Seeds, Variety "White Cos"

Dose (Mrad)	0.0		0.49		0.98		2.0		5.0			
Dose Rate Mrad/hr	-		0.175		0.175		0.175		0.175			
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	100	100	0	0	0	0	0	0	0	0		
2	-	100	0	0	0	0	0	0	0	0		
3	-	100	20	20	0	0	0	0	0	0		
4	-	100	35	35	0	0	0	0	0	0		
5	-	100	20	75	2	2	0	0	0	0		
6	-	100	15	90	9	11	0	0	0	0		
7	-	100	4	94	4	15	0	0	0	0		
8	-	100	3	97	4	19	0	0	0	0		
9	-	100	0	97	4	23	0	0	0	0		
10	-	100	0	97	5	28	0	0	0	0		
11	-	100	0	97	8	36	0	0	0	0		
12	-	100	0	97	8	44	0	0	0	0		
13	-	100	0	97	8	52	0	0	0	0		
14	-	100	0	97	10	62	0	0	0	0		
15	-	100	0	97	7	69	0	0	0	0		
16	-	100	0	97	5	74	0	0	0	0		
17	-	100	0	97	4	78	0	0	0	0		
18	-	100	0	97	2	80	0	0	0	0		
19	-	100	0	97	2	82	0	0	0	0		
20	-	100	0	97	1	83	0	0	0	0		
21					3	86						

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 6-2.12
The Effects of Gamma Radiation Without Storage on the Germination of Lettuce Seeds, Variety "White Cos".

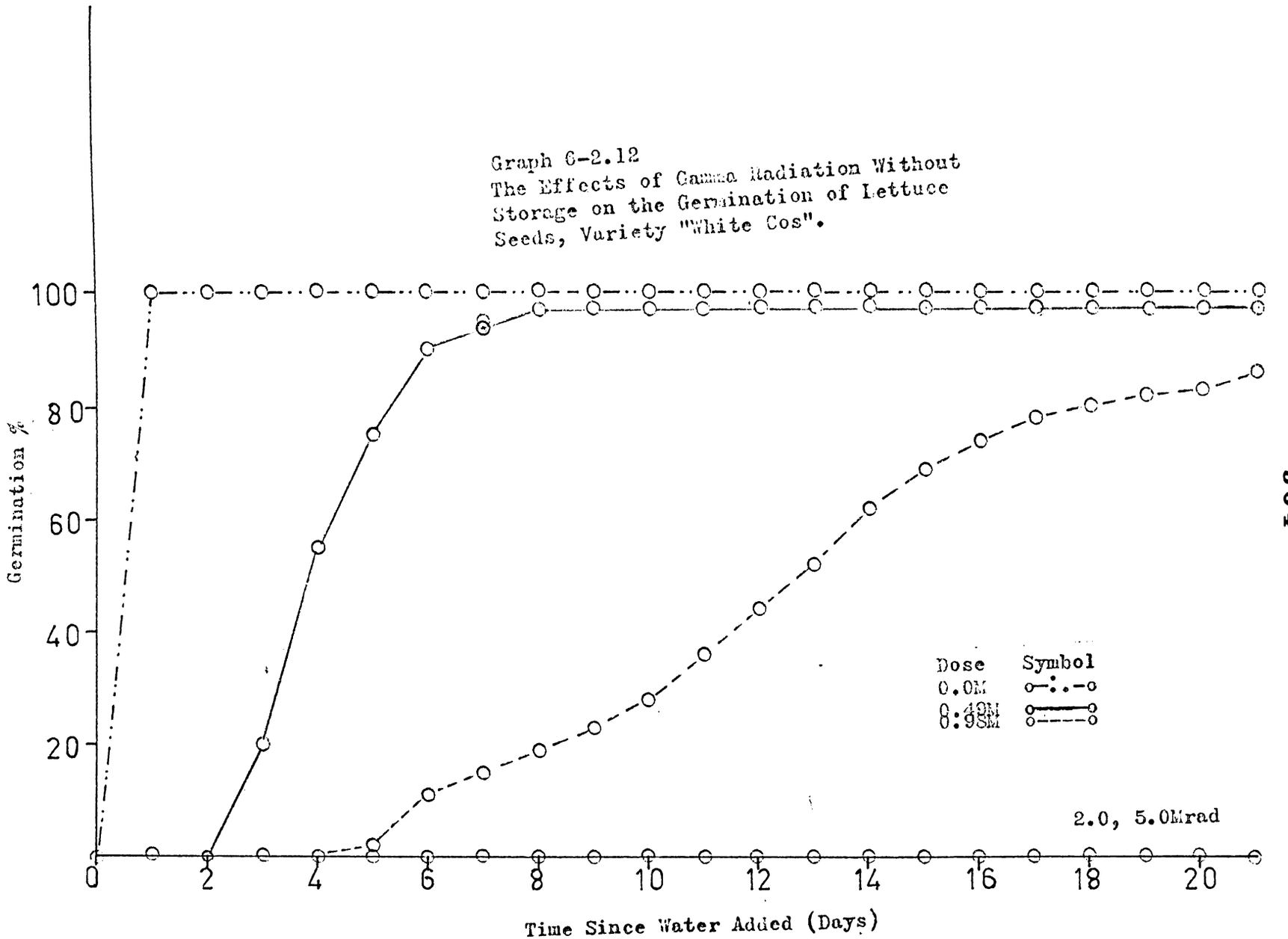


Table 6-2.13

The Effects of Gamma-Radiation Without Storage on the Germination of Lettuce Seeds, Variety "Great Lakes".

Dose (Mrad)	0.0		0.49		0.98		2.0		5.0		32.3	
Dose Rate Mrad/hr	-		0.175		0.175		0.175		0.175		0.175	
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	98	98	0	0	0	0	0	0	0	0	0	0
2	2	100	2	2	0	0	0	0	0	0	0	0
3		100	21	23	0	0	0	0	0	0	0	0
4		100	36	59	0	0	0	0	0	0	0	0
5		100	18	77	3	3	0	0	0	0	0	0
6		100	14	91	10	13	0	0	0	0	0	0
7		100	5	96	5	18	0	0	0	0	0	0
8		100	2	98	4	22	0	0	0	0	0	0
9		100	0	98	3	25	0	0	0	0	0	0
10		100	0	98	4	29	0	0	0	0	0	0
11		100	0	98	7	36	0	0	0	0	0	0
12		100	0	98	7	43	0	0	0	0	0	0
13		100	0	98	9	52	0	0	0	0	0	0
14		100	0	98	12	64	0	0	0	0	0	0
15		100	0	98	7	71	0	0	0	0	0	0
16		100	0	98	6	77	0	0	0	0	0	0
17		100	0	98	5	82	0	0	0	0	0	0
18		100	0	98	5	87	0	0	0	0	0	0
19		100	0	98	0	87	0	0	0	0	0	0
20		100	0	98	1	88	0	0	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Graph G-2.13
The Effects of Gamma Radiation Without Storage on the Germination of Lettuce Seeds, Variety "Great Lakes".

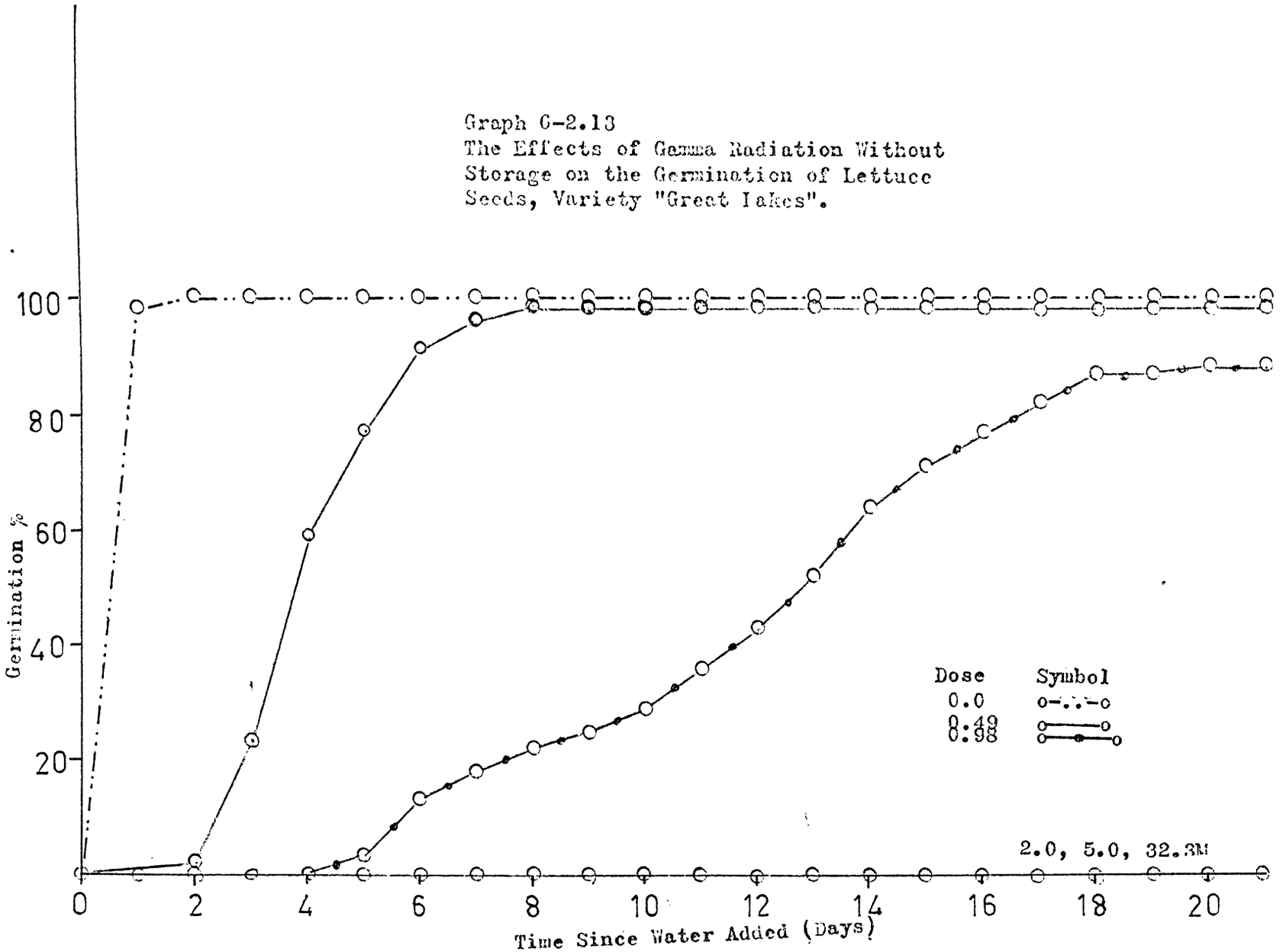
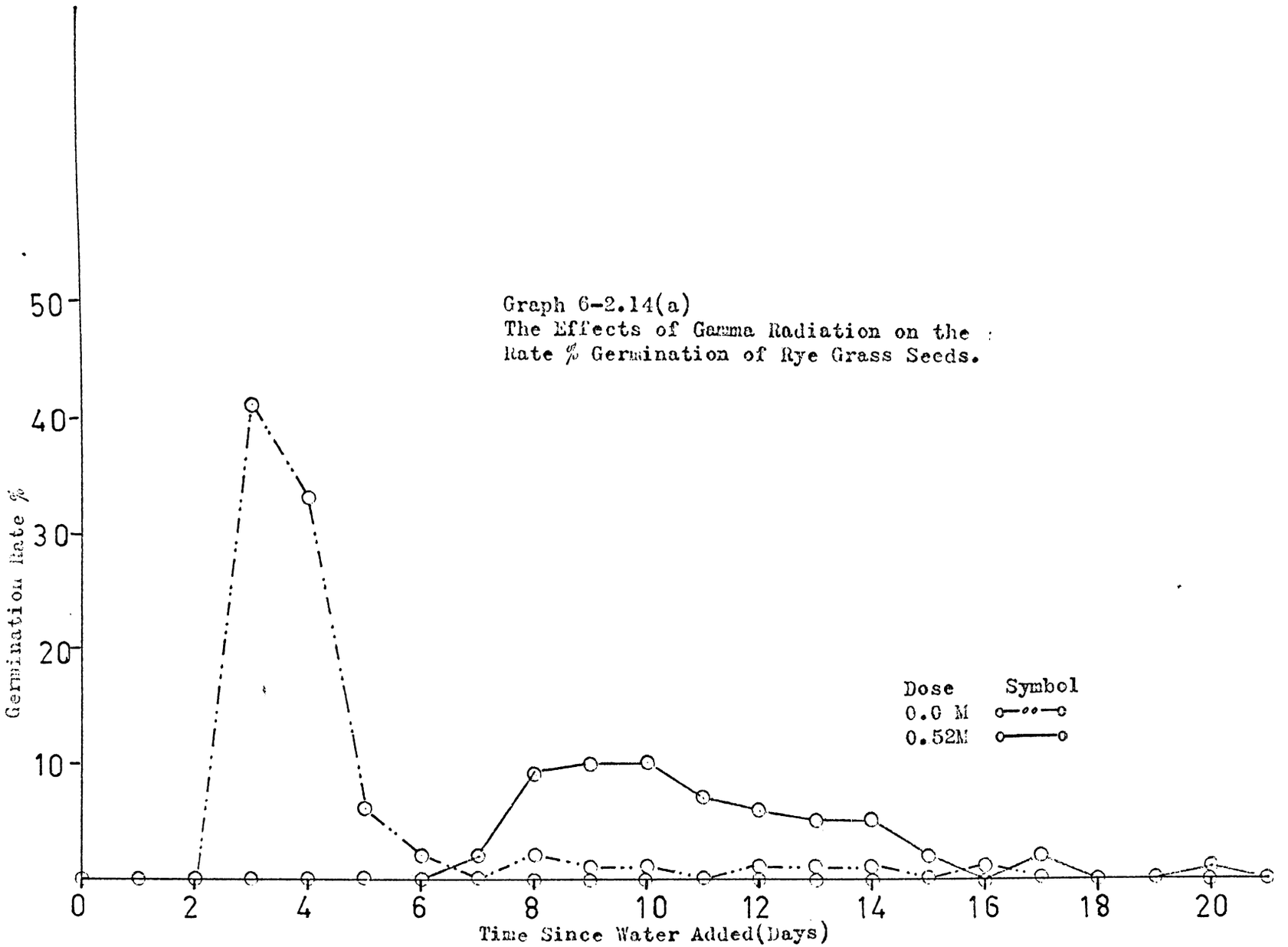


Table 6-2.14

The Effects of Gamma-Radiation Without Storage on the Germination of Ryegrass Seeds

Dose (Mrad)	0.0		0.52		2.0		5.0		10.0			
Dose Rate Mrad/hr	-		0.175		0.175		0.175		0.175			
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	41	41	0	0	0	0	0	0	0	0		
4	33	74	0	0	0	0	0	0	0	0		
5	6	80	0	0	0	0	0	0	0	0		
6	2	82	0	0	0	0	0	0	0	0		
7	0	82	2	2	0	0	0	0	0	0		
8	2	84	9	11	0	0	0	0	0	0		
9	1	85	10	21	0	0	0	0	0	0		
10	1	86	10	31	0	0	0	0	0	0		
11	0	86	7	38	0	0	0	0	0	0		
12	1	87	6	44	0	0	0	0	0	0		
13	1	88	5	49	0	0	0	0	0	0		
14	1	89	5	54	0	0	0	0	0	0		
15	0	89	2	56	0	0	0	0	0	0		
16	1	90	0	56	0	0	0	0	0	0		
17	0	90	2	58	0	0	0	0	0	0		
18	0	90	0	58	0	0	0	0	0	0		
19	0	90	0	58	0	0	0	0	0	0		
20	0	90	1	59	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.


Graph 6-2.14(a)
The Effects of Gamma Radiation on the Rate % Germination of Rye Grass Seeds.
Germination Rate %
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
Time Since Water Added(Days)
Dose
Symbol
0.0 M
0.52M

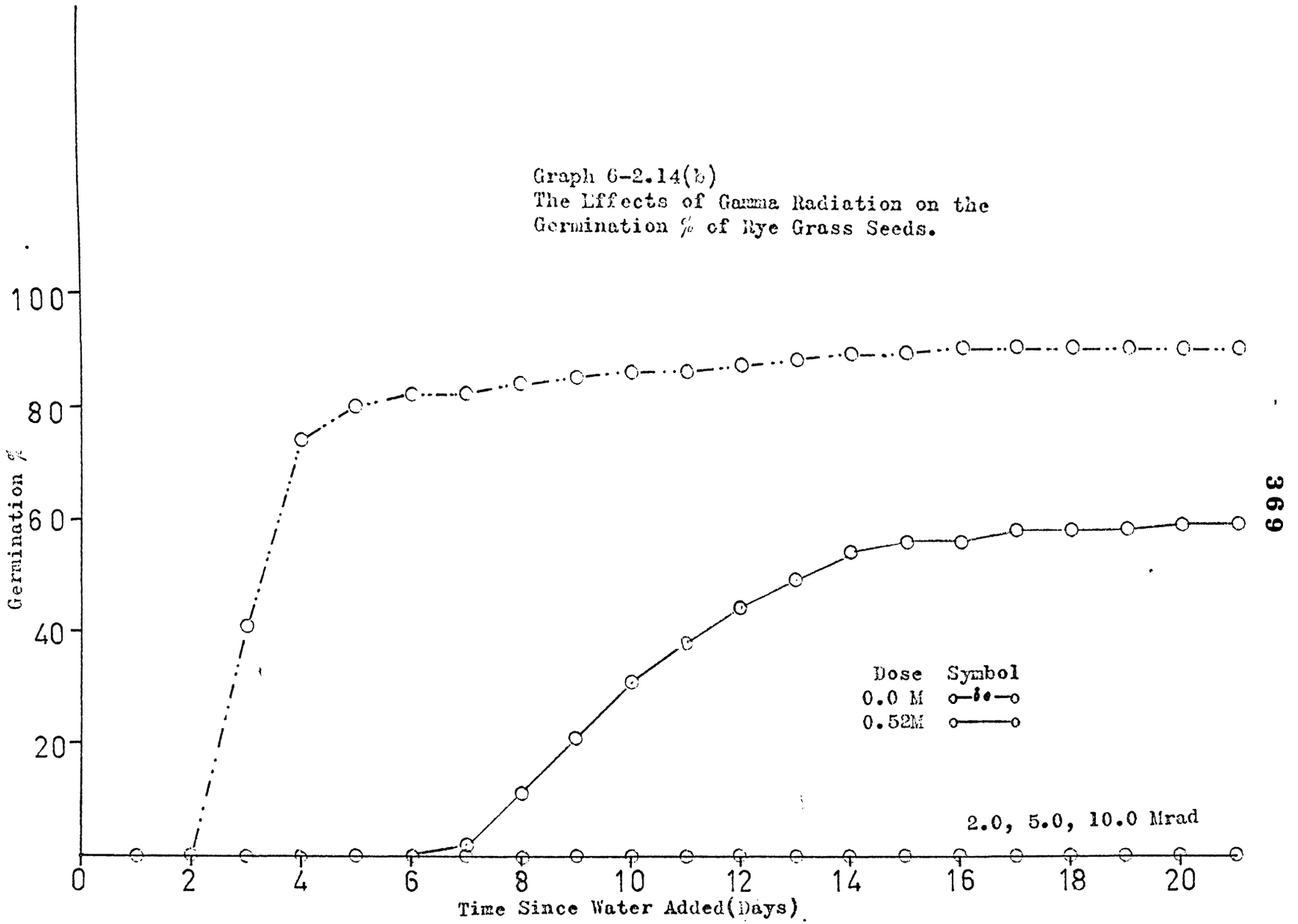

Graph 6-2.14(b)
The Effects of Gamma Radiation on the
Germination % of Rye Grass Seeds.
Germination %
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
Time Since Water Added(Days)
Dose Symbol
0.0 M
0.52M
2.0, 5.0, 10.0 Mrad

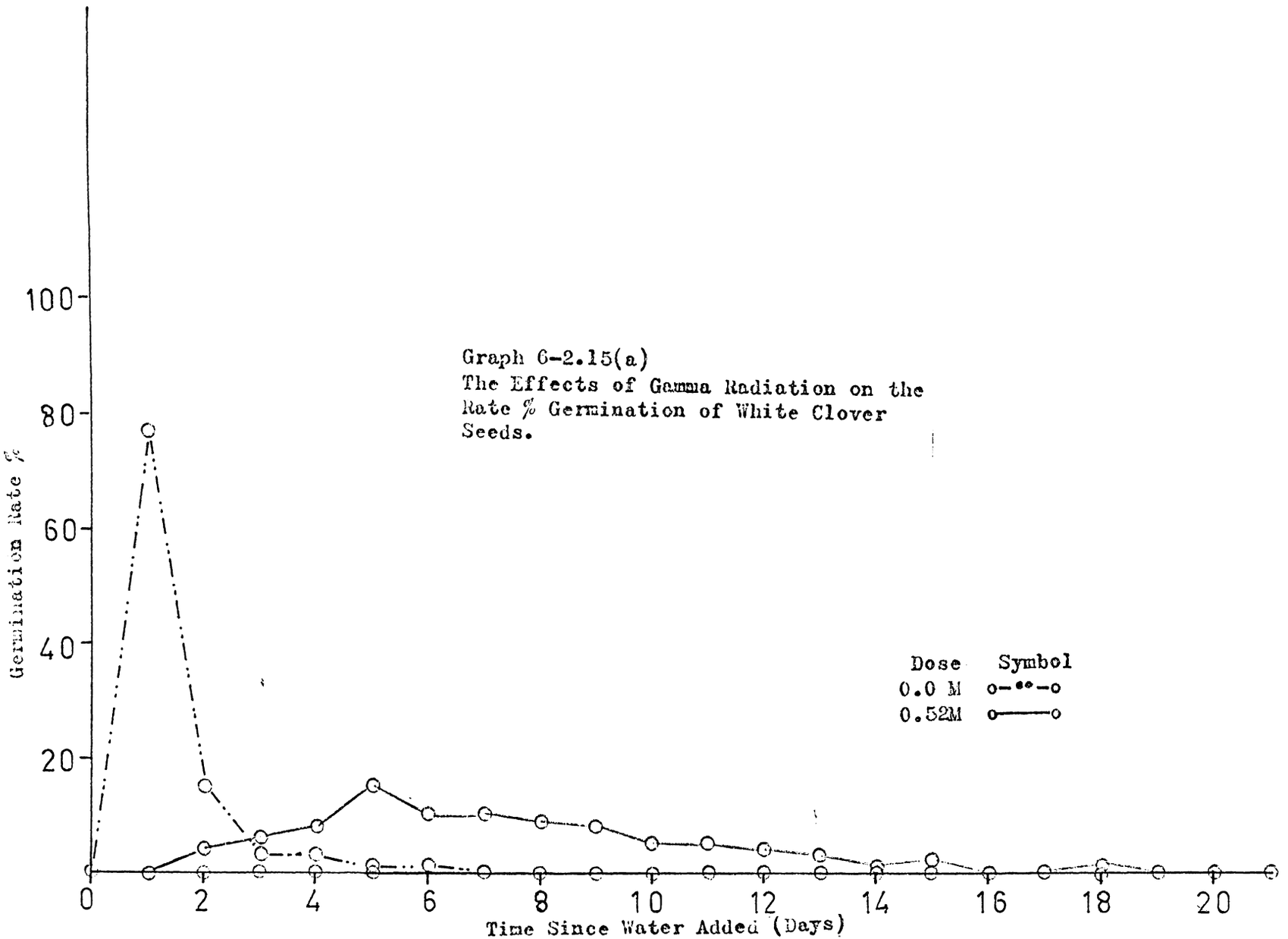
Table 6-2.15

The Effects of Gamma-Radiation Without Storage on the Germination of White Clover Seeds

Dose (Mrad)	0.0		0.52		2.0		5.0		10.0			
Dose Rate Mrad/hr	-		0.175		0.175		0.175		0.175			
Time Exposed (hrs)												
Time Stored Mths												
R.H.												
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	77	77	0	0	0	0	0	0	0	0		
2	15	92	4	4	0	0	0	0	0	0		
3	3	95	6	10	0	0	0	0	0	0		
4	3	98	8	18	0	0	0	0	0	0		
5	1	99	15	33	0	0	0	0	0	0		
6	1	100	10	43	0	0	0	0	0	0		
7	-	100	10	53	0	0	0	0	0	0		
8	-	100	9	62	0	0	0	0	0	0		
9	-	100	8	70	0	0	0	0	0	0		
10	-	100	5	75	0	0	0	0	0	0		
11	-	100	5	80	0	0	0	0	0	0		
12	-	100	4	84	0	0	0	0	0	0		
13	-	100	3	87	0	0	0	0	0	0		
14	-	100	1	88	0	0	0	0	0	0		
15	-	100	2	90	0	0	0	0	0	0		
16	-	100	0	90	0	0	0	0	0	0		
17	-	100	0	90	0	0	0	0	0	0		
18	-	100	1	91	0	0	0	0	0	0		
19	-	100	0	91	0	0	0	0	0	0		
20	-	100	0	91	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Graph 6-2.15(a)
The Effects of Gamma Radiation on the Rate % Germination of White Clover Seeds.



Graph 6-2.15(b)
The Effects of Gamma Radiation on the
Germination % of White Clover Seeds.

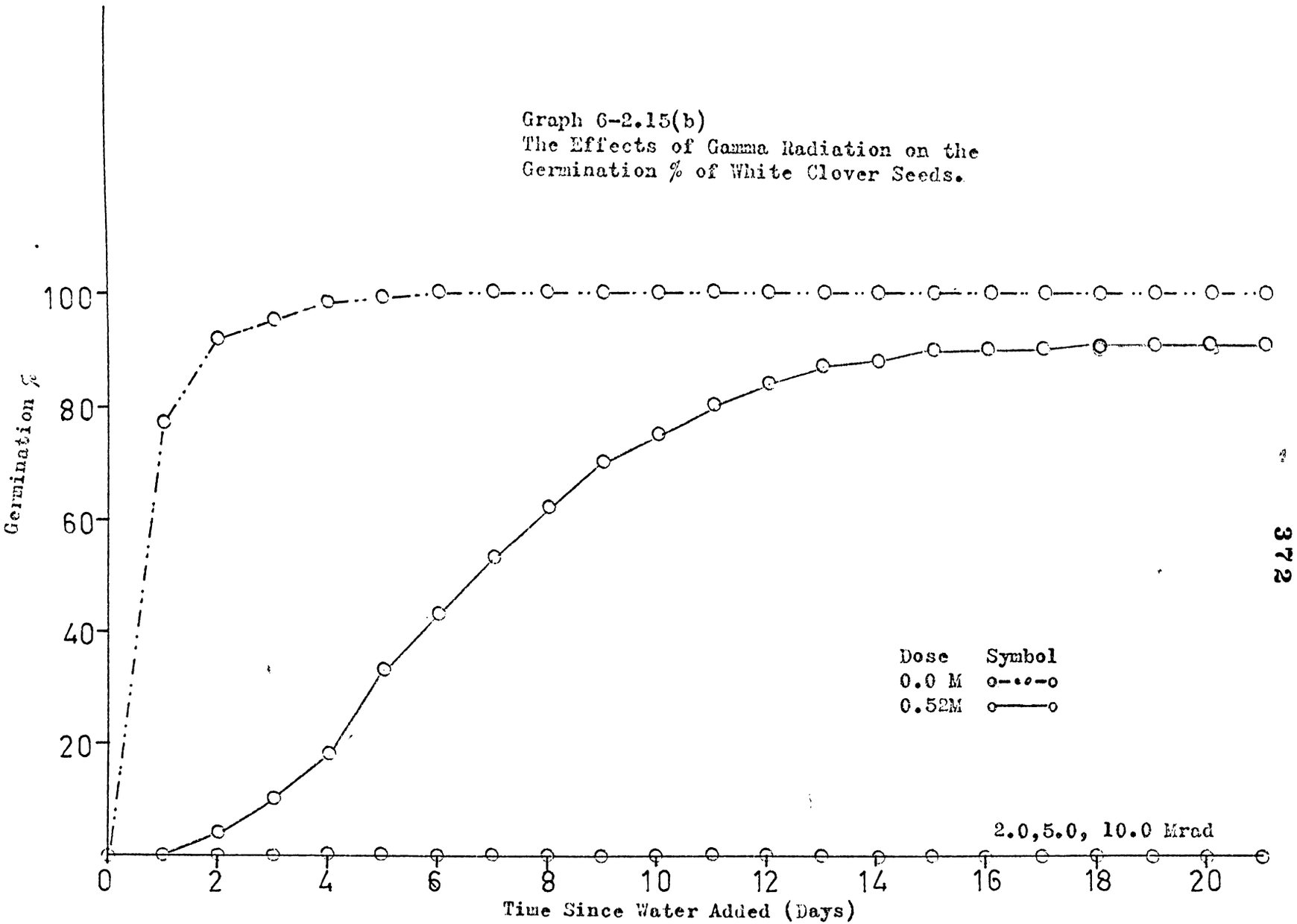


Table 7-3.1

The Effects of Storage at Various Relative Humidities for 2 months on the Germination of Broad Bean Seeds.

Seed	Broad Bean (Vicia faba)											
Dose (Mrad)	0.0		0.33		5.1							
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2 months		2 months							
R.H.	-		23%		9%							
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0						
2	0	0	0	0	0	0						
3	0	0	0	0	0	0						
4	70	70	30	30	0	0						
5	25	95	8	38	0	0						
6	5	100	10	48	0	0						
7	-	100	12	60	0	0						
8	-	100	2	62	0	0						
9	-	100	6	68	0	0						
10	-	100	2	70	0	0						
11	-	100	0	70	0	0						
12	-	100	0	70	0	0						
13	-	100	2	72	0	0						
14	-	100	0	72	0	0						
15	-	100	0	72	0	0						
16	-	100	2	74	0	0						
17	-	100	2	76	0	0						
18	-	100	4	80	0	0						
19	-	100	0	80	0	0						
20	-	100	0	80	0	0						

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 7-3.1
The Effects of Storage at Various
Relative Humidities on the Germination
% of Broad Bean Seeds (2 Months).

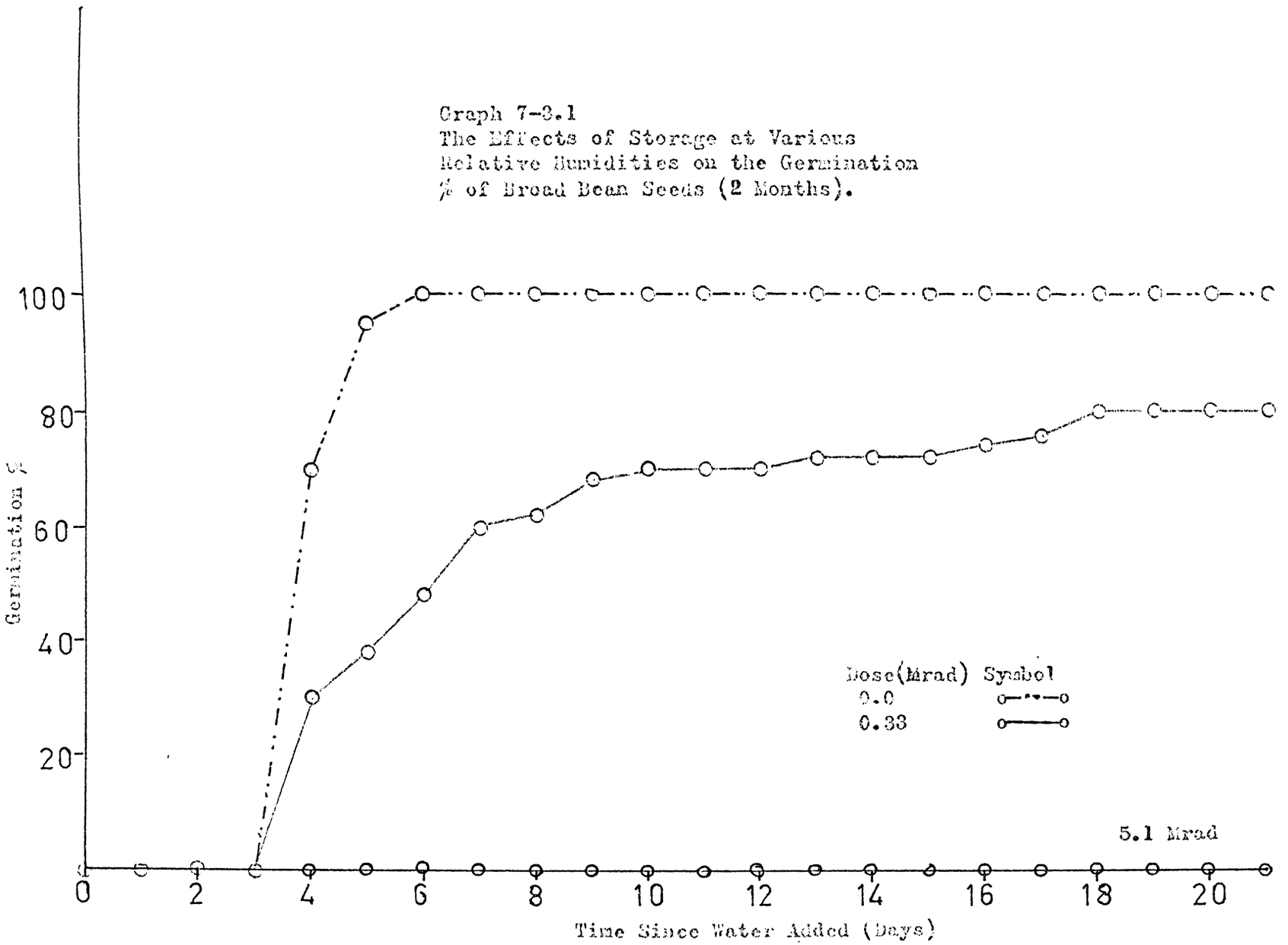


Table 7-3.2

The Effects of Storage at Various Relative Humidities for Eight Months on the Germination of Irradiated Broad Bean Seeds.

Seeds	Broad Bean											
Dose (Mrad)	0.0		0.33		5.1							
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		8 months		8 months							
R.H.	-		93%		9%							
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	10	10	0	0	0	0						
2	10	20	0	0	0	0						
3	15	35	0	0	0	0						
4	45	80	0	0	0	0						
5	5	85	0	0	0	0						
6	0	85	0	0	0	0						
7	5	90	0	0	0	0						
8	0	90	0	0	0	0						
9	0	90	0	0	0	0						
10	0	90	0	0	0	0						
11	0	90	0	0	0	0						
12	0	90	0	0	0	0						
13	0	90	0	0	0	0						
14	0	90	0	0	0	0						
15	0	90	0	0	0	0						
16	0	90	0	0	0	0						
17	0	90	0	0	0	0						
18	0	90	0	0	0	0						
19	0	90	0	0	0	0						
20	0	90	0	0	0	0						

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.3

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Broad Bean Seeds

Seed	Broad Bean											
Dose (Mrad)	0.0		0.33		0.33		5.1		5.1			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12 months		12 months		12 months		12 months			
R.H.	-		23		93		9		76			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	10	10	0	0	0	0	0	0	0	0		
4	50	60	0	0	0	0	0	0	0	0		
5	5	65	0	0	0	0	0	0	0	0		
6	5	70	0	0	0	0	0	0	0	0		
7	8	78	0	0	0	0	0	0	0	0		
8	2	80	0	0	0	0	0	0	0	0		
9	0	80	0	0	0	0	0	0	0	0		
10	0	80	0	0	0	0	0	0	0	0		
11	0	80	0	0	0	0	0	0	0	0		
12	0	80	0	0	0	0	0	0	0	0		
13	0	80	0	0	0	0	0	0	0	0		
14	0	80	0	0	0	0	0	0	0	0		
15	0	80	0	0	0	0	0	0	0	0		
16	0	80	0	0	0	0	0	0	0	0		
17	0	80	0	0	0	0	0	0	0	0		
18	0	80	0	0	0	0	0	0	0	0		
19	0	80	0	0	0	0	0	0	0	0		
20	0	80	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.4

The Effects of Storage at Room Relative Humidities for 15 Months on the Germination of Irradiated Broad Bean Seeds.

Seeds	Broad Bean (Vicia faba)											
Dose (Mrad)	0.0		0.1		1.0		3.3					
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	15 months		15 months		15 months		15 months					
R.H.	Room humidities											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0				
2	0	0	0	0	0	0	0	0				
3	10	10	0	0	0	0	0	0				
4	10	20	0	0	0	0	0	0				
5	30	50	30	30	0	0	0	0				
6	30	80	20	50	0	0	0	0				
7	15	95	20	70	0	0	0	0				
8	5	100	15	85	0	0	0	0				
9	-	100	5	90	0	0	0	0				
10	-	100	5	95	0	0	0	0				
11	-	100	5	100	0	0	0	0				
12	-	100	-	100	0	0	0	0				
13	-	100	-	100	0	0	0	0				
14	-	100	-	100	0	0	0	0				
15	-	100	-	100	0	0	0	0				
16	-	100	-	100	0	0	0	0				
17	-	100	-	100	0	0	0	0				
18	-	100	-	100	0	0	0	0				
19	-	100	-	100	0	0	0	0				
20	-	100	-	100	0	0	0	0				

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.5

The Effects of Storage at Room Relative Humidities for 18 Months on the Germination of Irradiated Broad Bean Seeds.

Seeds	Broad Bean											
Dose (Mrad)	0.0		1.0		3.3							
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	18 months		18 months		18 months							
R.H.	Room humidities											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0						
2	0	0	0	0	0	0						
3	0	0	0	0	0	0						
4	0	0	0	0	0	0						
5	0	0	0	0	0	0						
6	0	0	0	0	0	0						
7	0	0	0	0	0	0						
8	10	10	0	0	0	0						
9	5	15	0	0	0	0						
10	5	20	0	0	0	0						
11	0	20	0	0	0	0						
12	0	20	0	0	0	0						
13	0	20	0	0	0	0						
14	0	20	0	0	0	0						
15	0	20	0	0	0	0						
16	0	20	0	0	0	0						
17	0	20	0	0	0	0						
18	0	20	0	0	0	0						
19	0	20	0	0	0	0						
20	0	20	0	0	0	0						

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.6

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Cucumber Seeds.

Seed	Cucumber											
Dose (Mrad)	0.0		0.51		0.51		5.1		5.1			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2 months		2 months		2 months		2 months			
R.H.	-		33		86		33		86			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	85	85	65	65	10	10	0	0	0	0		
3	15	100	20	85	75	85	0	0	0	0		
4	-	100	5	90	0	85	0	0	0	0		
5	-	100	10	100	0	85	0	0	0	0		
6	-	100	-	100	0	85	0	0	0	0		
7	-	100	-	100	0	85	0	0	0	0		
8	-	100	-	100	0	85	0	0	0	0		
9	-	100	-	100	0	85	0	0	0	0		
10	-	100	-	100	0	85	0	0	0	0		
11	-	100	-	100	0	85	0	0	0	0		
12	-	100	-	100	0	85	0	0	0	0		
13	-	100	-	100	0	85	0	0	0	0		
14	-	100	-	100	0	85	0	0	0	0		
15	-	100	-	100	0	85	0	0	0	0		
16	-	100	-	100	0	85	0	0	0	0		
17	-	100	-	100	0	85	0	0	0	0		
18	-	100	-	100	0	85	0	0	0	0		
19	-	100	-	100	0	85	0	0	0	0		
20	-	100	-	100	0	85	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 7-8.6
The Effects of Storage at Various Relative Humidities for 2 Months on The Germination % of Irradiated Cucumber Seeds.

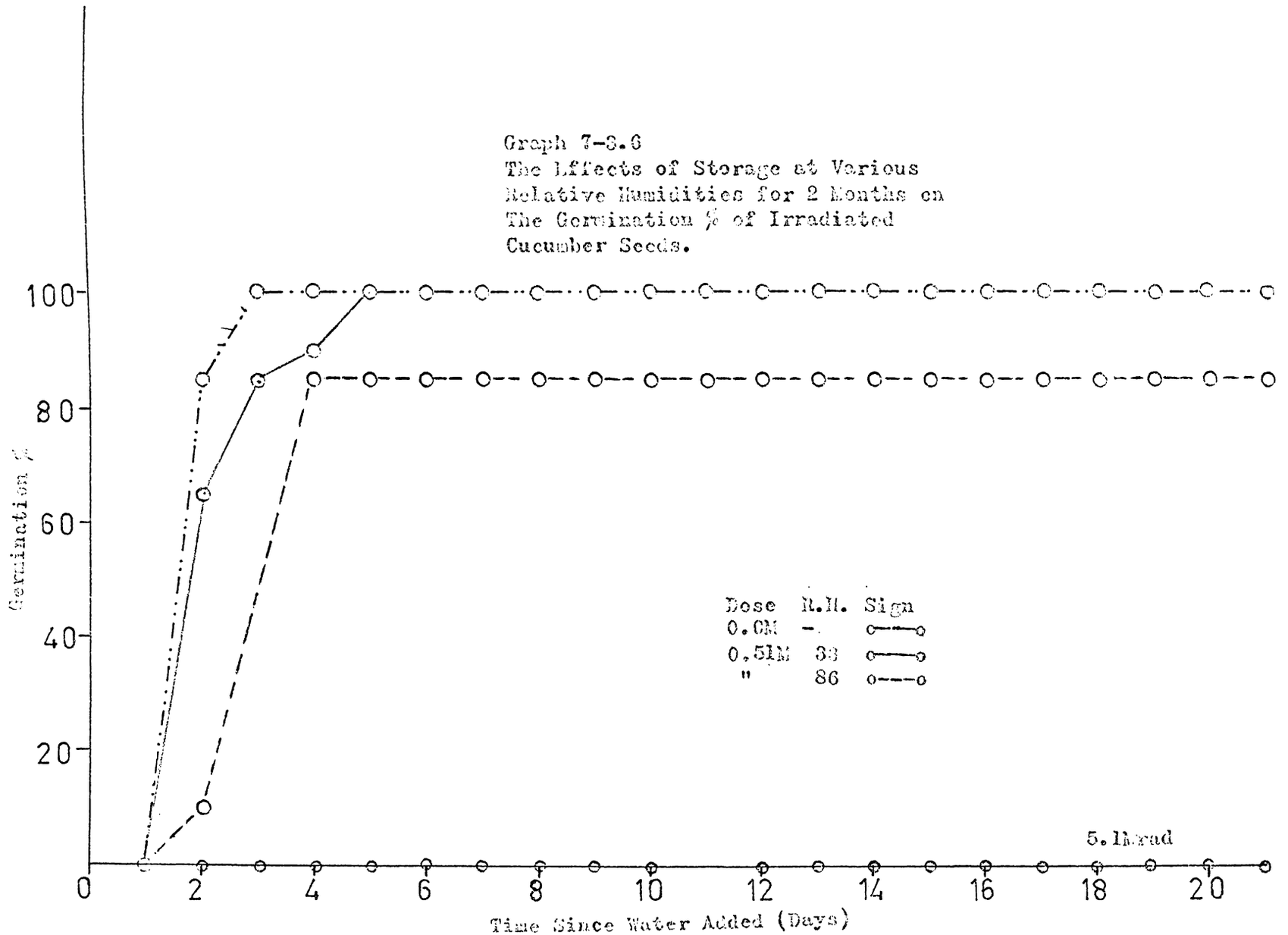


Table 7-3.7

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Cucumber Seeds

Seed	Cucumber											
Dose (Mrad)	0.0		0.51		0.51		5.1		5.1			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12 months		12 months		12 months		12 months			
R.H.	-		33		86		33		86			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	84	84	8	8	0	0	0	0	0	0		
3	8	92	76	84	0	0	0	0	0	0		
4	8	100	8	92	0	0	0	0	0	0		
5	-	100	0	92	0	0	0	0	0	0		
6	-	100	0	92	0	0	0	0	0	0		
7	-	100	2	94	0	0	0	0	0	0		
8	-	100	2	96	0	0	0	0	0	0		
9	-	100	0	96	0	0	0	0	0	0		
10	-	100	0	96	0	0	0	0	0	0		
11	-	100	0	96	0	0	0	0	0	0		
12	-	100	0	96	0	0	0	0	0	0		
13	-	100	2	98	0	0	0	0	0	0		
14	-	100	0	98	0	0	0	0	0	0		
15	-	100	0	98	0	0	0	0	0	0		
16	-	100	0	98	0	0	0	0	0	0		
17	-	100	0	98	0	0	0	0	0	0		
18	-	100	0	98	0	0	0	0	0	0		
19	-	100	0	98	0	0	0	0	0	0		
20	-	100	0	98	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.8

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Egg Plant Seeds.

Seeds	Egg Plant											
Dose (Mrad)	0.0		0.51		0.51		0.51		5.00		5.00	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2 months		2 months		2 months		2 months		2 months	
R.H.	-		9		55		97		33		93	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	1	1
2	0	0	0	0	0	0	0	0	0	0	2	3
3	0	0	0	0	1	1	0	0	0	0	0	3
4	0	0	0	0	0	1	0	0	0	0	0	3
5	1	1	0	0	0	1	0	0	0	0	0	3
6	7	8	0	0	0	1	0	0	0	0	0	3
7	15	23	0	0	0	1	0	0	0	0	0	3
8	20	43	0	0	0	1	0	0	0	0	0	3
9	11	54	3	3	0	1	0	0	0	0	0	3
10	8	62	3	6	1	2	2	2	0	0	0	3
11	1	63	6	12	4	6	4	6	0	0	0	3
12	4	67	5	17	5	11	0	6	0	0	0	3
13	5	72	6	23	5	16	3	9	0	0	0	3
14	1	73	6	29	5	21	3	12	0	0	0	3
15	1	74	4	34	4	25	2	14	0	0	0	3
16	1	75	4	38	4	29	2	16	0	0	0	3
17	0	75	2	40	2	31	2	18	0	0	0	3
18	1	76	3	43	3	34	0	18	0	0	0	3
19	2	78	1	44	1	35	1	19	0	0	0	3
20	3	81	0	44	1	36	0	19	0	0	0	3

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.9

The Effects of Storage at Various Relative Humidities for 8 Months on the Germination of Irradiated Egg Plant Seeds

Seeds	Egg Plant											
Dose (Mrad)	0.0		0.51		0.51		0.51		5.0		5.0	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		8 months		8 months		8 months		8 months		8 months	
R.H.	-		9		55		97		33		93	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	1	1	1	1	0	0	1	1
2	0	0	0	0	0	1	0	1	1	1	0	1
3	0	0	0	0	0	1	0	1	1	2	0	1
4	1	1	0	0	0	1	0	1	1	3	0	1
5	1	2	0	0	0	1	0	1	0	3	0	1
6	2	4	0	0	0	1	0	1	0	3	0	1
7	2	6	0	0	0	1	0	1	0	3	0	1
8	5	11	0	0	0	1	0	1	0	3	0	1
9	4	15	0	0	0	1	0	1	0	3	0	1
10	23	38	0	0	0	1	0	1	0	3	0	1
11	9	47	0	0	1	2	0	1	0	3	0	1
12	8	55	0	0	0	2	0	1	0	3	0	1
13	3	58	1	1	0	2	0	1	0	3	0	1
14	4	62	4	5	2	4	0	1	0	3	0	1
15	2	64	5	10	2	6	0	1	0	3	0	1
16	1	65	9	19	4	10	0	1	0	3	0	1
17	3	68	6	25	1	11	0	1	0	3	0	1
18	0	68	3	28	2	13	0	1	0	3	0	1
19	1	69	3	31	1	14	0	1	0	3	0	1
20	0	69	0	31	0	14	0	1	0	3	0	1

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.10

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Egg Plant Seeds.

Seeds — Egg Plant

Dose (Mrad)	0.0		0.51		0.51		0.51		5.0		5.0	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12 months		12 months		12 months		12 months		12 months	
R.H.	-		9		55		97		33		93	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	2	2	3	3
2	0	0	0	0	0	0	0	0	0	2	1	4
3	0	0	0	0	0	0	0	0	1	3	0	4
4	0	0	0	0	0	0	0	0	1	4	0	4
5	1	1	0	0	0	0	0	0	0	4	0	4
6	3	4	0	0	0	0	0	0	0	4	0	4
7	7	11	0	0	0	0	0	0	0	4	0	4
8	9	20	0	0	0	0	0	0	0	4	0	4
9	2	22	0	0	0	0	0	0	0	4	0	4
10	3	25	0	0	0	0	0	0	0	4	0	4
11	10	35	1	1	0	0	0	0	0	4	0	4
12	14	49	1	2	1	1	0	0	0	4	0	4
13	2	51	2	4	1	2	0	0	0	4	0	4
14	5	56	4	8	6	8	0	0	0	4	0	4
15	8	64	6	14	9	17	0	0	0	4	0	4
16	6	70	3	17	5	22	0	0	0	4	0	4
17	5	75	3	20	3	25	0	0	0	4	0	4
18	3	78	2	22	3	28	0	0	0	4	0	4
19	3	81	2	24	2	30	0	0	0	4	1	5
20	1	82	1	25	0	30	0	0	0	4	0	5

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.11

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Oat Seeds.

Seeds	Oat											
Dose (Mrad)	0.0		0.51		0.51		5.10		5.10		5.10	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2 months		2 months		2 months		2 months		2 months	
R.H.	-		33		76		33		55		76	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	29	29	0	0	4	4	0	0	0	0	0	0
3	21	50	2	2	6	10	0	0	0	0	0	0
4	8	58	1	3	10	20	0	0	0	0	0	0
5	2	60	2	5	1	21	0	0	0	0	0	0
6	1	61	0	5	0	21	0	0	0	0	0	0
7	4	65	1	6	1	22	0	0	0	0	0	0
8	0	65	2	8	0	22	0	0	0	0	0	0
9	0	65	2	10	0	22	0	0	0	0	0	0
10	0	65	0	10	2	24	0	0	0	0	0	0
11	1	66	0	10	0	24	0	0	0	0	0	0
12	0	66	2	12	1	25	0	0	0	0	0	0
13	1	67	0	12	1	26	0	0	0	0	0	0
14	0	67	0	12	0	26	0	0	0	0	0	0
15	0	67	0	12	2	28	0	0	0	0	0	0
16	0	67	0	12	1	29	0	0	0	0	0	0
17	0	67	0	12	0	29	0	0	0	0	0	0
18	0	67	0	12	0	29	0	0	0	0	0	0
19	0	67	0	12	1	30	0	0	0	0	0	0
20	0	67	0	12	0	30	0	0	0	0	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 7-3.11
The Effects of Storage at Various Relative Humidities for 2 Months on the Germination % of Irradiated Oat Seeds.

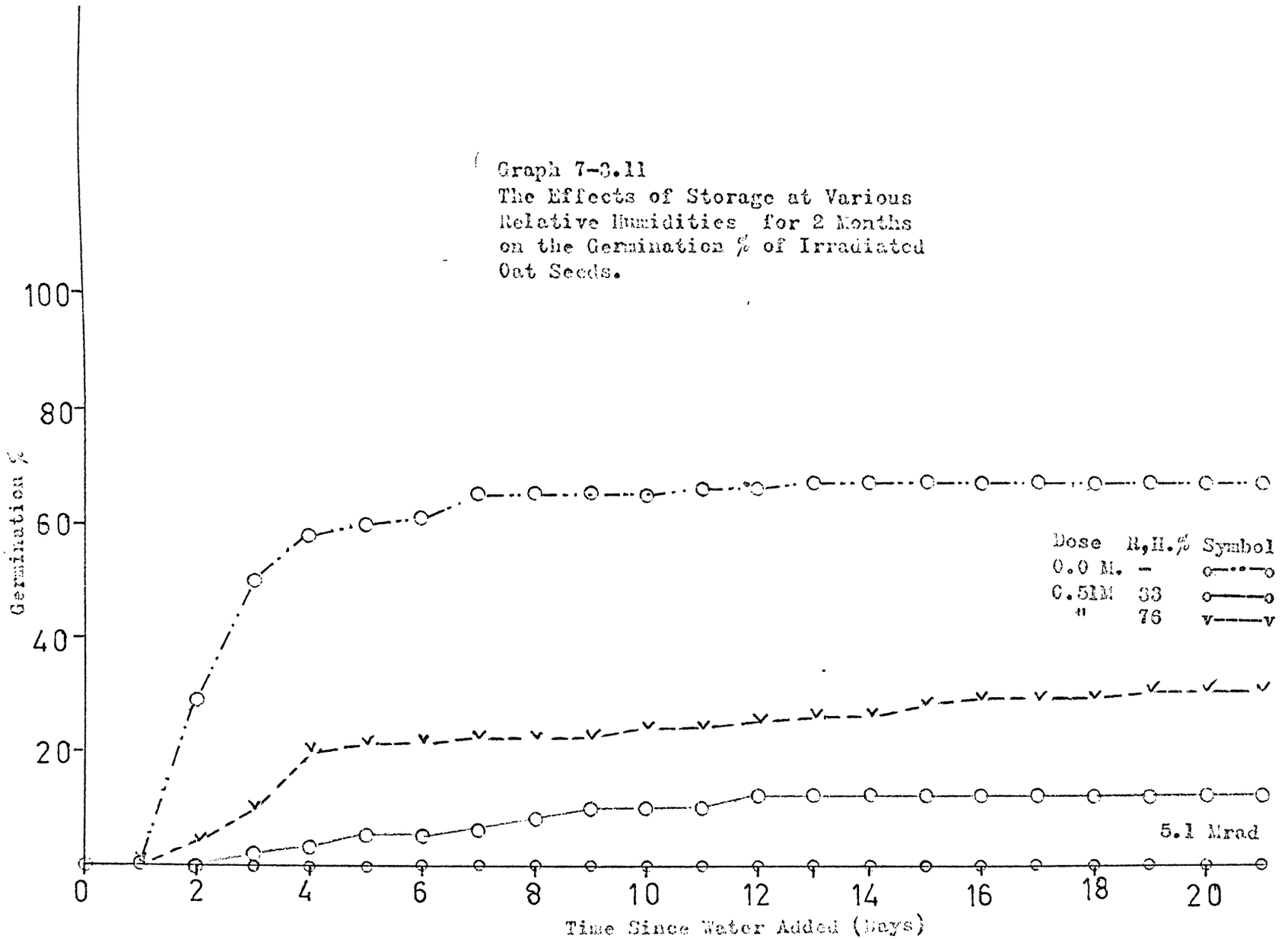


Table 7-3.12

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Oat Seeds.

Seeds: Oat

Dose (Mrad)	0.0		0.51		0.51		5.1		5.1		5.1	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12 months		12 months		12 months		12 months		12months	
R.H.	-		33		76		33		55		76	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	6	6	0	0	0	0	0	0	0	0	0	0
3	11	17	16	16	0	0	0	0	0	0	0	0
4	2	19	4	20	0	0	0	0	0	0	0	0
5	4	23	1	21	0	0	0	0	0	0	0	0
6	3	26	1	22	0	0	0	0	0	0	0	0
7	2	28	1	23	0	0	0	0	0	0	0	0
8	2	30	1	24	0	0	0	0	0	0	0	0
9	0	30	0	24	0	0	0	0	0	0	0	0
10	0	30	0	24	0	0	0	0	0	0	0	0
11	0	30	0	24	0	0	0	0	0	0	0	0
12	0	30	0	24	0	0	0	0	0	0	0	0
13	0	30	0	24	0	0	0	0	0	0	0	0
14	0	30	0	24	0	0	0	0	0	0	0	0
15	0	30	0	24	0	0	0	0	0	0	0	0
16	0	30	0	24	0	0	0	0	0	0	0	0
17	0	30	0	24	0	0	0	0	0	0	0	0
18	0	30	0	24	0	0	0	0	0	0	0	0
19	0	30	0	24	0	0	0	0	0	0	0	0
20	0	30	0	24	0	0	0	0	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Graph 7-3.12
The effects of Storage at Various R.H. for 12 Months on the Germination of Irradiated Oat Seeds.

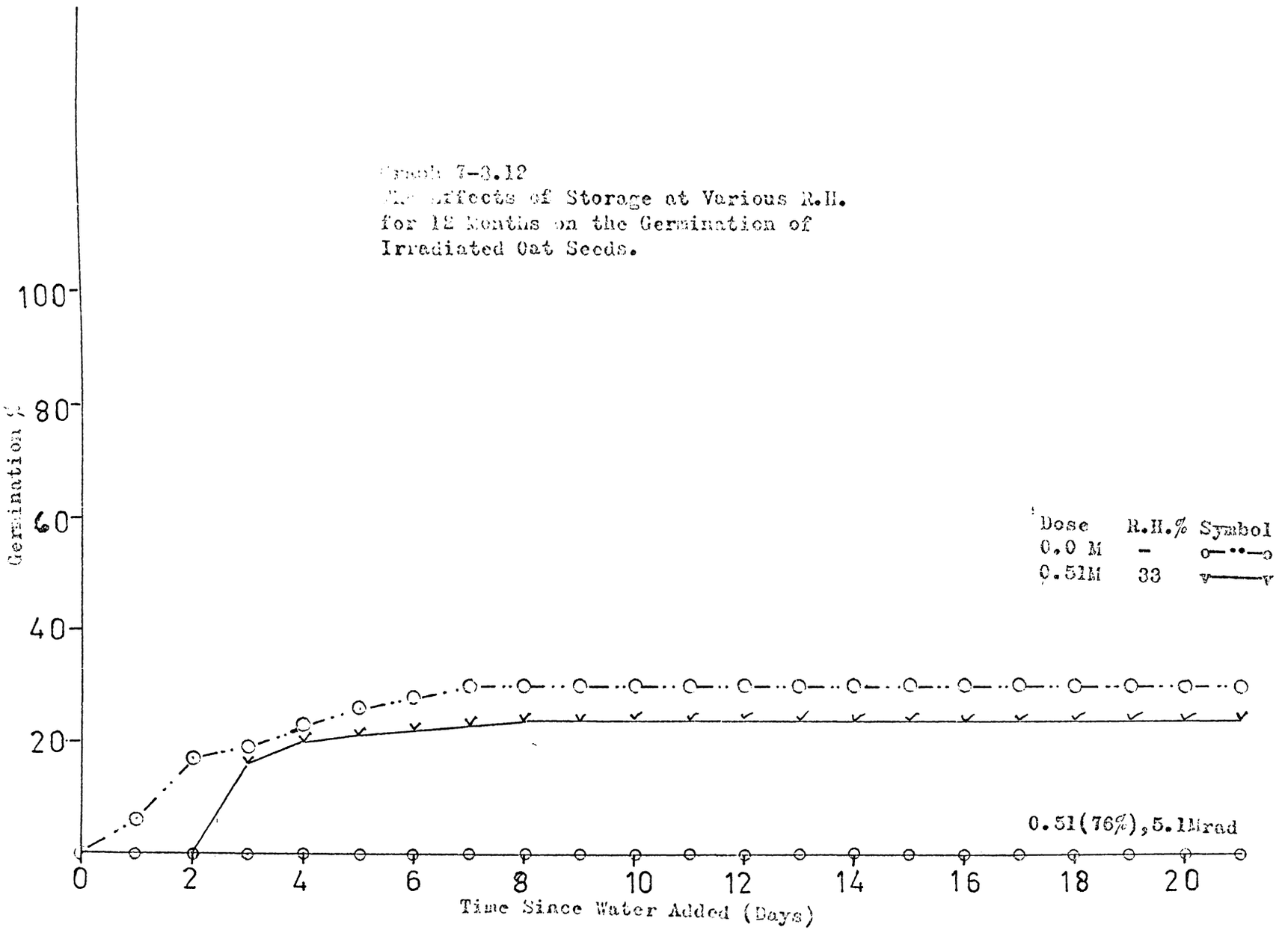


Table 7-3.13

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Lettuce Seeds (Variety "Webbs Wonderful")

Seed	Lettuce - Var. "Webbs Wonderful											
Dose (Mrad)	0.0		0.51		0.51		0.51		0.51		0.51	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2 months		2 months		2 months		2 months		2 months	
R.H.	-		9		23		45		55		76	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	91	91	0	0	0	0	0	0	0	0	0	0
2	9	100	0	0	0	0	0	0	0	0	0	0
3	-	100	4	4	20	20	12	12	3	3	1	1
4	-	100	8	12	4	24	9	21	4	7	1	2
5	-	100	1	13	10	34	9	30	3	10	6	8
6	-	100	0	13	5	39	2	32	1	11	8	16
7	-	100	8	21	5	44	5	37	0	11	3	19
8	-	100	2	23	5	49	5	42	2	13	0	19
9	-	100	1	24	1	50	0	42	4	17	0	19
10	-	100	3	27	3	53	3	45	1	18	7	26
11	-	100	0	27	2	55	2	47	0	18	3	29
12	-	100	0	27	1	56	1	48	0	18	0	29
13	-	100	0	27	3	59	0	48	3	21	2	31
14	-	100	2	29	1	60	0	48	0	21	2	33
15	-	100	2	31	0	60	0	48	0	21	1	34
16	-	100	0	31	0	60	0	48	0	21	1	35
17	-	100	2	33	0	60	0	48	1	22	1	36
18	-	100	1	34	0	60	0	48	0	22	0	36
19	-	100	1	35	0	60	0	48	0	22	2	38
20	-	100	0	35	1	61	0	48	1	23	2	40

* (a) % Germinated previous 24 hours

* (b) Total % germination.

/continued...

Table 7-3.13 continued

The Effects of Storage at Various Relative Humidities for 1 Months on the Germination of Irradiated Lettuce Seeds (Variety "Webbs Wonderful")

Seeds	Lettuce Var. "Webbs Wonderful"											
Dose (Mrad)	0.51											
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	2 months											
R.H.	97											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0										
2	0	0										
3	0	0										
4	2	2										
5	5	7										
6	7	14										
7	9	23										
8	5	28										
9	8	36										
10	0	36										
11	5	41										
12	2	43										
13	0	43										
14	1	44										
15	1	45										
16	2	47										
17	2	49										
18	0	49										
19	0	49										
20	1	50										

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 7-3.13
The Effects of Storage at Various Relative Humidities for 2 Months on The Germination % of Irradiated Lettuce Seeds (Webbs Wonderful).

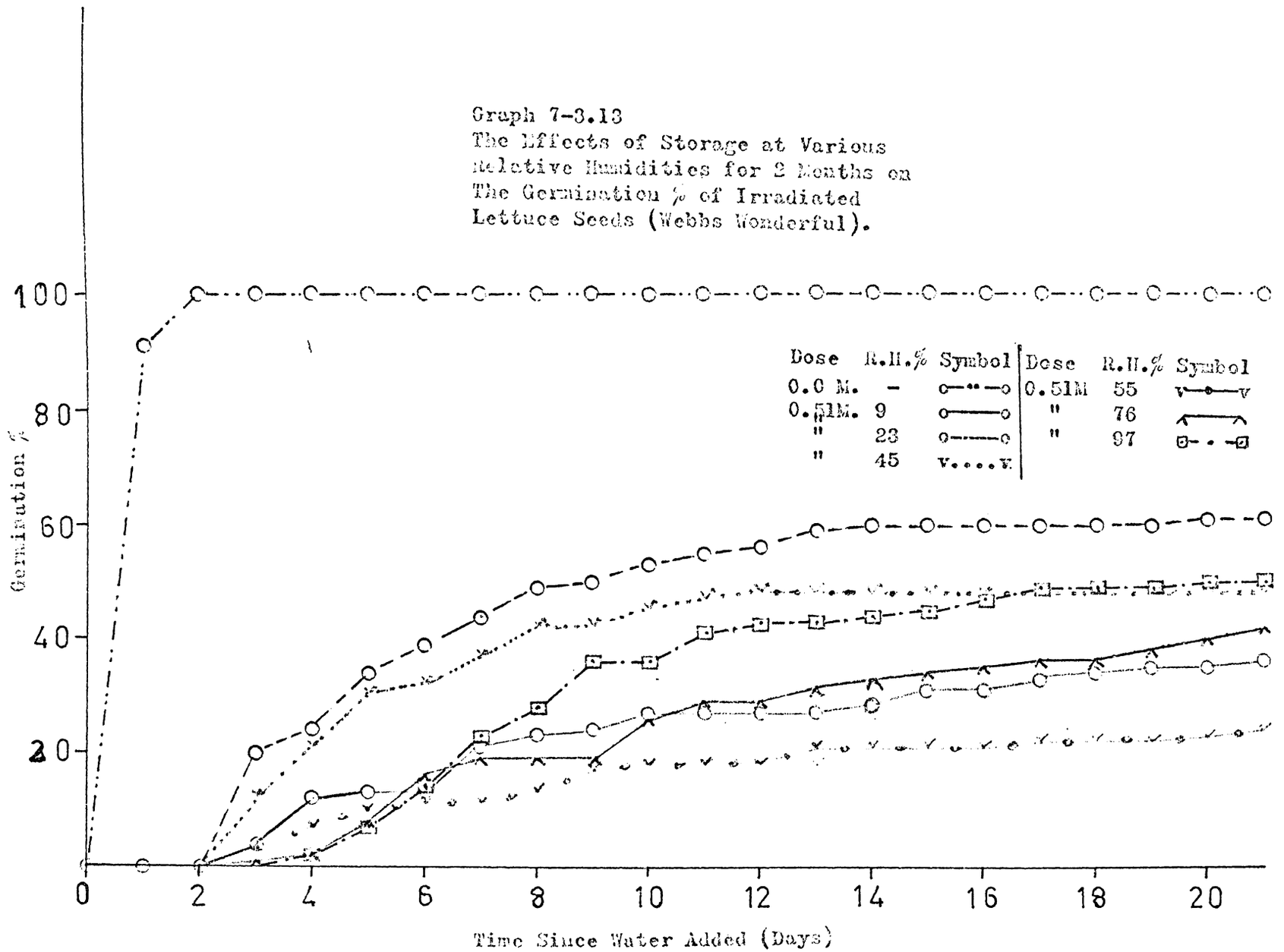


Table 7-3.14

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Lettuce Seeds (Variety "Webbs Wonderful")

Seeds — Lettuce Var. "Webbs Wonderful" (Lactuca sativa)

Dose (Mrad)	0.0		5.0		5.0		5.0		5.0		5.0	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2 months		2 months		2 months		2 months		2 months	
R.H.	-		9		23		45		55		76	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	91	91	0	0	0	0	0	0	0	0	0	0
2	9	100	0	0	0	0	0	0	0	0	0	0
3	-	100	0	0	0	0	0	0	0	0	0	0
4	-	100	0	0	0	0	0	0	0	0	0	0
5	-	100	0	0	0	0	0	0	0	0	0	0
6	-	100	0	0	0	0	0	0	0	0	0	0
7	-	100	0	0	0	0	0	0	0	0	0	0
8	-	100	0	0	0	0	0	0	0	0	0	0
9	-	100	0	0	0	0	0	0	0	0	0	0
10	-	100	0	0	0	0	0	0	0	0	0	0
11	-	100	0	0	0	0	0	0	0	0	0	0
12	-	100	0	0	0	0	0	0	0	0	0	0
13	-	100	0	0	0	0	0	0	0	0	0	0
14	-	100	0	0	0	0	0	0	0	0	0	0
15	-	100	0	0	2	2	0	0	0	0	0	0
16	-	100	0	0	0	2	0	0	0	0	0	0
17	-	100	0	0	0	2	0	0	0	0	1	1
18	-	100	0	0	0	2	0	0	0	0	0	1
19	-	100	0	0	1	3	0	0	0	0	0	1
20	-	100	0	0	0	3	0	0	0	0	0	1

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued....

Table 7-3.14 continued

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Lettuce Seeds (Variety "Webbs Wonderful")

Seeds	Lettuce Var. "Webbs Wonderful" (Lactuca sativa)											
Dose (Mrad)	5.0											
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	2 months											
R.H.	97											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0										
2	0	0										
3	0	0										
4	0	0										
5	0	0										
6	0	0										
7	0	0										
8	0	0										
9	0	0										
10	0	0										
11	0	0										
12	0	0										
13	0	0										
14	0	0										
15	0	0										
16	0	0										
17	0	0										
18	0	0										
19	0	0										
20	0	0										

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.15

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Lettuce Seeds (Variety "Webbs Wonderful).

Seed	Lettuce Var. "Webbs Wonderful" (Lactuca sativa)											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2 months		2 months		2 months		2 months			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	38	38	0	0	0	0	0	0	0	0	0	0
3	56	94	0	0	0	0	0	0	0	0	0	0
4	5	99	0	0	0	0	0	0	0	0	0	0
5	0	99	0	0	0	0	0	0	0	0	0	0
6	0	99	0	0	0	0	0	0	0	0	0	0
7	0	99	0	0	0	0	0	0	0	0	0	0
8	1	100	0	0	0	0	0	0	0	0	0	0
9	-	100	0	0	0	0	0	0	0	0	0	0
10	-	100	0	0	0	0	0	0	0	0	0	0
11	-	100	0	0	0	0	0	0	0	0	0	0
12	-	100	0	0	0	0	0	0	0	0	0	0
13	-	100	0	0	0	0	0	0	0	0	0	0
14	-	100	0	0	0	0	0	0	0	0	0	0
15	-	100	0	0	0	0	0	0	0	0	0	0
16	-	100	0	0	0	0	0	0	0	0	0	0
17	-	100	0	0	0	0	0	0	0	0	0	0
18	-	100	0	0	0	0	0	0	0	0	0	0
19	-	100	0	0	0	0	0	0	0	0	0	0
20	-	100	0	0	0	0	0	0	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.16

The Effects of Storage at Various Relative Humidities for 4 Months on the Germination of Irradiated Lettuce Seeds (Variety "Webbs Wonderful")

Seeds — Lettuce Var. "Webbs Wonderful" (Lactuca sativa)

Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		4 months		4 months		4 months		4 months			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	91	91	0	0	0	0	0	0	0	0		
2	9	100	0	0	0	0	0	0	0	0		
3	-	100	0	0	0	0	0	0	0	0		
4	-	100	0	0	0	0	0	0	0	0		
5	-	100	0	0	0	0	0	0	0	0		
6	-	100	0	0	0	0	0	0	0	0		
7	-	100	0	0	0	0	0	0	0	0		
8	-	100	0	0	0	0	0	0	0	0		
9	-	100	0	0	0	0	0	0	0	0		
10	-	100	0	0	0	0	0	0	0	0		
11	-	100	0	0	0	0	0	0	0	0		
12	-	100	0	0	0	0	0	0	0	0		
13	-	100	0	0	0	0	1	1	0	0		
14	-	100	0	0	0	0	0	1	0	0		
15	-	100	0	0	0	0	0	1	0	0		
16	-	100	0	0	0	0	0	1	0	0		
17	-	100	0	0	0	0	0	1	0	0		
18	-	100	0	0	0	0	0	1	0	0		
19	-	100	0	0	0	0	0	1	0	0		
20	-	100	0	0	0	0	0	1	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.17

The Effects of Storage at Various Relative Humidities for 8 Months on the Germination of Irradiated Lettuce Seeds, Variety "Webbs Wonderful".

Seeds	Lettuce (Var. "Webbs Wonderful")											
Dose (Mrad)	0.0		0.51		0.51		0.51		0.51		0.51	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		8 months		8 months		8 months		8 months		8 months	
R.H.	-		9		23		45		55		76	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	69	69	0	0	0	0	0	0	0	0	0	0
2	28	86	1	1	3	3	2	2	0	0	0	0
3	2	99	5	6	14	17	15	17	2	2	0	0
4	1	100	39	45	42	59	20	37	14	16	0	0
5	0	100	39	84	24	83	52	89	64	80	0	0
6	-	100	2	86	3	86	1	90	1	81	0	0
7	-	100	3	89	2	88	2	92	2	83	0	0
8	-	100	3	92	3	91	2	94	5	88	0	0
9	-	100	5	97	2	93	3	97	7	95	0	0
10	-	100	1	98	0	93	1	98	4	99	0	0
11	-	100	0	98	0	93	0	98	0	99	0	0
12	-	100	0	98	0	93	0	98	0	99	0	0
13	-	100	0	98	0	93	0	98	0	99	0	0
14	-	100	0	98	0	93	0	98	0	99	0	0
15	-	100	0	98	0	93	0	98	0	99	0	0
16	-	100	0	98	0	93	0	98	0	99	0	0
17	-	100	0	98	0	93	0	98	0	99	0	0
18	-	100	0	98	0	93	0	98	0	99	0	0
19	-	100	0	98	0	93	0	98	0	99	0	0
20	-	100	0	98	0	93	0	98	0	99	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

/continued...

Table 7-3.17 continued

The Effects of Storage at Various Relative Humidities for 8 Months on the Germination of Irradiated Lettuce Seeds, Variety "Webbs Wonderful".

Dose (Mrad)	0.51											
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	8 months											
R.H.	97											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0										
2	0	0										
3	0	0										
4	0	0										
5	0	0										
6	0	0										
7	0	0										
8	0	0										
9	0	0										
10	0	0										
11	0	0										
12	0	0										
13	0	0										
14	0	0										
15	0	0										
16	0	0										
17	0	0										
18	0	0										
19	0	0										
20	0	0										

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 7-3.17
The Effects of Storage at Various Relative Humidities for 8 Months on the Germination % of Irradiated Lettuce Seeds (Webbs Wonderful).

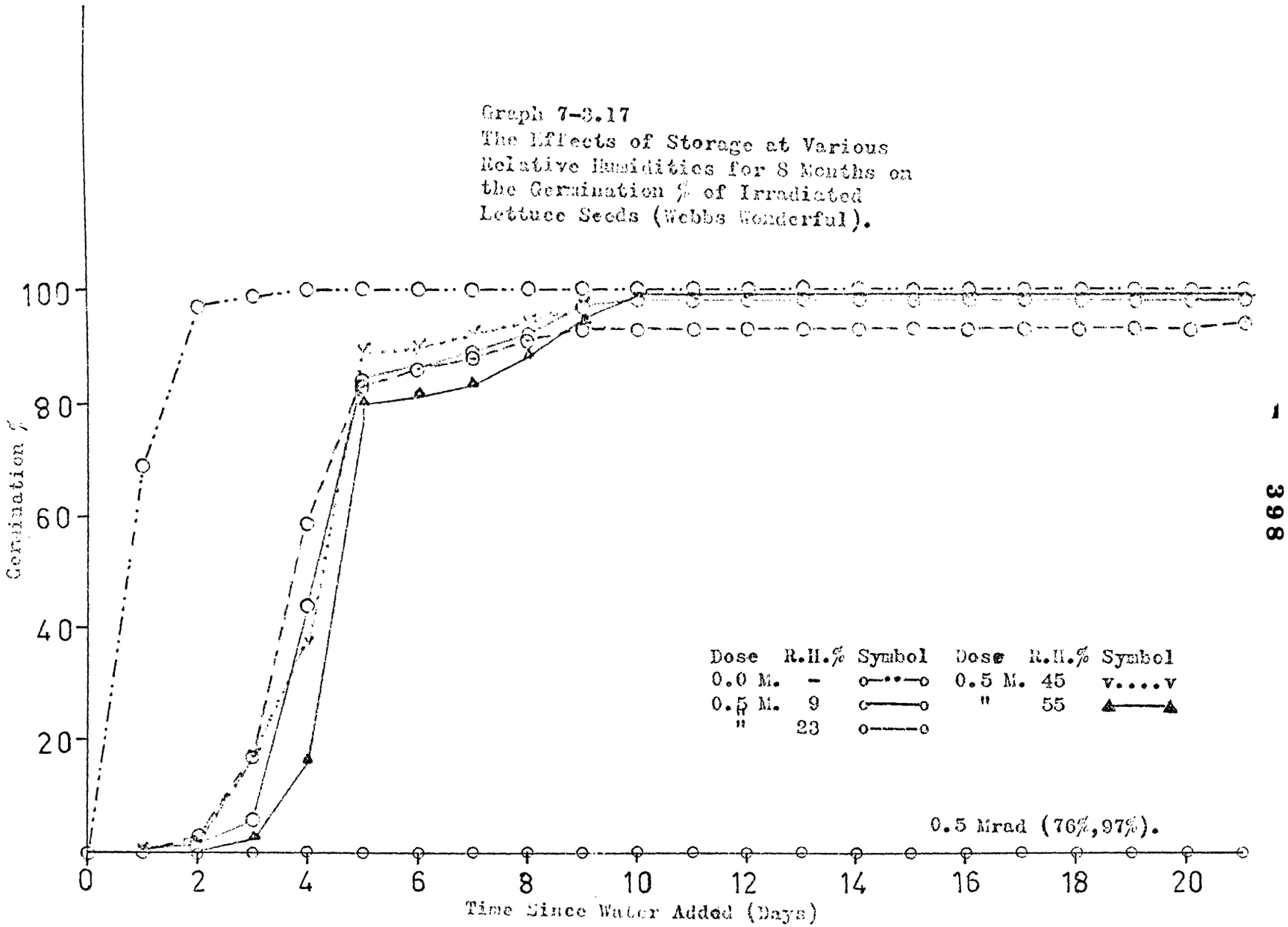


Table 7-3.18

The Effects of Storage at Various Relative Humidities for 8 Months on the Germination of Irradiated Lettuce Seeds (Var. "Webbs Wonderful")

Seeds	Lettuce (Var. "Webbs Wonderful")											
Dose (Mrad)	0.0		5.0		5.0		5.0		5.0		5.0	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		8 months		8 months		8 months		8 months		8 months	
R.H.	-		9		23		45		55		76	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	69	69	0	0	0	0	0	0	0	0	0	0
2	28	97	0	0	0	0	0	0	0	0	0	0
3	2	99	0	0	0	0	0	0	0	0	0	0
4	1	100	0	0	0	0	0	0	0	0	0	0
5	-	100	0	0	0	0	0	0	0	0	0	0
6	-	100	0	0	0	0	0	0	0	0	0	0
7	-	100	0	0	0	0	1	1	0	0	0	0
8	-	100	0	0	1	1	0	1	0	0	0	0
9	-	100	0	0	0	1	0	1	0	0	0	0
10	-	100	1	1	0	1	0	0	0	0	0	0
11	-	100	0	1	1	2	0	1	0	0	0	0
12	-	100	0	1	0	2	0	1	0	0	0	0
13	-	100	0	1	0	2	0	1	0	0	0	0
14	-	100	0	1	0	2	0	1	0	0	0	0
15	-	100	0	1	0	2	1	2	0	0	0	0
16	-	100	0	1	0	2	0	2	0	0	0	0
17	-	100	0	1	0	2	0	2	0	0	0	0
18	-	100	0	1	0	2	0	2	0	0	0	0
19	-	100	0	1	0	2	0	2	0	0	0	0
20	-	100	0	1	0	2	0	2	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued....

Table 7-3.18 continued

The Effects of Storage at Various Relative Humidities for 8 Months on the Germination of Irradiated Lettuce Seeds (Var. "Webbs Wonderful")

Seeds	Lettuce (Var. "Webbs Wonderful")											
Dose (Mrad)	5.0											
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	8 months											
R.H.	97											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0										
2	0	0										
3	0	0										
4	0	0										
5	0	0										
6	0	0										
7	0	0										
8	0	0										
9	0	0										
10	0	0										
11	0	0										
12	0	0										
13	0	0										
14	0	0										
15	0	0										
16	0	0										
17	0	0										
18	0	0										
19	0	0										
20	0	0										

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.19

The Effects of Storage at Various Relative Humidities for 10 Months on the Germination of Irradiated Lettuce Seeds, Variety "Webbs Wonderful"

Seeds	Lettuce (Var. "Webbs Wonderful")											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		10 months		10 months		10 months		10 months			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	69	69	0	0	0	0	0	0	0	0		
2	28	97	0	0	0	0	0	0	0	0		
3	2	99	0	0	0	0	0	0	0	0		
4	1	100	0	0	0	0	0	0	0	0		
5	-	100	0	0	0	0	0	0	0	0		
6	-	100	0	0	0	0	0	0	0	0		
7	-	100	0	0	0	0	0	0	0	0		
8	-	100	0	0	0	0	0	0	0	0		
9	-	100	0	0	0	0	0	0	0	0		
10	-	100	0	0	0	0	0	0	0	0		
11	-	100	0	0	0	0	0	0	0	0		
12	-	100	0	0	0	0	0	0	0	0		
13	-	100	0	0	0	0	0	0	0	0		
14	-	100	0	0	0	0	0	0	0	0		
15	-	100	0	0	0	0	0	0	0	0		
16	-	100	0	0	0	0	0	0	0	0		
17	-	100	0	0	0	0	0	0	0	0		
18	-	100	0	0	0	0	0	0	0	0		
19	-	100	0	0	0	0	0	0	0	0		
20	-	100	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.20

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Lettuce Seeds, Variety "Webbs Wonderful"

Seeds	Lettuce (Var. "Webbs Wonderful")											
Dose (Mrad)	0.0		0.51		0.51		0.51		0.51		0.51	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12 months		12 months		12 months		12 months		12 months	
R.H.	-		9		23		45		55		76	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	78	78	0	0	0	0	0	0	0	0	0	0
2	10	88	0	0	0	0	0	0	0	0	0	0
3	8	96	0	0	0	0	2	2	0	0	0	0
4	1	97	12	12	18	18	46	48	13	13	0	0
5	1	98	6	18	12	30	17	65	9	22	0	0
6	0	98	32	50	23	53	16	81	11	33	0	0
7	0	98	5	55	4	57	7	88	16	49	0	0
8	0	98	7	62	16	73	2	90	9	58	0	0
9	0	98	2	64	0	73	1	91	3	61	0	0
10	0	98	1	65	1	74	1	92	2	63	0	0
11	0	98	0	65	1	75	1	93	3	66	0	0
12	0	98	0	65	1	76	0	93	2	68	0	0
13	0	98	10	75	6	82	1	94	1	69	0	0
14	0	98	9	84	5	87	0	94	2	71	0	0
15	0	98	8	92	1	88	0	94	3	74	0	0
16	0	98	1	93	0	88	0	94	2	76	0	0
17	0	98	0	93	1	89	0	94	1	77	0	0
18	0	98	1	94	1	90	0	94	1	78	0	0
19	0	98	1	95	2	92	0	94	1	79	0	0
20	0	98	2	97	0	92	0	94	3	82	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

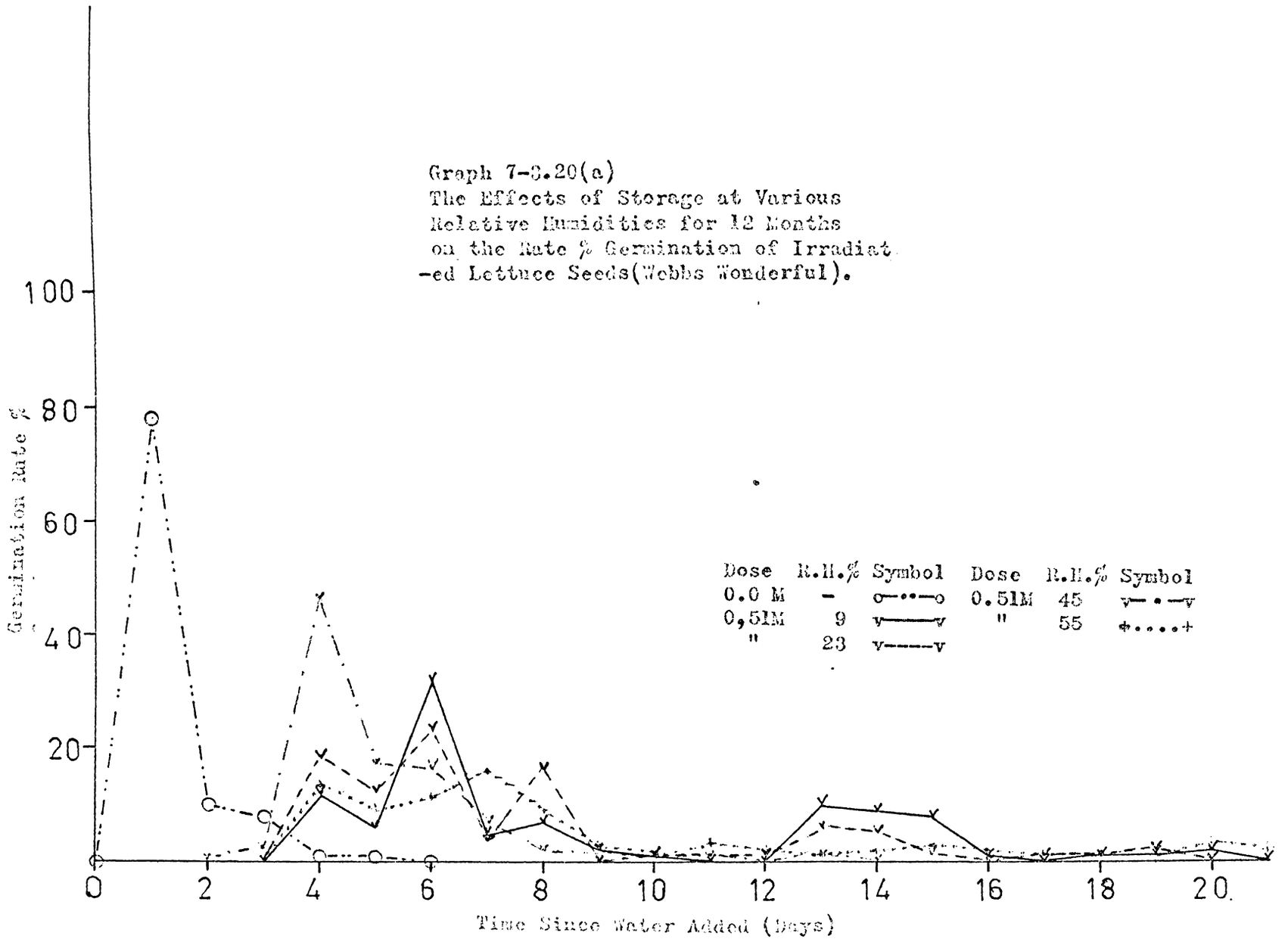
Table 7-3.20 continued

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Lettuce Seeds, Variety "Webbs Wonderful".

Seeds	Lettuce (var. "Webbs Wonderful")											
Dose (Mrad)	0.51											
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	12 months											
R.H.	97											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0										
2	0	0										
3	0	0										
4	0	0										
5	0	0										
6	0	0										
7	0	0										
8	0	0										
9	0	0										
10	0	0										
11	0	0										
12	0	0										
13	0	0										
14	0	0										
15	0	0										
16	0	0										
17	0	0										
18	0	0										
19	0	0										
20	0	0										

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Graph 7-3.20(a)
The Effects of Storage at Various Relative Humidities for 12 Months on the Rate % Germination of Irradiat -ed Lettuce Seeds(Webbs Wonderful).



Graph 7-3.20(b)
The Effects of Storage at Various Relative Humidities for 12 Months on the Germination % of Irradiated Lettuce Seeds(Webbs Wonderful).

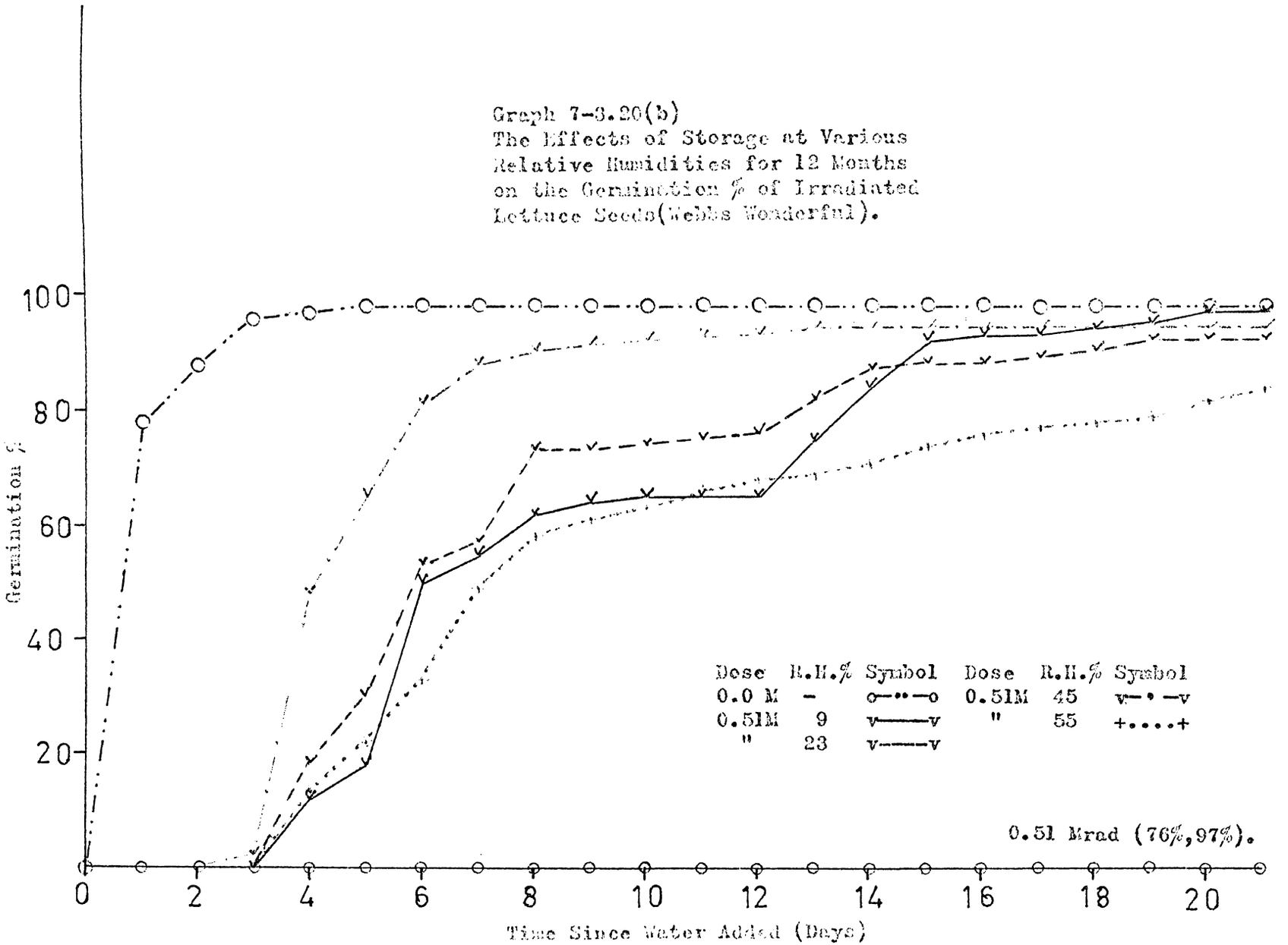


Table 7-3.21

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Lettuce Seeds, Variety "Webbs Wonderful".

Seeds	Lettuce (Var. "Webbs Wonderful")											
Dose (Mrad)	0.0		5.0		5.0		5.0		5.0		5.0	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12 months		12 months		12 months		12 months		12 months	
R.H.	-		9		23		45		55		76	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	78	78	0	0	0	0	0	0	0	0	0	0
2	10	88	0	0	0	0	0	0	0	0	0	0
3	8	96	0	0	0	0	0	0	0	0	0	0
4	1	97	0	0	0	0	0	0	0	0	0	0
5	1	98	0	0	0	0	0	0	0	0	0	0
6	0	98	0	0	0	0	0	0	0	0	0	0
7	0	98	0	0	0	0	0	0	0	0	0	0
8	0	98	0	0	0	0	0	0	0	0	0	0
9	0	98	0	0	0	0	0	0	0	0	0	0
10	0	98	0	0	0	0	0	0	0	0	0	0
11	0	98	0	0	0	0	1	1	0	0	0	0
12	0	98	0	0	0	0	0	1	0	0	0	0
13	0	98	0	0	0	0	0	1	0	0	0	0
14	0	98	0	0	0	0	0	1	0	0	0	0
15	0	98	0	0	0	0	0	1	0	0	0	0
16	0	98	0	0	0	0	0	1	0	0	0	0
17	0	98	0	0	0	0	0	1	0	0	0	0
18	0	98	0	0	0	0	0	1	0	0	0	0
19	0	98	0	0	0	0	0	1	0	0	0	0
20	0	98	0	0	0	0	0	1	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued....

Table 7-3.21 continued.

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Lettuce Seeds, Variety "Webbs Wonderful".

Seeds	Lettuce (Var. "Webbs Wonderful")											
Dose (Mrad)	5.0											
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	12 months											
R.H.	97											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0										
2	0	0										
3	0	0										
4	0	0										
5	0	0										
6	0	0										
7	0	0										
8	0	0										
9	0	0										
10	0	0										
11	0	0										
12	0	0										
13	0	0										
14	0	0										
15	0	0										
16	0	0										
17	0	0										
18	0	0										
19	0	0										
20	0	0										

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.22

The Effects of Storage at Various Relative Humidities for 14 Months on the Germination of Irradiated Lettuce Seeds, Variety "Webbs Wonderful"

Seeds	Lettuce (Var. "Webbs Wonderful")											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		14 months		14 months		14 months		14 months			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	78	78	0	0	0	0	0	0	0	0		
2	10	88	0	0	0	0	0	0	0	0		
3	8	96	0	0	0	0	0	0	0	0		
4	1	97	0	0	0	0	0	0	0	0		
5	1	98	0	0	0	0	0	0	0	0		
6	0	98	0	0	0	0	0	0	0	0		
7	0	98	0	0	0	0	0	0	0	0		
8	0	98	0	0	0	0	0	0	0	0		
9	0	98	0	0	0	0	0	0	0	0		
10	0	98	0	0	0	0	0	0	0	0		
11	0	98	0	0	0	0	0	0	0	0		
12	0	98	0	0	0	0	0	0	0	0		
13	0	98	0	0	0	0	0	0	0	0		
14	0	98	0	0	0	0	0	0	0	0		
15	0	98	0	0	0	0	0	0	0	0		
16	0	98	0	0	0	0	0	0	0	0		
17	0	98	0	0	0	0	0	0	0	0		
18	0	98	0	0	0	0	0	0	0	0		
19	0	98	0	0	0	0	0	0	0	0		
20	0	98	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.23

The Effects of Storage at Room Relative Humidities for 15 months on the Germination of Irradiated Lettuce Seeds, Variety "Webbs Wonderful".

Seeds	Lettuce (Var. "Webbs Wonderful")											
Dose (Mrad)	0.0		0.51		0.98		2.0		5.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		15 months		15 months		15 months		15 months			
R.H.			Room humidities									
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	98	98	1	1	0	0	0	0	0	0		
3	0	98	0	1	0	0	0	0	0	0		
4	0	98	0	1	0	0	0	0	0	0		
5	0	98	0	1	0	0	0	0	0	0		
6	1	99	1	2	0	0	0	0	0	0		
7	0	99	0	2	0	0	0	0	0	0		
8	0	99	0	2	0	0	0	0	0	0		
9	0	99	0	2	0	0	0	0	0	0		
10	0	99	0	2	0	0	0	0	0	0		
11	0	99	1	3	0	0	0	0	0	0		
12	0	99	0	3	0	0	0	0	0	0		
13	0	99	0	3	0	0	0	0	0	0		
14	0	99	0	3	0	0	0	0	0	0		
15	0	99	0	3	0	0	0	0	0	0		
16	0	99	1	4	0	0	0	0	0	0		
17	0	99	0	4	0	0	0	0	0	0		
18	0	99	0	4	0	0	0	0	0	0		
19	0	99	0	4	0	0	0	0	0	0		
20	0	99	0	4	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.24

The Effects of Storage at Room Relative Humidities for 24 Months on the Germination of Irradiated Lettuce Seeds, Variety "Webbs Wonderful"

Seeds	Lettuce (Var. "Webbs Wonderful")											
Dose (Mrad)	0.0		2.0		5.0							
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		24 months		24 months							
R.H.	-		Room humidities									
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0						
2	0	0	0	0	0	0						
3	3	3	0	0	0	0						
4	69	72	0	0	0	0						
5	10	82	0	0	0	0						
6	3	85	0	0	0	0						
7	2	87	0	0	0	0						
8	1	88	0	0	0	0						
9	0	88	0	0	0	0						
10	0	88	0	0	0	0						
11	2	90	0	0	0	0						
12	0	90	0	0	0	0						
13	0	90	0	0	0	0						
14	0	90	0	0	0	0						
15	0	90	0	0	0	0						
16	0	90	0	0	0	0						
17	0	90	0	0	0	0						
18	0	90	0	0	0	0						
19	0	90	0	0	0	0						
20	0	90	0	0	0	0						

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.25

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Lettuce Seeds Variety "Great Lakes".

Seeds — Lettuce Var. "Great Lakes" (Lactuca sativa)

Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2 months		2 months		2 months		2 months			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	55	55	0	0	0	0	0	0	0	0		
3	37	92	0	0	0	0	0	0	0	0		
4	3	95	0	0	0	0	0	0	0	0		
5	0	95	0	0	0	0	0	0	0	0		
6	2	97	0	0	0	0	0	0	0	0		
7	0	97	0	0	0	0	0	0	0	0		
8	1	98	0	0	0	0	0	0	0	0		
9	0	98	0	0	0	0	0	0	0	0		
10	2	100	0	0	0	0	0	0	0	0		
11	-	100	0	0	0	0	0	0	0	0		
12	-	100	0	0	0	0	0	0	0	0		
13	-	100	0	0	0	0	0	0	0	0		
14	-	100	0	0	0	0	0	0	0	0		
15	-	100	0	0	0	0	0	0	0	0		
16	-	100	0	0	0	0	0	0	0	0		
17	-	100	0	0	0	0	0	0	0	0		
18	-	100	0	0	0	0	0	0	0	0		
19	-	100	0	0	0	0	0	0	0	0		
20	-	100	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.26

The Effects of Storage at Various Relative Humidities for 4 Months on the Germination of Irradiated Lettuce Seeds (Variety "Great Lakes")

Seeds	Lettuce (Var. "Great Lakes")											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		4 months		4 months		4 months		4 months			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	95	95	0	0	0	0	0	0	0	0		
2	5	100	0	0	0	0	0	0	0	0		
3	-	100	0	0	0	0	0	0	0	0		
4	-	100	0	0	0	0	0	0	0	0		
5	-	100	0	0	0	0	0	0	0	0		
6	-	100	0	0	0	0	0	0	0	0		
7	-	100	0	0	0	0	0	0	0	0		
8	-	100	0	0	0	0	0	0	0	0		
9	-	100	0	0	0	0	0	0	0	0		
10	-	100	0	0	0	0	0	0	0	0		
11	-	100	0	0	0	0	0	0	0	0		
12	-	100	0	0	0	0	0	0	0	0		
13	-	100	0	0	0	0	0	0	0	0		
14	-	100	0	0	0	0	0	0	0	0		
15	-	100	0	0	0	0	0	0	0	0		
16	-	100	0	0	0	0	0	0	0	0		
17	-	100	0	0	0	0	0	0	0	0		
18	-	100	0	0	0	0	0	0	0	0		
19	-	100	0	0	0	0	0	0	0	0		
20	-	100	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.27

The Effects of Storage at Various Relative Humidities for 10 Months on the Germination of Irradiated Lettuce (Variety "Great Lakes") Seeds.

Seeds	Lettuce (var. "Great Lakes")											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		10 months		10 months		10 months		10 months			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	81	81	0	0	0	0	0	0	0	0		
2	11	92	0	0	0	0	0	0	0	0		
3	4	96	0	0	0	0	0	0	0	0		
4	3	99	0	0	0	0	0	0	0	0		
5	0	99	0	0	0	0	0	0	0	0		
6	0	99	0	0	0	0	0	0	0	0		
7	0	99	0	0	0	0	0	0	0	0		
8	0	99	0	0	0	0	0	0	0	0		
9	0	99	0	0	0	0	0	0	0	0		
10	0	99	0	0	0	0	0	0	0	0		
11	0	99	0	0	0	0	0	0	0	0		
12	0	99	0	0	0	0	0	0	0	0		
13	0	99	0	0	0	0	0	0	0	0		
14	0	99	0	0	0	0	0	0	0	0		
15	0	99	0	0	0	0	0	0	0	0		
16	0	99	0	0	0	0	0	0	0	0		
17	0	99	0	0	0	0	0	0	0	0		
18	0	99	0	0	0	0	0	0	0	0		
19	0	99	0	0	0	0	0	0	0	0		
20	0	99	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.28

The Effects of Storage at Various Relative Humidities for 14 Months on the Germination of Irradiated Lettuce Seeds, Variety "Great Lakes"

Seeds	Lettuce (Var. "Great Lakes")											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		14 months		14 months		14 months		14 months			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	43	43	0	0	0	0	0	0	0	0		
2	43	86	0	0	0	0	0	0	0	0		
3	10	96	0	0	0	0	0	0	0	0		
4	1	97	0	0	0	0	0	0	0	0		
5	0	97	0	0	0	0	0	0	0	0		
6	0	97	0	0	0	0	0	0	0	0		
7	0	97	0	0	0	0	0	0	0	0		
8	0	97	0	0	0	0	0	0	0	0		
9	0	97	0	0	0	0	0	0	0	0		
10	0	97	0	0	0	0	0	0	0	0		
11	0	97	0	0	0	0	0	0	0	0		
12	0	97	0	0	0	0	0	0	0	0		
13	0	97	0	0	0	0	0	0	0	0		
14	0	97	0	0	0	0	0	0	0	0		
15	0	97	0	0	0	0	0	0	0	0		
16	0	97	0	0	0	0	0	0	0	0		
17	0	97	0	0	0	0	0	0	0	0		
18	0	97	0	0	0	0	0	0	0	0		
19	0	97	0	0	0	0	0	0	0	0		
20	0	97	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.29

The Effects of Storage at Room Relative Humidities for 15 Months on the Germination of Irradiated Lettuce Seeds, Variety "Great Lakes".

Seeds	Lettuce (Var. "Great Lakes")											
Dose (Mrad)	0.0		0.49		0.98		2.0		5.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		15 months		15 months		15 months		15 months			
R.H.	-				Room Humidities							
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	99	99	0	0	0	0	0	0	0	0		
3	0	99	0	0	0	0	0	0	0	0		
4	1	100	0	0	0	0	0	0	0	0		
5	-	100	0	0	0	0	0	0	0	0		
6	-	100	0	0	0	0	0	0	0	0		
7	-	100	0	0	0	0	0	0	0	0		
8	-	100	0	0	0	0	0	0	0	0		
9	-	100	0	0	0	0	0	0	0	0		
10	-	100	0	0	0	0	0	0	0	0		
11	-	100	0	0	0	0	0	0	0	0		
12	-	100	0	0	0	0	0	0	0	0		
13	-	100	0	0	0	0	0	0	0	0		
14	-	100	0	0	0	0	0	0	0	0		
15	-	100	0	0	0	0	0	0	0	0		
16	-	100	0	0	0	0	0	0	0	0		
17	-	100	0	0	0	0	0	0	0	0		
18	-	100	0	0	0	0	0	0	0	0		
19	-	100	0	0	0	0	0	0	0	0		
20	-	100	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.30

The Effects of Storage at Room Relative Humidities for 24 Months on the Germination of Irradiated Lettuce Seeds, Varieties "White Cos" and "Great Lakes"

Seed	White Cos						Great Lakes					
Dose (Mrad)	0.0		2.0		5.0		0.0		2.0		5.0	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		24		24		-		24		24	
R.H.	-		Room				-		Room			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	7	7	0	0	0	0
2	0	0	0	0	0	0	76	83	0	0	0	0
3	0	0	0	0	0	0	8	91	0	0	0	0
4	0	0	0	0	0	0	3	94	0	0	0	0
5	1	1	0	0	0	0	3	97	0	0	0	0
6	0	1	0	0	0	0	1	98	0	0	0	0
7	1	2	0	0	0	0	1	99	0	0	0	0
8	1	3	0	0	0	0	1	100	0	0	0	0
9	0	3	0	0	0	0	-	100	0	0	0	0
10	0	3	0	0	0	0	-	100	0	0	0	0
11	0	3	0	0	0	0	-	100	0	0	0	0
12	0	3	0	0	0	0	-	100	0	0	0	0
13	0	3	0	0	0	0	-	100	0	0	0	0
14	0	3	0	0	0	0	-	100	0	0	0	0
15	0	3	0	0	0	0	-	100	0	0	0	0
16	0	3	0	0	0	0	-	100	0	0	0	0
17	0	3	0	0	0	0	-	100	0	0	0	0
18	0	3	0	0	0	0	-	100	0	0	0	0
19	0	3	0	0	0	0	-	100	0	0	0	0
20	0	3	0	0	0	0	-	100	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.31

The Effects of Storage at Room Relative Humidities for 15 Months on the Germination of Irradiated Lettuce Seeds, Variety "White Cos"

Seeds	Lettuce (var. "White Cos")											
Dose (Mrad)	0.0		0.49		0.98		2.0		5.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		15		15		15		15			
R.H.	Room Humidities											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	0	0	0	0	0	0	0	0	0	0		
4	0	0	0	0	0	0	0	0	0	0		
5	0	0	0	0	0	0	0	0	0	0		
6	0	0	0	0	0	0	0	0	0	0		
7	0	0	0	0	0	0	0	0	0	0		
8	0	0	0	0	0	0	0	0	0	0		
9	0	0	0	0	0	0	0	0	0	0		
10	1	1	0	0	0	0	0	0	0	0		
11	0	1	0	0	0	0	0	0	0	0		
12	0	1	0	0	0	0	0	0	0	0		
13	0	1	0	0	0	0	0	0	0	0		
14	0	1	0	0	0	0	0	0	0	0		
15	0	1	0	0	0	0	0	0	0	0		
16	0	1	0	0	0	0	0	0	0	0		
17	0	1	0	0	0	0	0	0	0	0		
18	0	1	0	0	0	0	0	0	0	0		
19	0	1	0	0	0	0	0	0	0	0		
20	0	1	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.32

The Effect of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Sinapis alba Seeds.

Seed	Mustard (Sinapis alba)											
Dose (Mrad)	0.0		0.50		0.50		0.50		0.50		0.50	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2		2		2		2		2	
R.H.	-		9		23		33		45		55	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	25	25	3	3	3	3	5	5	10	10	11	11
2	62	87	48	51	41	44	52	57	61	71	52	63
3	7	94	25	76	26	70	21	78	12	83	15	78
4	5	99	5	81	13	83	7	85	11	94	9	87
5	0	99	2	83	1	84	3	88	0	94	5	92
6	1	100	1	84	5	89	2	90	2	96	3	95
7	-	100	0	84	1	90	0	90	1	97	2	97
8	-	100	0	84	0	90	0	90	0	97	0	97
9	-	100	0	84	0	90	0	90	0	97	0	97
10	-	100	0	84	0	90	3	93	0	97	0	97
11	-	100	0	84	0	90	0	93	1	98	0	97
12	-	100	0	84	0	90	0	93	0	98	0	97
13	-	100	0	84	1	91	0	93	1	99	0	97
14	-	100	1	85	0	91	1	94	0	99	0	97
15	-	100	0	85	0	91	0	94	0	99	0	97
16	-	100	0	85	0	91	0	94	0	99	0	97
17	-	100	0	85	0	91	0	94	0	99	0	97
18	-	100	0	85	0	91	0	94	0	99	0	97
19	-	100	0	85	0	91	0	94	0	99	0	97
20	-	100	0	85	0	91	0	94	0	99	0	97

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

Table 7-3.32 continued

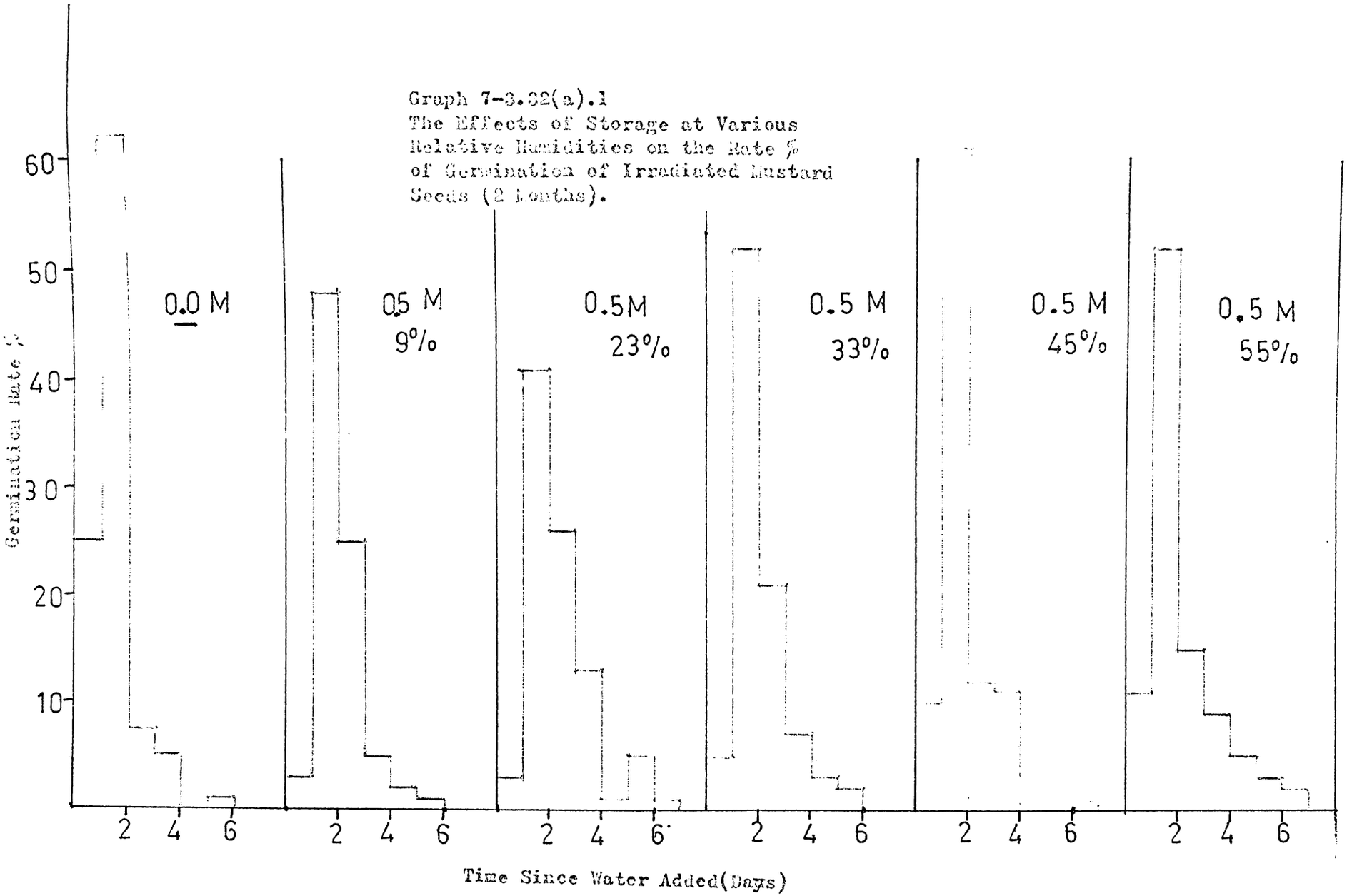
The Effect of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Sinapis alba Seeds.

Seed	Mustard (Sinapis alba)											
Dose (Mrad)	0.50		0.50		0.50		0.50		0.50			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	2		2		2		2		2			
R.H.	66		76		86		93		97			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	2	2	6	6	0	0	0	0	2	2		
2	29	31	24	30	32	32	4	4	14	16		
3	30	61	33	63	27	59	5	9	20	36		
4	14	75	13	76	15	74	9	18	16	52		
5	5	80	7	83	3	77	21	39	4	56		
6	5	85	4	87	7	84	9	48	12	68		
7	2	87	0	87	3	87	8	56	9	77		
8	0	87	1	88	1	88	2	58	3	80		
9	0	87	2	90	0	88	1	59	3	83		
10	0	87	0	90	0	88	0	59	0	83		
11	0	87	0	90	0	88	0	59	0	83		
12	0	87	0	90	0	88	1	60	0	83		
13	0	87	0	90	0	88	0	60	0	83		
14	0	87	1	91	0	88	1	61	1	84		
15	0	87	0	91	0	88	3	64	1	84		
16	0	87	0	91	0	88	1	65	0	84		
17	0	87	0	91	0	88	0	65	0	84		
18	0	87	0	91	0	88	0	65	0	84		
19	0	87	0	91	0	88	0	65	0	84		
20	0	87	0	91	0	88	0	65	0	84		

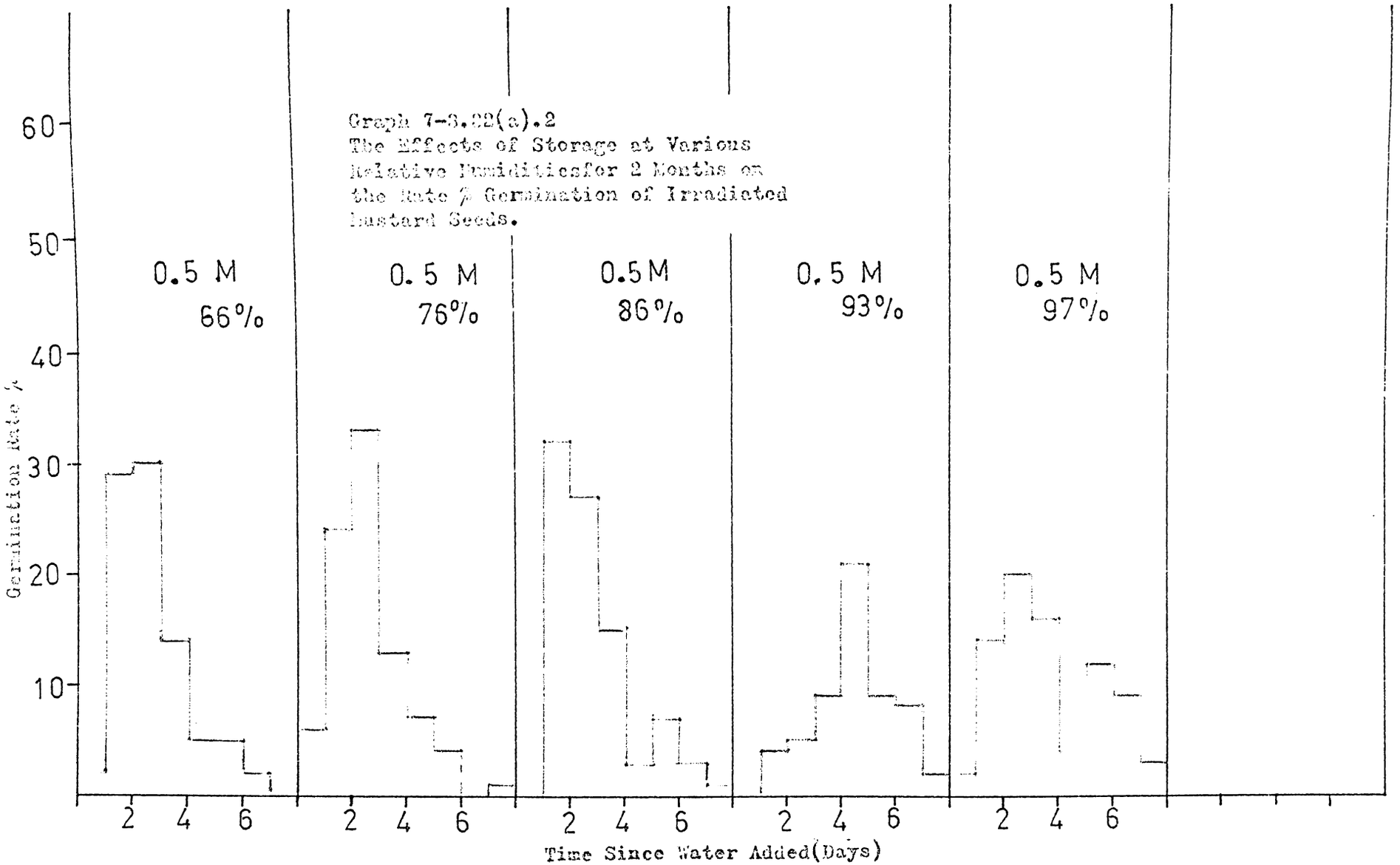
* (a) % Germinated previous 24 hours

* (b) Total % germination.

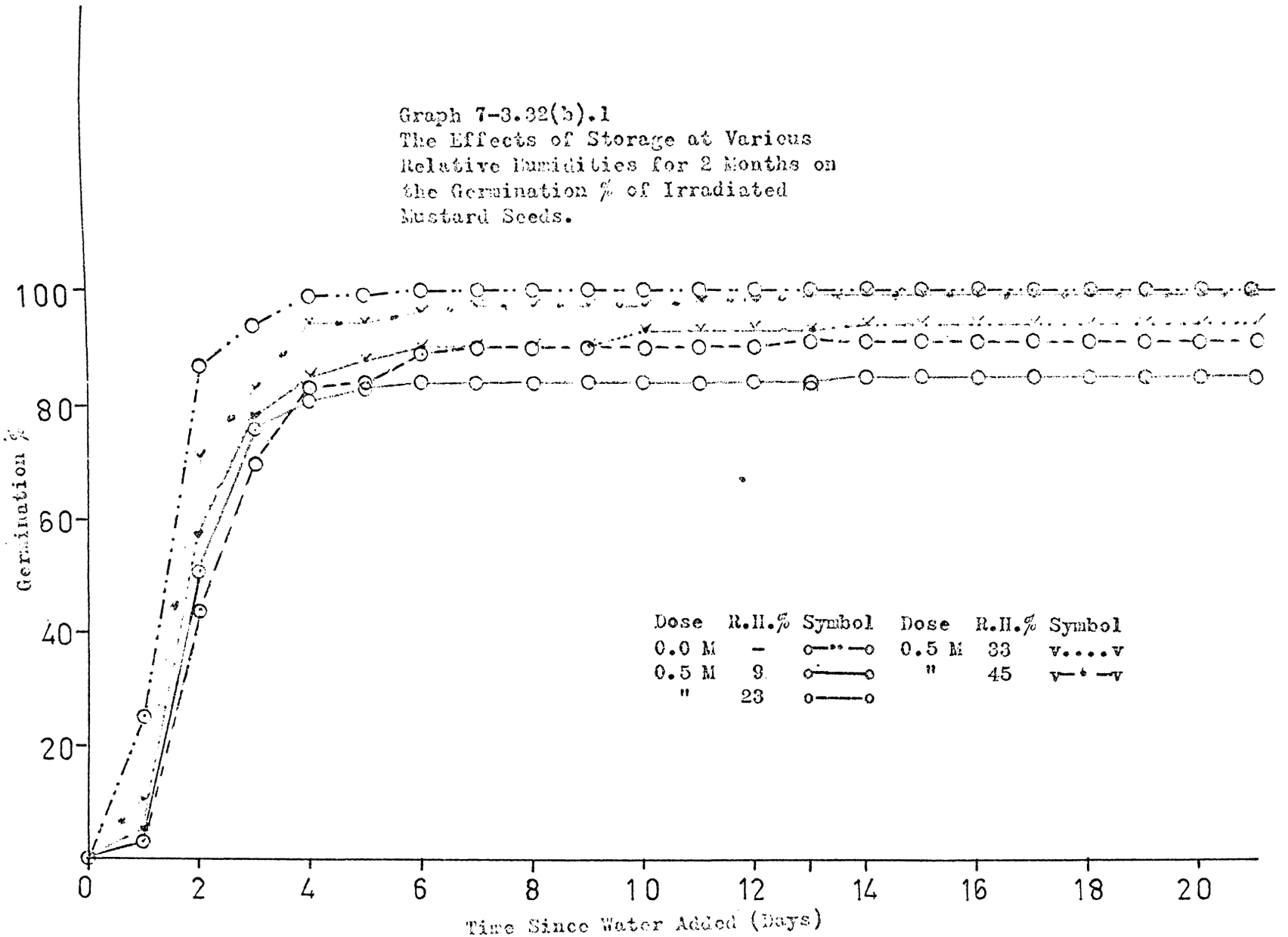
Graph 7-3.32(a).1
The Effects of Storage at Various Relative Humidities on the Rate % of Germination of Irradiated Mustard Seeds (2 Months).



Graph 7-3.22(a).2
The Effects of Storage at Various Relative Humiditiesfor 2 Months on the Rate % Germination of Irradiated Mustard Seeds.



Graph 7-3.32(b).1
The Effects of Storage at Various Relative Humidities for 2 Months on the Germination % of Irradiated Mustard Seeds.



Graph 7-3.32(b).2
The Effects of Storage at Various Relative Humidities for 2 Months on the Germination % of Irradiated Mustard Seeds.

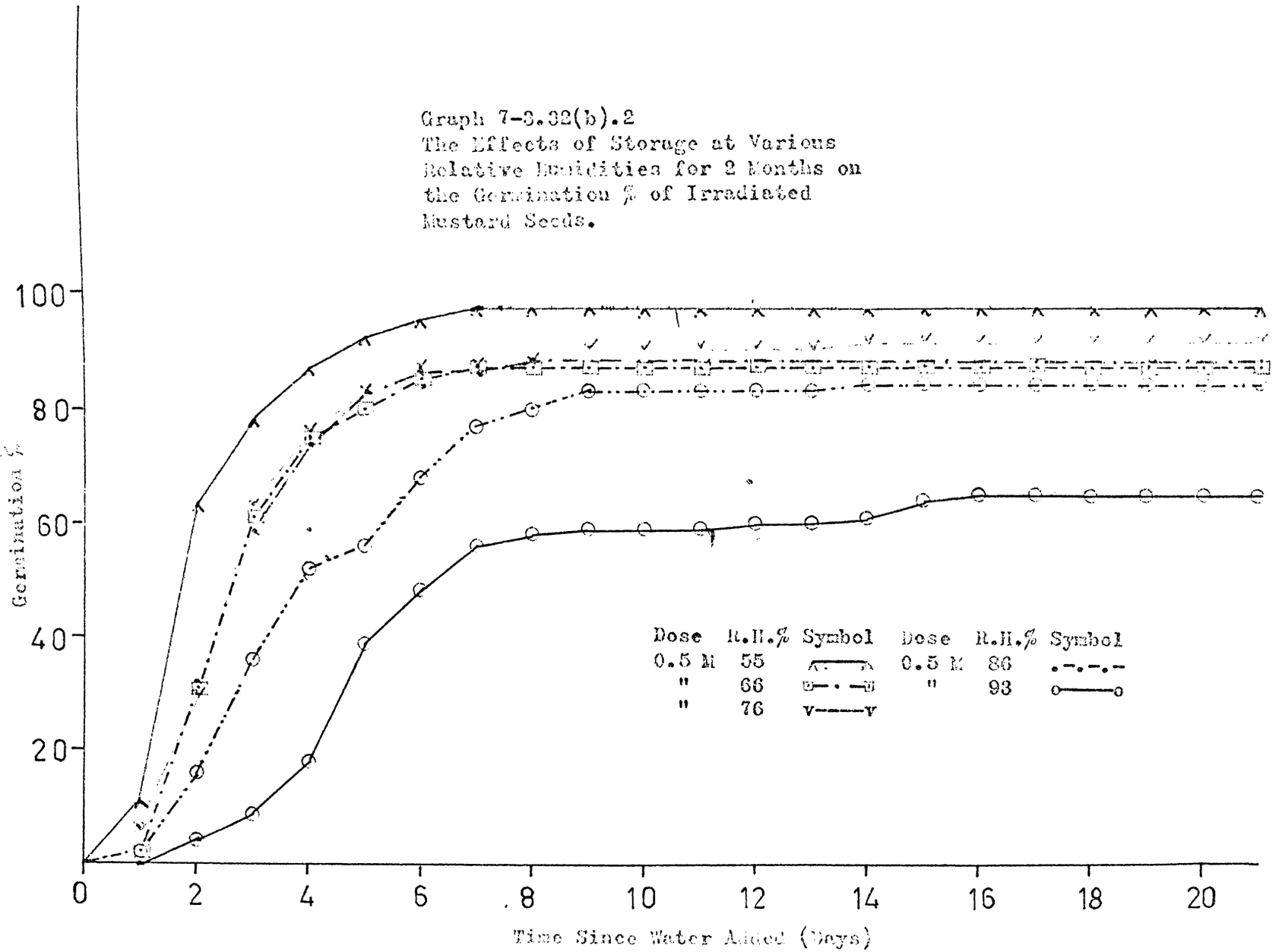


Table 7-3.33

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Sinapis alba Seeds.

Seeds — Mustard (Sinapis alba)

Dose (Mrad)	0.0		4.38		4.38		4.38		4.38		4.38	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2		2		2		2		2	
R.H.	-		9		23		33		45		66	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	25	25	0	0	0	0	1	1	0	0	0	0
2	62	87	0	0	0	0	0	1	0	0	0	0
3	7	94	0	0	0	0	0	1	0	0	0	0
4	5	99	0	0	0	0	0	1	0	0	0	0
5	0	99	0	0	0	0	0	1	0	0	0	0
6	1	100	0	0	0	0	0	1	0	0	0	0
7	-	100	0	0	0	0	0	1	0	0	0	0
8	-	100	0	0	0	0	0	1	0	0	0	0
9	-	100	0	0	0	0	0	1	0	0	0	0
10	-	100	0	0	0	0	0	1	0	0	0	0
11	-	100	0	0	0	0	0	1	0	0	0	0
12	-	100	0	0	0	0	0	1	0	0	0	0
13	-	100	0	0	0	0	0	1	0	0	0	0
14	-	100	0	0	0	0	0	1	0	0	0	0
15	-	100	0	0	0	0	0	1	0	0	0	0
16	-	100	0	0	0	0	0	1	0	0	0	0
17	-	100	0	0	0	0	0	1	0	0	0	0
18	-	100	0	0	0	0	0	1	0	0	0	0
19	-	100	0	0	0	0	0	1	0	0	0	0
20	-	100	0	0	0	0	0	1	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

Table 7-3.33 continued

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Sinapis alba Seeds.

Seed	Mustard (Sinapis alba)											
Dose (Mrad)	4.38		4.38		4.38							
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	2		2		2							
R.H.	86		93		97							
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	1	1						
2	0	0	0	0	0	1						
3	0	0	0	0	0	1						
4	0	0	0	0	0	1						
5	0	0	0	0	0	1						
6	0	0	0	0	0	1						
7	0	0	0	0	0	1						
8	0	0	0	0	0	1						
9	0	0	0	0	0	1						
10	0	0	0	0	0	1						
11	0	0	0	0	0	1						
12	0	0	0	0	0	1						
13	0	0	0	0	0	1						
14	0	0	0	0	0	1						
15	0	0	0	0	0	1						
16	0	0	0	0	0	1						
17	0	0	0	0	0	1						
18	0	0	0	0	0	1						
19	0	0	0	0	0	1						
20	0	0	0	0	0	1						

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.34

The Effects of Storage at Various Relative Humidities for 2 Months on the Storage of Irradiated Sinapis alba Seeds.

Seed	Mustard (Sinapis alba)											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2		2		2		2			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	1	1	0	0	0	0	0	0	0	0		
2	68	69	0	0	0	0	0	0	0	0		
3	12	81	0	0	0	0	0	0	0	0		
4	3	84	0	0	0	0	0	0	0	0		
5	4	88	0	0	0	0	0	0	0	0		
6	4	92	0	0	0	0	0	0	0	0		
7	0	92	0	0	0	0	0	0	0	0		
8	0	92	0	0	0	0	0	0	0	0		
9	1	93	0	0	0	0	0	0	0	0		
10	0	93	0	0	0	0	0	0	0	0		
11	1	94	0	0	0	0	0	0	0	0		
12	0	94	0	0	0	0	0	0	0	0		
13	0	94	0	0	0	0	0	0	0	0		
14	0	94	0	0	0	0	0	0	0	0		
15	0	94	0	0	0	0	0	0	0	0		
16	1	95	0	0	0	0	0	0	0	0		
17	0	95	0	0	0	0	0	0	0	0		
18	0	95	0	0	0	0	0	0	0	0		
19	0	95	0	0	0	0	0	0	0	0		
20	0	95	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.35

The Effects of Storage at Various Relative Humidities for 4 Months on the Germination of Mustard Seeds.

Seed	Mustard (Sinapis alba)											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		4		4		4		4			
R.H.	-		23		45		66		93**			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	25	25	0	0	0	0	0	0	0	0		
2	62	87	0	0	0	0	0	0	0	0		
3	7	94	0	0	0	0	0	0	0	0		
4	5	99	0	0	0	0	0	0	0	0		
5	0	99	0	0	0	0	0	0	0	0		
6	1	100	0	0	0	0	0	0	0	0		
7	0	100	0	0	0	0	0	0	0	0		
8	-	100	0	0	0	0	0	0	0	0		
9	-	100	0	0	0	0	0	0	0	0		
10	-	100	0	0	0	0	0	0	0	0		
11	-	100	0	0	0	0	0	0	0	0		
12	-	100	0	0	0	0	0	0	0	0		
13	-	100	0	0	0	0	0	0	0	0		
14	-	100	0	0	0	0	0	0	0	0		
15	-	100	0	0	0	0	0	0	0	0		
16	-	100	0	0	0	0	0	0	0	0		
17	-	100	0	0	0	0	0	0	0	0		
18	-	100	0	0	0	0	0	0	0	0		
19	-	100	0	0	0	0	0	0	0	0		
20	-	100	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

** These seeds were covered in a thin layer of green mould when removed from the jar.

Table 7-3.36

The Effects of Storage at Various Relative Humidities for 8 Months on the Germination of Irradiated Mustard Seeds.

Seeds	Mustard											
Dose (Mrad)	0.0		0.5		0.5		0.5		0.5		0.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		8		8		8		8		8	
R.H.	-		9		23		33		45		55	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	70	70	4	4	30	30	1	1	3	3	2	2
2	14	84	39	43	23	53	50	51	48	51	39	41
3	7	91	23	66	9	62	15	66	21	72	31	72
4	0	91	7	73	16	78	6	72	9	81	6	78
5	3	94	11	84	2	80	11	83	8	89	8	86
6	0	94	1	85	3	83	2	85	0	89	0	86
7	0	94	2	87	1	84	2	87	1	90	0	86
8	0	94	3	90	3	87	4	91	1	91	2	88
9	1	95	1	91	2	89	3	94	3	94	2	90
10	0	95	1	92	1	90	4	98	1	95	4	94
11	1	96	1	93	1	91	0	98	1	96	1	95
12	0	96	1	94	0	91	2	100	0	96	0	95
13	0	96	0	94	0	91	-	100	1	97	0	95
14	0	96	0	94	0	91	-	100	0	97	0	95
15	0	96	0	94	0	91	-	100	0	97	0	95
16	0	96	0	94	0	91	-	100	0	97	0	95
17	0	96	0	94	0	91	-	100	0	97	1	96
18	0	96	0	94	0	91	-	100	0	97	0	96
19	0	96	0	94	0	91	-	100	0	97	0	96
20	0	96	0	94	0	91	-	100	0	97	0	96

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

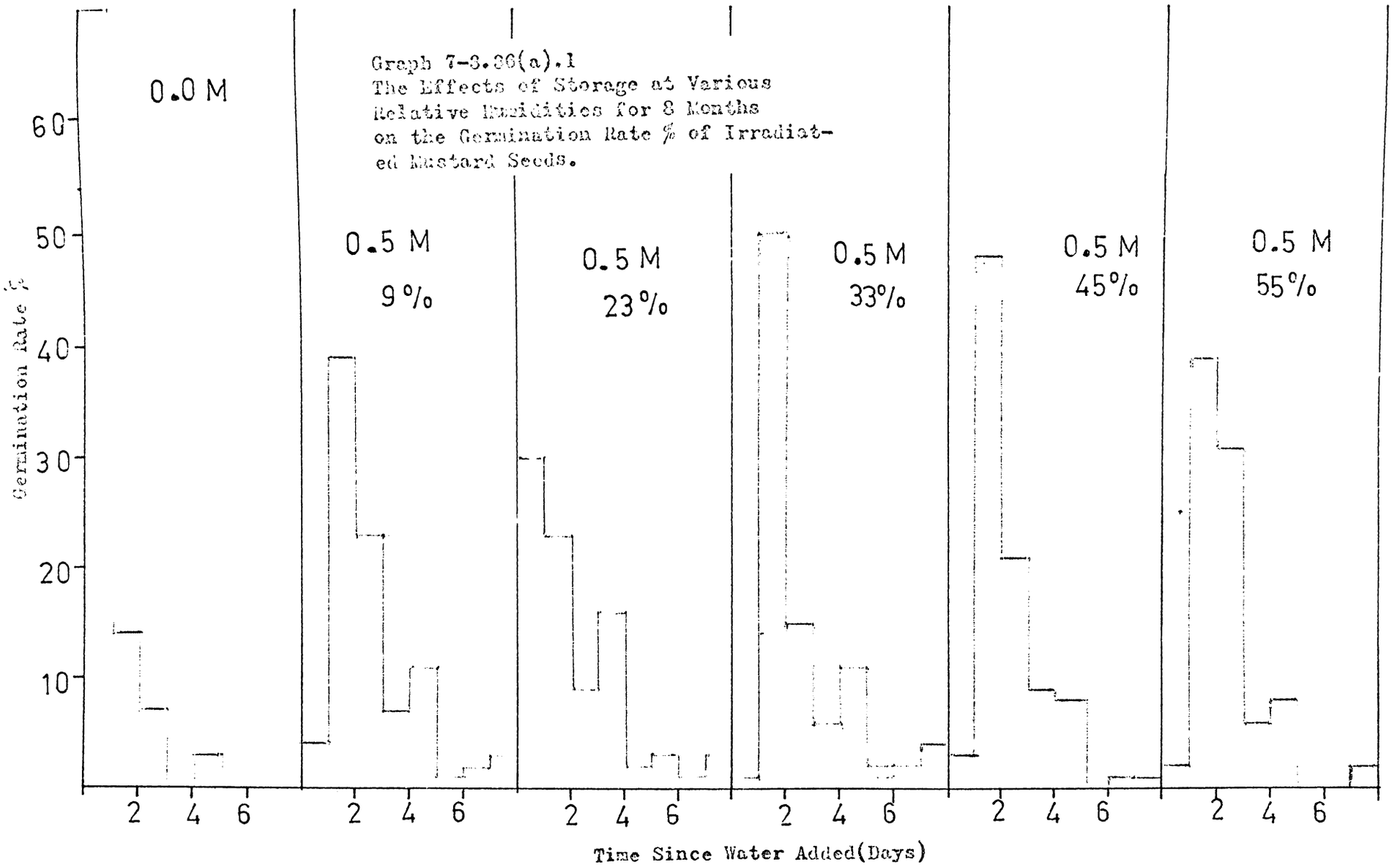
Table 7-3.36 continued

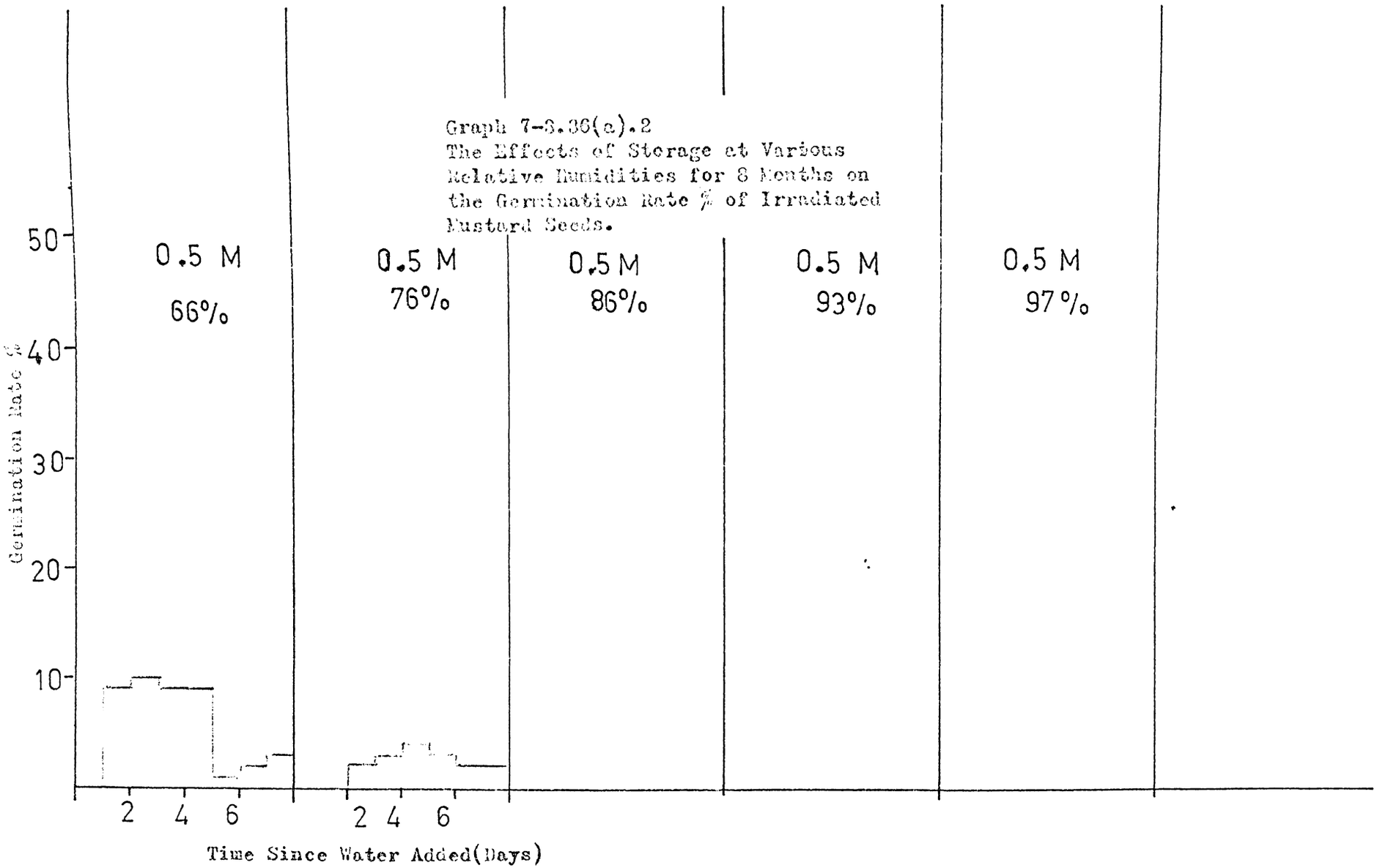
The Effects of Storage at Various Relative Humidities for 8 Months on the Germination of Irradiated Mustard Seeds.

Seeds	Mustard											
Dose (Mrad)	0.5		0.5		0.5		0.5		0.5			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	8		8		8		8		8			
R.H.	66		76		86		93		97			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	9	9	0	0	0	0	0	0	0	0		
3	10	19	2	2	0	0	0	0	0	0		
4	9	28	3	5	0	0	0	0	0	0		
5	9	37	4	9	0	0	0	0	0	0		
6	1	38	3	12	0	0	0	0	0	0		
7	2	40	2	14	0	0	0	0	0	0		
8	3	43	2	16	0	0	0	0	0	0		
9	6	49	4	20	0	0	0	0	0	0		
10	6	55	9	29	0	0	0	0	0	0		
11	15	70	9	38	0	0	0	0	0	0		
12	1	71	13	51	0	0	0	0	0	0		
13	5	76	7	58	0	0	0	0	0	0		
14	2	78	4	62	0	0	0	0	0	0		
15	2	80	7	69	0	0	0	0	0	0		
16	1	81	1	70	0	0	0	0	0	0		
17	0	81	2	72	0	0	0	0	0	0		
18	0	81	1	73	0	0	0	0	0	0		
19	1	82	2	75	0	0	0	0	0	0		
20	0	82	1	76	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

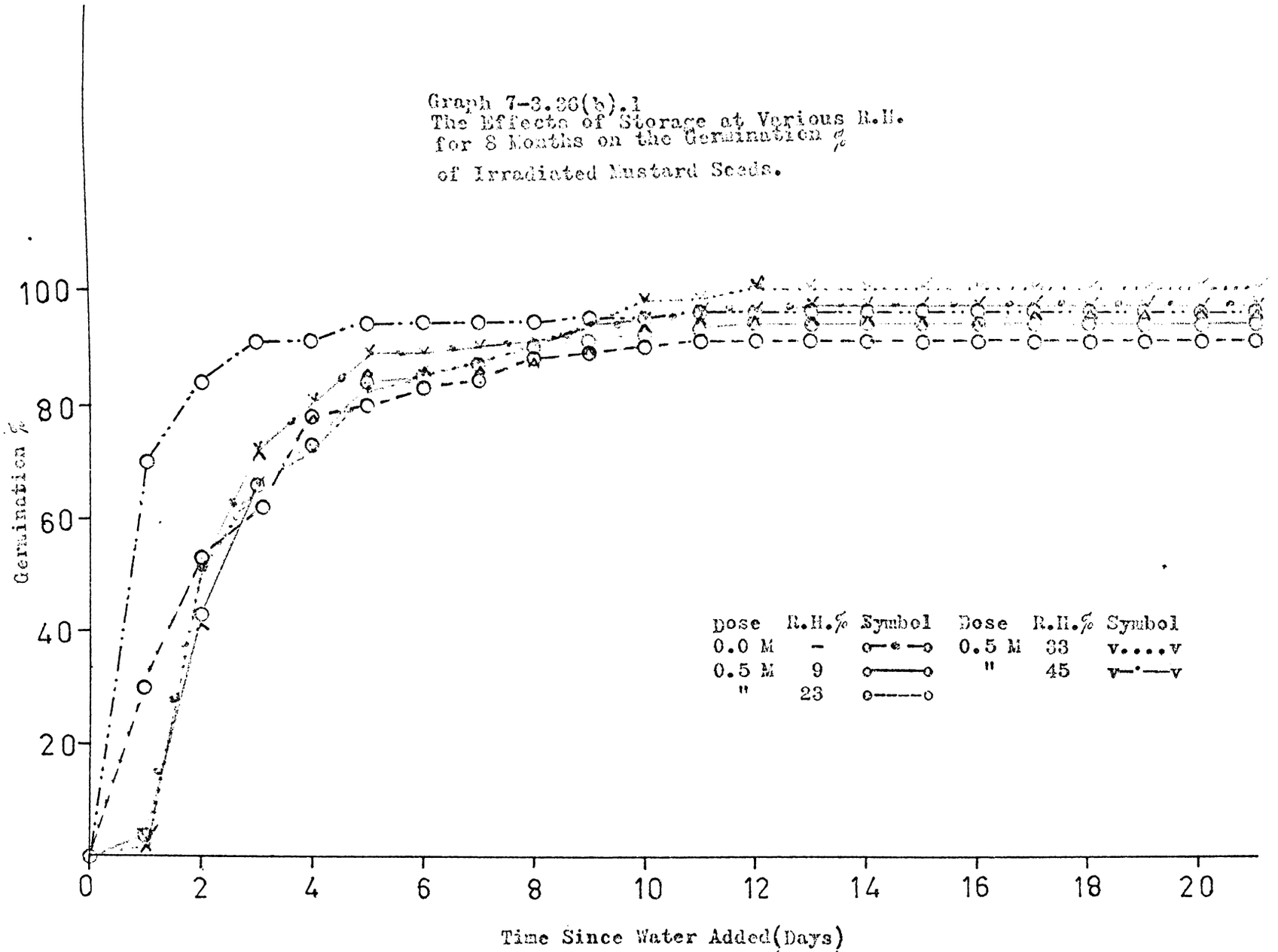
Graph 7-3.36(a).1
The Effects of Storage at Various Relative Humidities for 8 Months on the Germination Rate % of Irradiated Mustard Seeds.





Graph 7-3.36(a).2
The Effects of Storage at Various Relative Humidities for 8 Months on the Germination Rate % of Irradiated Mustard Seeds.

Graph 7-3.36(b).1
The Effects of Storage at Various R.H. for 8 Months on the Germination % of Irradiated Mustard Seeds.



Graph 7-3.36(b).2.
The Effects of Storage at Various Relative Humidities for 8 Months on the Germination % of Irradiated Mustard Seeds.

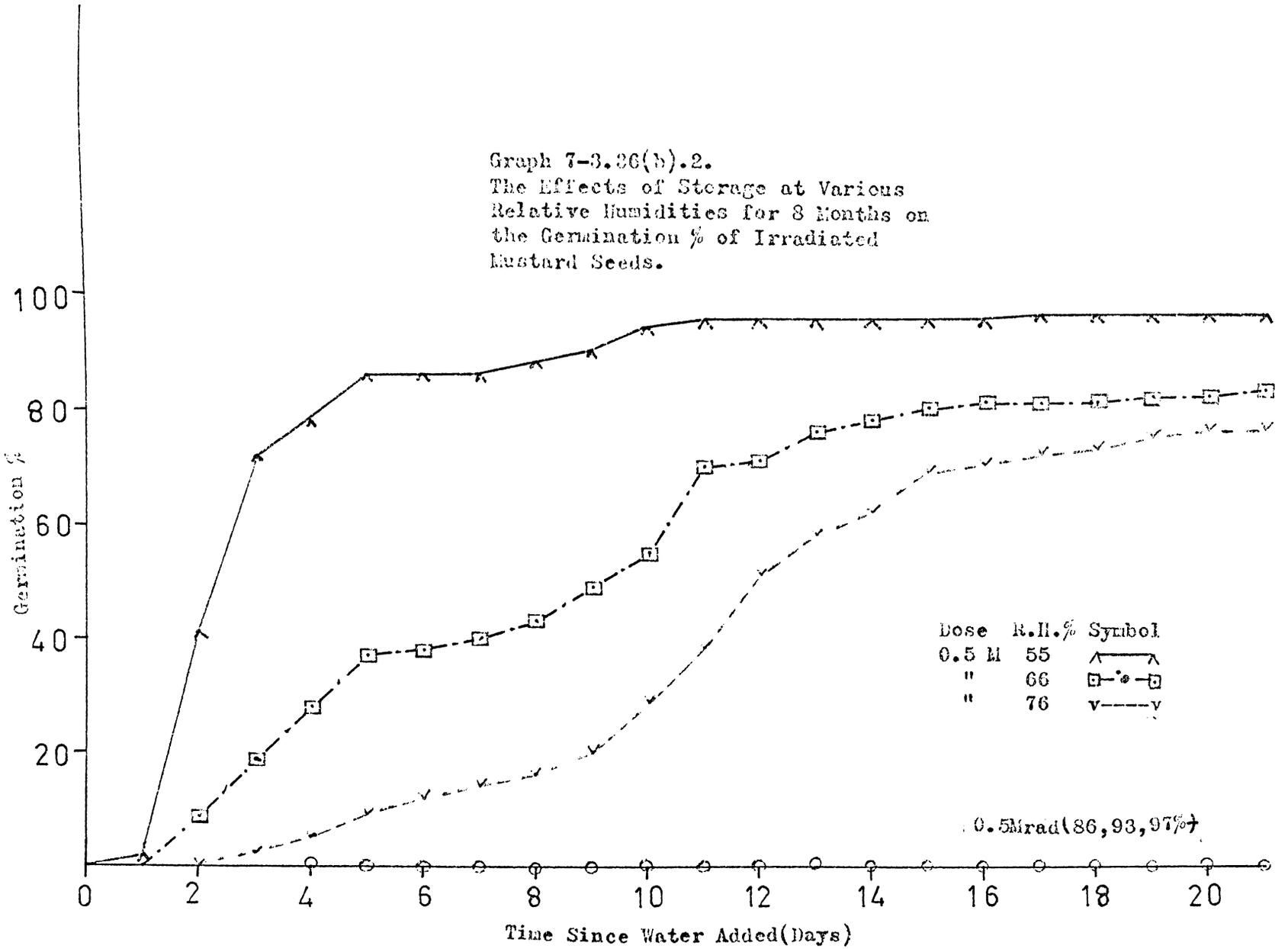


Table 7-3.37

The Effects of Storage at Various Relative Humidities for 8 Months on the Germination of Irradiated Mustard Seeds.

Seeds	Mustard											
Dose (Mrad)	0.0		4.38		4.38		4.38		4.38		4.38	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		8		8		8		8		8	
R.H.	-		9		23		33		45		66	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	70	70	2	2	2	2	1	1	1	1	0	0
2	14	84	0	2	0	2	0	1	0	1	0	0
3	7	91	1	3	0	2	0	1	0	1	0	0
4	0	91	0	3	0	2	0	1	0	1	0	0
5	3	94	0	3	0	2	0	1	0	1	0	0
6	0	94	0	3	0	2	0	1	0	1	0	0
7	0	94	0	3	0	2	0	1	0	1	0	0
8	0	94	0	3	0	2	0	1	0	1	0	0
9	1	95	0	3	0	2	0	1	0	1	0	0
10	0	95	0	3	0	2	0	1	0	1	0	0
11	1	96	0	3	0	2	0	1	0	1	0	0
12	0	96	0	3	0	2	0	1	0	1	0	0
13	0	96	0	3	0	2	0	1	0	1	0	0
14	0	96	0	3	0	2	0	1	0	1	0	0
15	0	96	0	3	0	2	0	1	0	1	0	0
16	0	96	0	3	0	2	0	1	0	1	0	0
17	0	96	0	3	1	3	0	1	0	1	0	0
18	0	96	0	3	1	4	0	1	0	1	0	0
19	0	96	0	3	0	4	0	1	0	1	0	0
20	0	96	0	3	0	4	0	1	0	1	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

Table 7-3.37 continued

The Effects of Storage at Various Relative Humidities for 8 Months on the Germination of Irradiated Mustard Seeds.

Seeds	Mustard											
Dose (Mrad)	4.38		4.38		4.38							
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	8		8		8							
R.H.	86		93		97							
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0						
2	0	0	0	0	0	0						
3	0	0	0	0	0	0						
4	0	0	0	0	0	0						
5	0	0	0	0	0	0						
6	0	0	0	0	0	0						
7	0	0	0	0	0	0						
8	0	0	0	0	0	0						
9	0	0	0	0	0	0						
10	0	0	0	0	0	0						
11	0	0	0	0	0	0						
12	0	0	0	0	0	0						
13	0	0	0	0	0	0						
14	0	0	0	0	0	0						
15	0	0	0	0	0	0						
16	0	0	0	0	0	0						
17	0	0	0	0	0	0						
18	0	0	0	0	0	0						
19	0	0	0	0	0	0						
20	0	0	0	0	0	0						

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.38

The Effects of Storage at Various Relative Humidities for 10 Months on the Germination of Irradiated Mustard Seeds.

Seeds	Mustard											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		10		10		10		10			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	70	70	2	2	0	0	0	0	0	0		
2	14	84	0	2	0	0	0	0	0	0		
3	7	91	0	2	0	0	0	0	0	0		
4	0	91	0	2	0	0	0	0	0	0		
5	3	94	0	2	0	0	0	0	0	0		
6	0	94	0	2	0	0	0	0	0	0		
7	0	94	0	2	0	0	0	0	0	0		
8	0	94	0	2	0	0	0	0	0	0		
9	1	95	0	2	0	0	0	0	0	0		
10	0	95	0	2	0	0	0	0	0	0		
11	1	96	0	2	0	0	0	0	0	0		
12	0	96	0	2	0	0	0	0	0	0		
13	0	96	0	2	0	0	0	0	0	0		
14	0	96	0	2	0	0	0	0	0	0		
15	0	96	0	2	0	0	0	0	0	0		
16	0	96	0	2	0	0	0	0	0	0		
17	0	96	0	2	0	0	0	0	0	0		
18	0	96	0	2	0	0	0	0	0	0		
19	0	96	0	2	0	0	0	0	0	0		
20	0	96	0	2	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.39

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Mustard Seeds.

Seeds	Mustard (Sinapis alba)											
Dose (Mrad)	0.0		0.5		0.5		0.5		0.5		0.5	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12		12		12		12		12	
R.H.	-		9		23		33		45		55	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	30	30	2	2	2	2	4	4	6	6	4	4
2	28	58	27	29	42	44	44	48	50	56	54	58
3	20	78	39	68	28	72	35	83	25	81	23	81
4	1	79	11	79	11	83	2	85	7	88	4	85
5	2	81	7	86	5	88	3	88	3	91	2	87
6	0	81	2	88	1	89	0	88	1	92	0	87
7	0	81	2	90	1	90	2	90	1	93	0	87
8	0	81	0	90	0	90	0	90	1	94	0	87
9	0	81	0	90	0	90	0	90	0	94	0	87
10	0	81	0	90	0	90	0	90	0	94	0	87
11	0	81	0	90	0	90	1	91	0	94	0	87
12	0	81	0	90	0	90	0	91	0	94	0	87
13	0	81	0	90	0	90	0	91	0	94	0	87
14	0	81	0	90	0	90	0	91	0	94	0	87
15	0	81	0	90	0	90	0	91	0	94	0	87
16	0	81	0	90	0	90	0	91	0	94	0	87
17	0	81	0	90	0	90	0	91	0	94	0	87
18	0	81	0	90	0	90	0	91	0	94	0	87
19	0	81	0	90	0	90	0	91	0	94	0	87
20	0	81	0	90	0	90	0	91	0	94	0	87

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

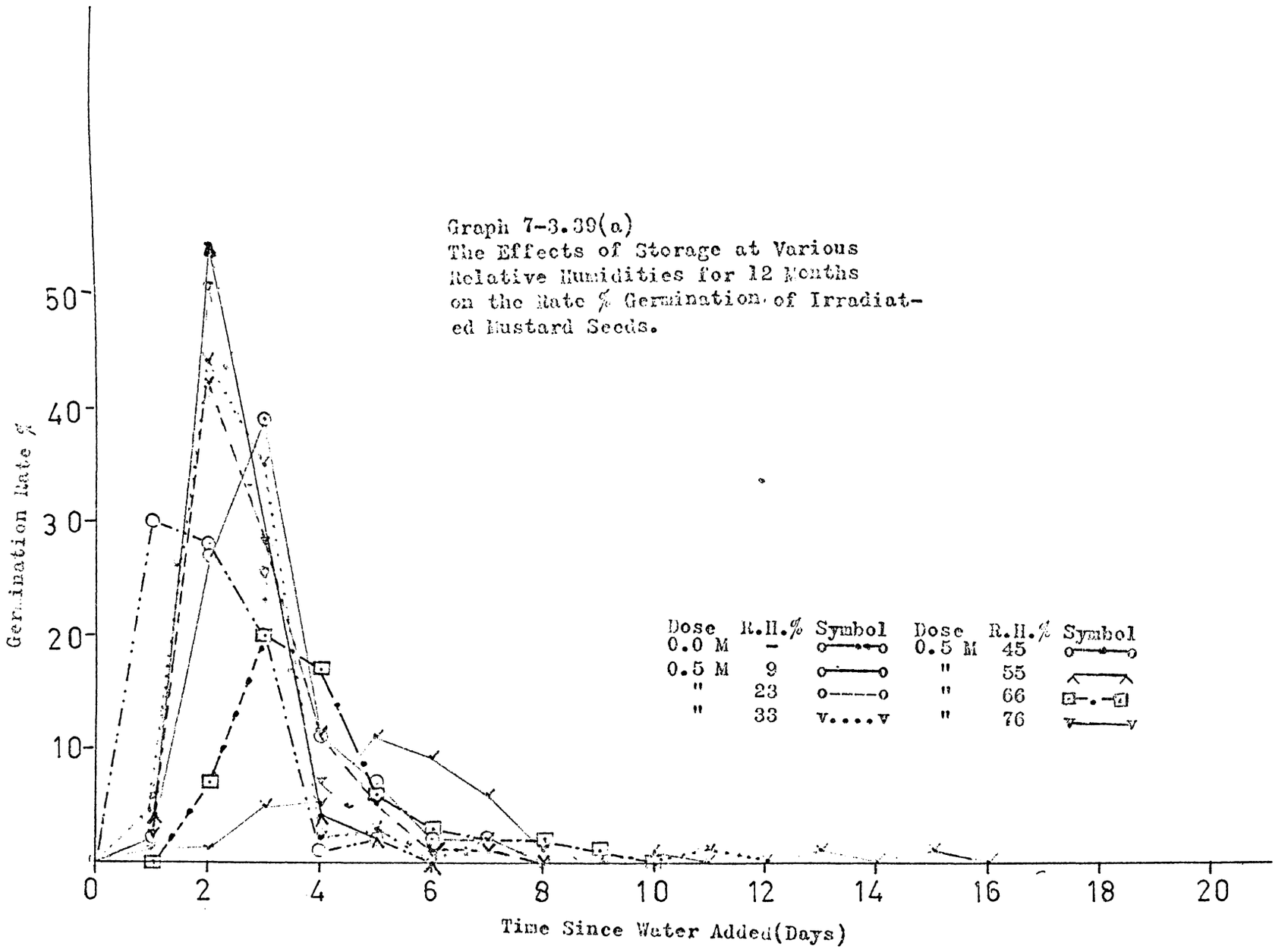
Table 7-3.39 continued

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Mustard Seeds.

Seeds	Mustard (Sinapis alba)											
Dose (Mrad)	0.5		0.5		0.5		0.5		0.5			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	12		12		12		12		12			
R.H.	66		76		86		93		97			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	1	1	0	0	0	0	0	0		
2	7	7	1	2	0	0	0	0	0	0		
3	20	27	5	7	0	0	0	0	0	0		
4	17	44	5	12	0	0	0	0	0	0		
5	6	50	11	23	0	0	0	0	0	0		
6	3	53	9	32	0	0	0	0	0	0		
7	2	55	6	38	0	0	0	0	0	0		
8	2	57	1	39	0	0	0	0	0	0		
9	1	58	0	39	0	0	0	0	0	0		
10	0	58	1	40	0	0	0	0	0	0		
11	0	58	0	40	0	0	0	0	0	0		
12	0	58	0	40	0	0	0	0	0	0		
13	0	58	1	41	0	0	0	0	0	0		
14	0	58	0	41	0	0	0	0	0	0		
15	0	58	1	42	0	0	0	0	0	0		
16	0	58	0	42	0	0	0	0	0	0		
17	0	58	0	42	0	0	0	0	0	0		
18	0	58	0	42	0	0	0	0	0	0		
19	0	58	0	42	0	0	0	0	0	0		
20	0	58	0	42	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Graph 7-3.39(a)
The Effects of Storage at Various Relative Humidities for 12 Months on the Rate % Germination of Irradiated Mustard Seeds.



Graph 7-3.39(b)
The Effects of Storage at Various Relative Humidities foe 12 Months on the Germination % of Irradiated Mustard Seeds.

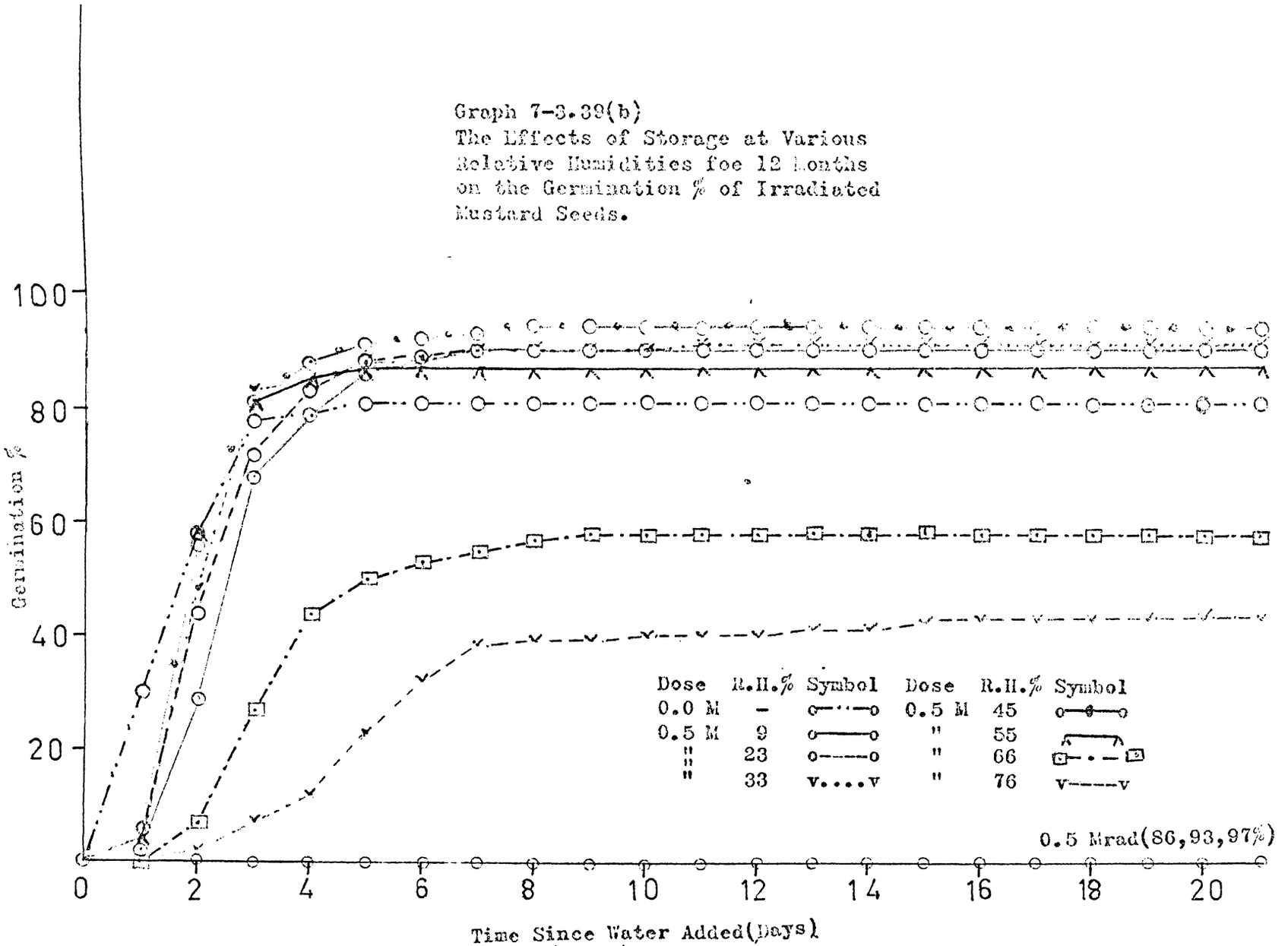


Table 7-3.40

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Mustard Seeds.

Seeds	Mustard											
Dose (Mrad)	0.0		4.38		4.38		4.38		4.38		4.38	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12		12		12		12		12	
R.H.	-		9		23		33		45		66	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	30	30	0	0	0	0	0	0	0	0	0	0
2	28	58	0	0	0	0	0	0	0	0	0	0
3	20	78	0	0	0	0	0	0	0	0	0	0
4	1	79	0	0	0	0	0	0	0	0	0	0
5	2	81	0	0	0	0	0	0	0	0	0	0
6	0	81	0	0	0	0	0	0	0	0	0	0
7	0	81	0	0	0	0	0	0	0	0	0	0
8	0	81	0	0	0	0	0	0	0	0	0	0
9	0	81	0	0	0	0	0	0	0	0	0	0
10	0	81	0	0	0	0	0	0	0	0	0	0
11	0	81	0	0	0	0	0	0	0	0	0	0
12	0	81	0	0	0	0	0	0	0	0	0	0
13	0	81	0	0	0	0	0	0	0	0	0	0
14	0	81	0	0	0	0	0	0	0	0	0	0
15	0	81	0	0	0	0	0	0	0	0	0	0
16	0	81	0	0	0	0	0	0	0	0	0	0
17	0	81	0	0	0	0	0	0	0	0	0	0
18	0	81	0	0	0	0	0	0	0	0	0	0
19	0	81	0	0	0	0	0	0	0	0	0	0
20	0	81	0	0	0	0	0	0	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

Table 7-3.40 continued

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Mustard Seeds.

Seeds	Mustard											
Dose (Mrad)	4.38		4.38		4.38							
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	12		12		12							
R.H.	93		97		86							
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0						
2	0	0	0	0	0	0						
3	0	0	0	0								
4	0	0	0	0								
5	0	0	0	0								
6	0	0	0	0								
7	0	0	0	0								
8	0	0	0	0								
9	0	0	0	0								
10	0	0	0	0								
11	0	0	0	0								
12	0	0	0	0								
13	0	0	0	0								
14	0	0	0	0								
15	0	0	0	0								
16	0	0	0	0								
17	0	0	0	0								
18	0	0	0	0								
19	0	0	0	0								
20	0	0	0	0								

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.41

The Effects of Storage at Various Relative Humidities for 14 Months on the Germination of Irradiated Mustard Seeds.

Seeds	Mustard (Sinapis alba)											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		14 months		14 months		14 months		14 months			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	30	30	0	0	0	0	0	0	0	0		
2	28	58	0	0	0	0	0	0	0	0		
3	20	78	0	0	0	0	0	0	0	0		
4	1	79	0	0	0	0	0	0	0	0		
5	2	81	0	0	0	0	0	0	0	0		
6	0	81	0	0	0	0	0	0	0	0		
7	0	81	0	0	0	0	0	0	0	0		
8	0	81	0	0	0	0	0	0	0	0		
9	0	81	0	0	0	0	0	0	0	0		
10	0	81	0	0	0	0	0	0	0	0		
11	0	81	0	0	0	0	0	0	0	0		
12	0	81	0	0	0	0	0	0	0	0		
13	0	81	0	0	0	0	0	0	0	0		
14	0	81	0	0	0	0	0	0	0	0		
15	0	81	0	0	0	0	0	0	0	0		
16	0	81	0	0	0	0	0	0	0	0		
17	0	81	0	0	0	0	0	0	0	0		
18	0	81	0	0	0	0	0	0	0	0		
19	0	81	0	0	0	0	0	0	0	0		
20	0	81	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.42

The Effects of Storage at Room Relative Humidities for 9 Months on the Germination of Irradiated White Mustard Seeds (Sinapis alba)

Seeds	White Mustard											
Dose (Mrad)	0.0		0.118		0.265		1.14		3.5		10.4	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		9		9		9		9		9	
R.H.	-		Room humidities									
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	84	84	83	83	57	57	1	1	0	0	1	1
3	7	91	5	88	26	83	3	4	0	0	0	1
4	1	92	1	89	6	89	2	6	0	0	0	1
5	0	92	1	90	1	90	13	19	0	0	0	1
6	1	93	2	92	2	92	8	27	0	0	0	1
7	0	93	1	93	0	92	9	36	0	0	0	1
8	0	93	0	93	2	94	4	40	0	0	0	1
9	1	94	0	93	0	94	4	44	0	0	0	1
10	1	95	0	93	0	94	3	47	0	0	0	1
11	0	95	2	95	0	94	2	49	0	0	0	1
12	0	95	0	95	0	94	2	51	0	0	0	1
13	0	95	0	95	0	94	1	52	0	0	0	1
14	0	95	0	95	0	94	0	52	0	0	0	1
15	1	96	0	95	0	94	1	53	0	0	0	1
16	0	96	0	95	1	95	0	53	0	0	0	1
17	0	96	1	96	1	96	0	53	0	0	0	1
18	0	96	0	96	0	96	0	53	0	0	0	1
19	0	96	0	96	0	96	1	54	0	0	0	1
20	0	96	0	96	0	96	0	54	0	0	0	1

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

Table 7-3.42 continued

The Effects of Storage at Room Relative Humidities for 9 Months on the Germination of Irradiated White Mustard Seeds (Sinapis alba)

Seeds	White Mustard											
Dose (Mrad)	10.8		30.7									
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	9		9									
R.H.	Room Humidities											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0								
2	2	2	0	0								
3	0	2	0	0								
4	0	2	0	0								
5	0	2	0	0								
6	0	2	0	0								
7	0	2	0	0								
8	0	2	0	0								
9	0	2	0	0								
10	0	2	0	0								
11	0	2	0	0								
12	0	2	0	0								
13	0	2	0	0								
14	0	2	0	0								
15	0	2	0	0								
16	0	2	0	0								
17	0	2	0	0								
18	0	2	0	0								
19	0	2	0	0								
20	0	2	0	0								

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.43

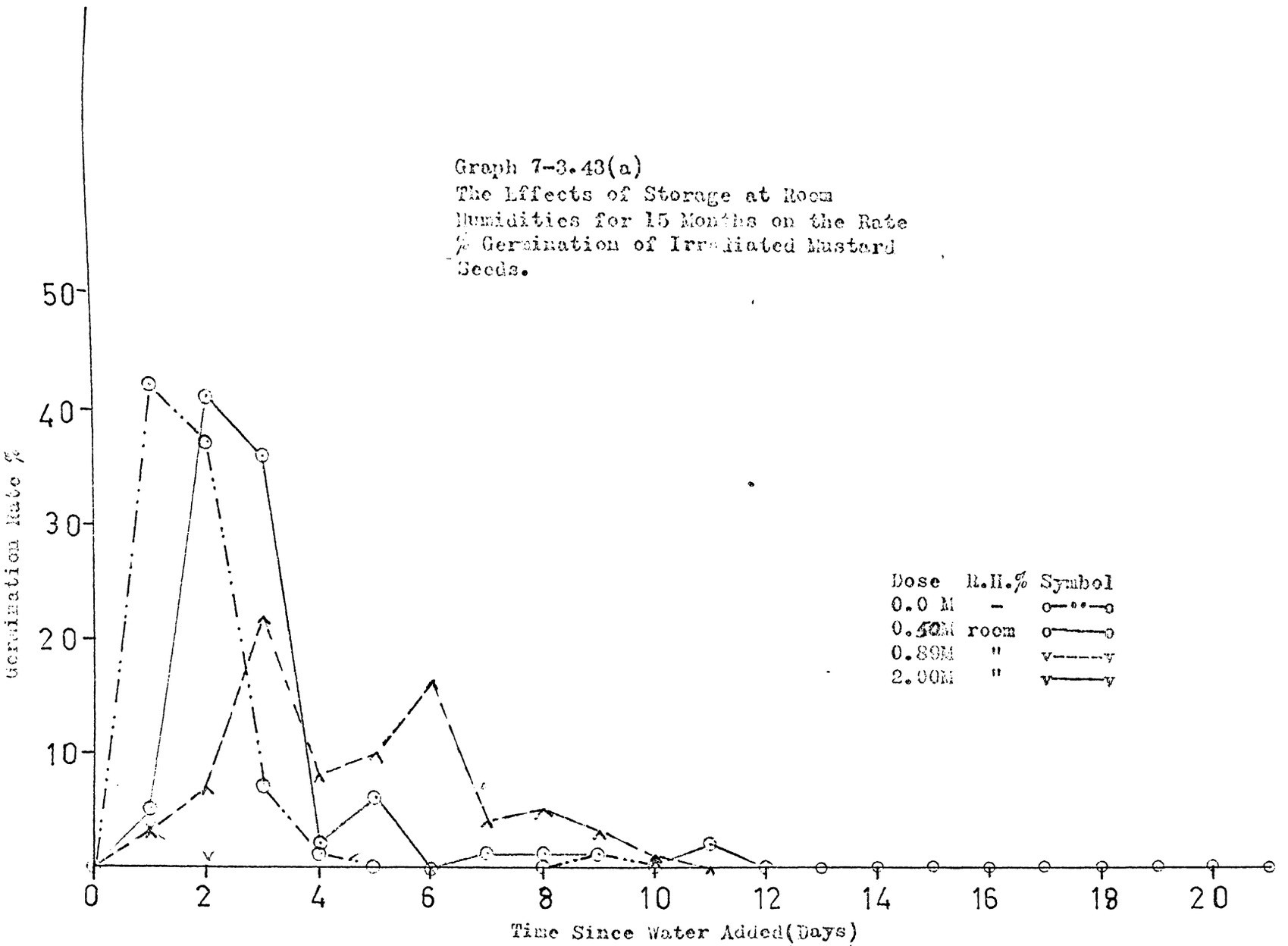
The Effects of Storage at Room Relative Humidities for 15 Months on the Germination of Irradiated Mustard Seeds.

Seeds	Mustard (Sinapis alba)											
Dose (Mrad)	0.0		0.50		2.0		4.5		5.0		9.0	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		15		15		15		15		15	
R.H.					Room Humidities							
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	42	42	5	5	3	3	0	0	0	0	0	0
2	37	79	41	46	0	3	0	0	0	0	0	0
3	7	86	36	82	0	3	0	0	0	0	0	0
4	1	87	2	84	0	3	0	0	0	0	0	0
5	0	87	6	90	0	3	0	0	0	0	0	0
6	0	87	0	90	0	3	0	0	0	0	0	0
7	0	87	1	91	0	3	0	0	0	0	0	0
8	0	87	1	92	0	3	0	0	0	0	0	0
9	1	88	1	93	0	3	0	0	0	0	0	0
10	0	88	0	93	0	3	0	0	0	0	0	0
11	0	88	2	95	0	3	0	0	0	0	0	0
12	0	88	0	95	0	3	0	0	0	0	0	0
13	0	88	0	95	0	3	0	0	0	0	0	0
14	0	88	0	95	0	3	0	0	0	0	0	0
15	0	88	0	95	0	3	0	0	0	0	0	0
16	0	88	0	95	0	3	0	0	0	0	0	0
17	0	88	0	95	0	3	0	0	0	0	0	0
18	0	88	0	95	0	3	0	0	0	0	0	0
19	0	88	0	95	0	3	0	0	0	0	0	0
20	0	88	0	95	0	3	0	0	0	0	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 7-3.43(a)
The Effects of Storage at Room Humidities for 15 Months on the Rate % Germination of Irradiated Mustard Seeds.



Graph 7-3.43(b)
The Effects of Storage at Room Humidities for 15 Months on the Germination % of Irradiated Mustard Seeds.

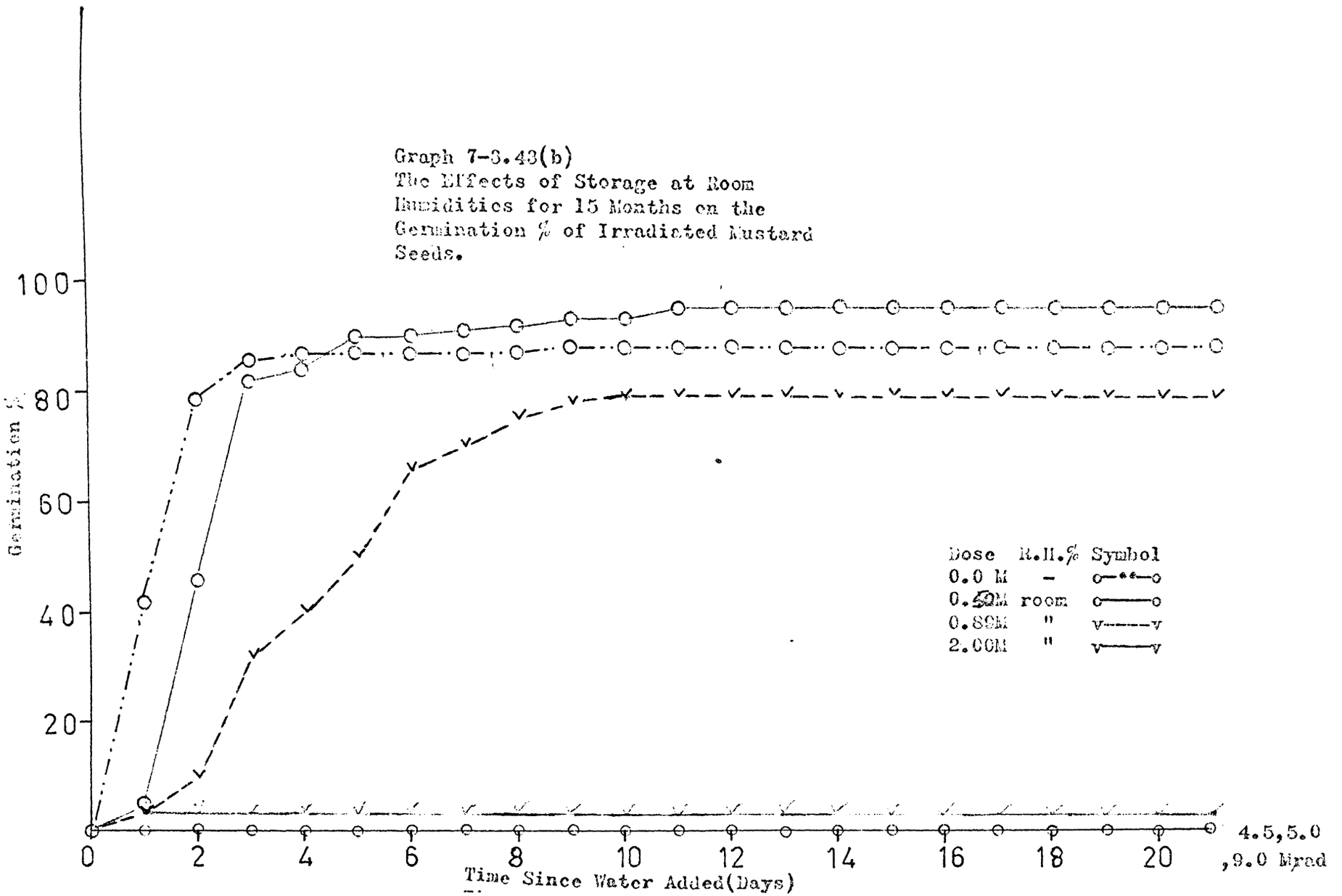


Table 7-3.44

The Effects of Storage at Room Relative Humidities for 24 Months on the Germination of Irradiated White Mustard Seed (Sinapis alba)

Seeds	Mustard											
Dose (Mrad)	0.0		2.0		4.5		5.0		9.0		32.3	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		24 months		24 months		24 months		24 months		24 months	
R.H.	-				Room Humidities							
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	68	68	0	0	0	0	0	0	0	0	0	0
2	17	85	0	0	0	0	0	0	0	0	0	0
3	5	90	0	0	0	0	0	0	0	0	0	0
4	2	92	0	0	0	0	0	0	0	0	0	0
5	0	92	0	0	0	0	0	0	0	0	0	0
6	1	93	0	0	0	0	0	0	0	0	0	0
7	0	93	0	0	0	0	0	0	0	0	0	0
8	1	94	0	0	0	0	0	0	0	0	0	0
9	0	94	0	0	0	0	0	0	0	0	0	0
10	0	94	0	0	0	0	0	0	0	0	0	0
11	0	94	0	0	0	0	0	0	0	0	0	0
12	0	94	0	0	0	0	0	0	0	0	0	0
13	0	94	0	0	0	0	0	0	0	0	0	0
14	0	94	0	0	0	0	0	0	0	0	0	0
15	0	94	0	0	0	0	0	0	0	0	0	0
16	0	94	0	0	0	0	0	0	0	0	0	0
17	0	94	0	0	0	0	0	0	0	0	0	0
18	0	94	0	0	0	0	0	0	0	0	0	0
19	0	94	0	0	0	0	0	0	0	0	0	0
20	0	94	0	0	0	0	0	0	0	0	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.45

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Onion Seeds.

Seed — Onion (Allium cepa)

Dose (Mrad)	0.0		0.49		0.49		5.0		5.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2		2		2		2			
R.H.	-		55		76		55		76			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	1	1	1	1	1	1	0	0	0	0		
2	4	5	0	1	0	1	0	0	0	0		
3	28	33	3	4	0	1	0	0	0	0		
4	33	66	8	12	2	3	0	0	0	0		
5	14	80	13	25	3	6	0	0	0	0		
6	3	83	14	39	6	12	0	0	0	0		
7	1	84	9	48	7	19	0	0	0	0		
8	1	85	7	55	3	22	0	0	0	0		
9	2	87	6	61	2	24	0	0	0	0		
10	0	87	1	62	2	26	0	0	0	0		
11	1	88	1	63	1	27	0	0	0	0		
12	1	89	2	65	1	28	0	0	0	0		
13	0	89	3	68	1	29	0	0	0	0		
14	1	90	1	69	2	31	0	0	0	0		
15	0	90	1	70	4	35	0	0	0	0		
16	0	90	2	72	1	36	0	0	0	0		
17	0	90	2	74	0	36	0	0	0	0		
18	0	90	3	77	1	37	0	0	0	0		
19	0	90	1	78	0	37	0	0	0	0		
20	0	90	2	80	0	37	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Graph 7-3.45
The Effects of Storage at Various Relative Humidities for 2 Months on the Germination % of Irradiated Onion Seeds.

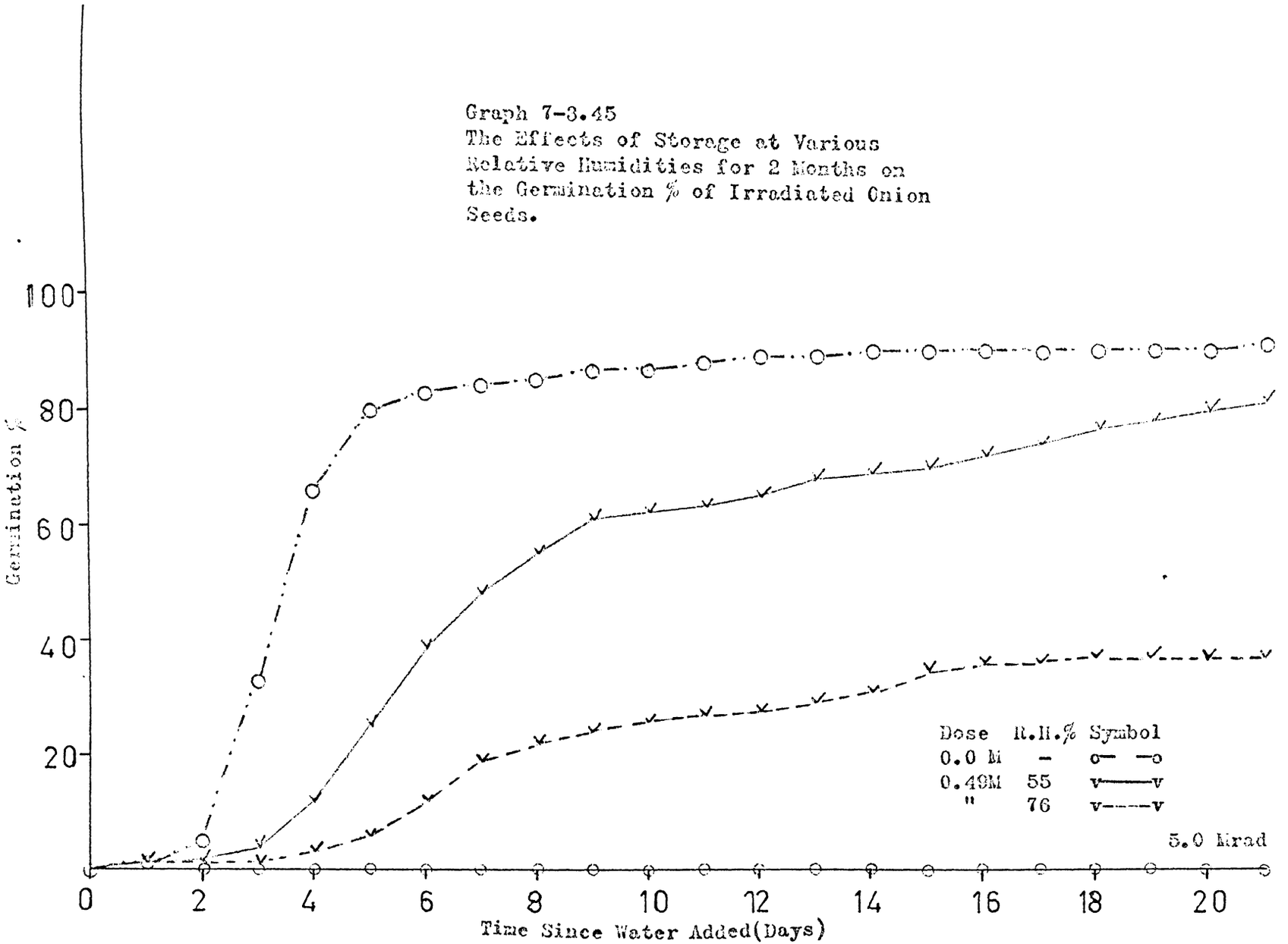


Table 7-3.46

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Onion Seeds.

Seed — Onion (*Allium cepa*)

Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2		2		2		2			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	0	0	0	0	0	0	0	0	0	0		
4	1	1	0	0	0	0	0	0	0	0		
5	2	3	0	0	0	0	0	0	0	0		
6	75	78	0	0	0	0	0	0	0	0		
7	1	79	0	0	0	0	0	0	0	0		
8	4	83	0	0	0	0	0	0	0	0		
9	3	86	0	0	0	0	0	0	0	0		
10	0	86	0	0	0	0	0	0	0	0		
11	2	88	0	0	0	0	0	0	0	0		
12	1	89	0	0	0	0	0	0	0	0		
13	1	90	0	0	0	0	0	0	0	0		
14	2	92	0	0	0	0	0	0	0	0		
15	0	92	0	0	0	0	0	0	0	0		
16	1	93	0	0	0	0	0	0	0	0		
17	0	93	0	0	0	0	0	0	0	0		
18	0	93	0	0	0	0	0	0	0	0		
19	0	93	0	0	0	0	0	0	0	0		
20	0	93	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.47

The Effects of Storage at Various Relative Humidities for 4 Months on the Germination of Irradiated Onion Seeds.

Seed	Onion (*Allium cepa*)											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		4		4		4		4			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	1	1	0	0	0	0	0	0	0	0		
2	4	5	0	0	0	0	0	0	0	0		
3	28	33	0	0	0	0	0	0	0	0		
4	33	66	0	0	0	0	0	0	0	0		
5	14	80	0	0	0	0	0	0	0	0		
6	3	83	0	0	0	0	0	0	0	0		
7	1	84	0	0	0	0	0	0	0	0		
8	1	85	0	0	0	0	0	0	0	0		
9	2	87	0	0	0	0	0	0	0	0		
10	0	87	0	0	0	0	0	0	0	0		
11	1	88	0	0	0	0	0	0	0	0		
12	1	89	0	0	0	0	0	0	0	0		
13	0	89	0	0	0	0	0	0	0	0		
14	1	90	0	0	0	0	0	0	0	0		
15	0	90	0	0	0	0	0	0	0	0		
16	0	90	0	0	0	0	0	0	0	0		
17	0	90	0	0	0	0	0	0	0	0		
18	0	90	0	0	0	0	0	0	0	0		
19	0	90	0	0	0	0	0	0	0	0		
20	0	90	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.48

The Effects of Storage at Various Relative Humidities for 8 Months on the Germination of Irradiated Onion Seeds.

Seeds	Onion											
Dose (Mrad)	0.0		0.49		0.49		5.0		5.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		8		8		8		8			
R.H.	-		55		76		55		76			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	10	10	0	0	0	0	0	0	0	0		
4	36	46	0	0	1	1	0	0	0	0		
5	30	76	9	9	0	1	0	0	0	0		
6	2	78	3	12	0	1	0	0	0	0		
7	5	83	6	18	0	1	0	0	0	0		
8	4	87	8	26	0	1	0	0	0	0		
9	3	90	9	35	0	1	0	0	0	0		
10	2	92	5	40	0	1	0	0	0	0		
11	0	92	5	45	0	1	0	0	0	0		
12	0	92	3	48	0	1	0	0	0	0		
13	0	92	0	48	0	1	0	0	0	0		
14	0	92	1	49	0	1	0	0	0	0		
15	0	92	1	50	0	1	0	0	0	0		
16	0	92	1	51	0	1	0	0	0	0		
17	0	92	2	53	0	1	0	0	0	0		
18	0	92	1	54	0	1	0	0	0	0		
19	0	92	0	54	0	1	0	0	0	0		
20	0	92	0	54	0	1	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 7-3.48
The Effects of Storage at Various Relative Humidities for 8 Months on the Germination % of Irradiated Onion Seeds.

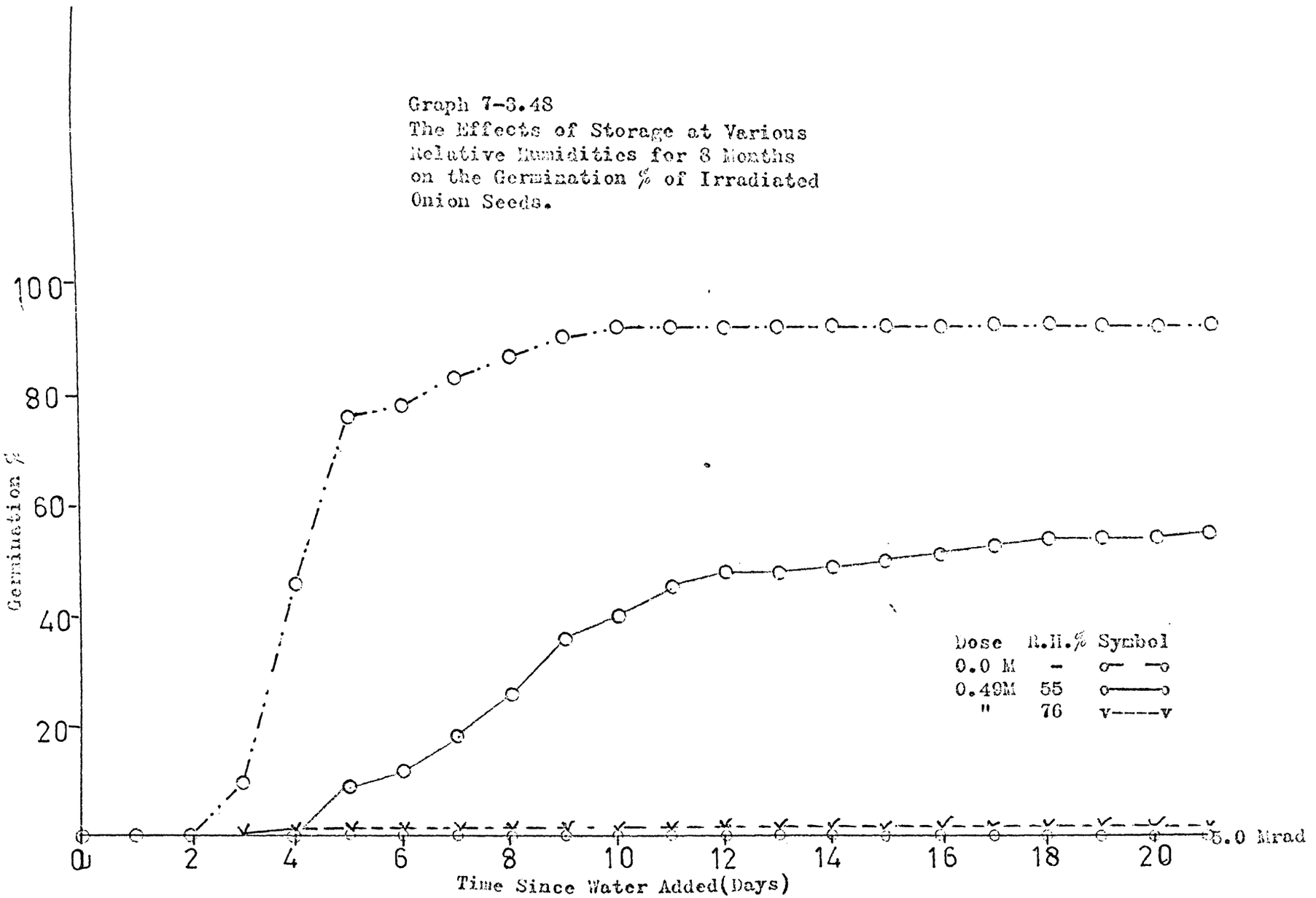


Table 7-3.49

The Effects of Storage at Various Relative Humidities for 10 Months on the Germination of Irradiated Onion Seeds.

Seeds	Onion											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		10		10		10		10			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	10	10	0	0	0	0	0	0	0	0		
4	36	46	0	0	0	0	0	0	0	0		
5	30	76	0	0	0	0	0	0	0	0		
6	2	78	0	0	0	0	0	0	0	0		
7	5	83	0	0	0	0	0	0	0	0		
8	4	87	0	0	0	0	0	0	0	0		
9	3	90	0	0	0	0	0	0	0	0		
10	2	92	0	0	0	0	0	0	0	0		
11	0	92	0	0	0	0	0	0	0	0		
12	0	92	0	0	0	0	0	0	0	0		
13	0	92	0	0	0	0	0	0	0	0		
14	0	92	0	0	0	0	0	0	0	0		
15	0	92	0	0	0	0	0	0	0	0		
16	0	92	0	0	0	0	0	0	0	0		
17	0	92	0	0	0	0	0	0	0	0		
18	0	92	0	0	0	0	0	0	0	0		
19	0	92	0	0	0	0	0	0	0	0		
20	0	92	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.50

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Onion Seeds.

Seeds	Onion											
Dose (Mrad)	0.0		0.49		0.49		05.0		5.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12		12		12		12			
R.H.	-		55		76		55		76			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	2	2	0	0	0	0	0	0	0	0		
3	6	8	0	0	0	0	0	0	0	0		
4	8	16	5	5	0	0	0	0	0	0		
5	7	23	9	14	0	0	0	0	0	0		
6	7	30	3	17	0	0	0	0	0	0		
7	6	36	2	19	0	0	0	0	0	0		
8	5	41	1	20	0	0	0	0	0	0		
9	4	45	1	21	0	0	0	0	0	0		
10	2	47	1	22	0	0	0	0	0	0		
11	0	47	3	25	0	0	0	0	0	0		
12	1	48	1	26	0	0	0	0	0	0		
13	0	48	0	26	0	0	0	0	0	0		
14	0	48	1	27	0	0	0	0	0	0		
15	2	50	0	27	0	0	0	0	0	0		
16	1	51	0	27	0	0	0	0	0	0		
17	0	51	0	27	0	0	0	0	0	0		
18	0	51	0	27	0	0	0	0	0	0		
19	0	51	0	27	0	0	0	0	0	0		
20	1	52	0	27	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Graph 7-3.50
The Effects of Storage at Various Relative Humidities for 12 Months on the Germination % of Irradiated Onion Seeds.

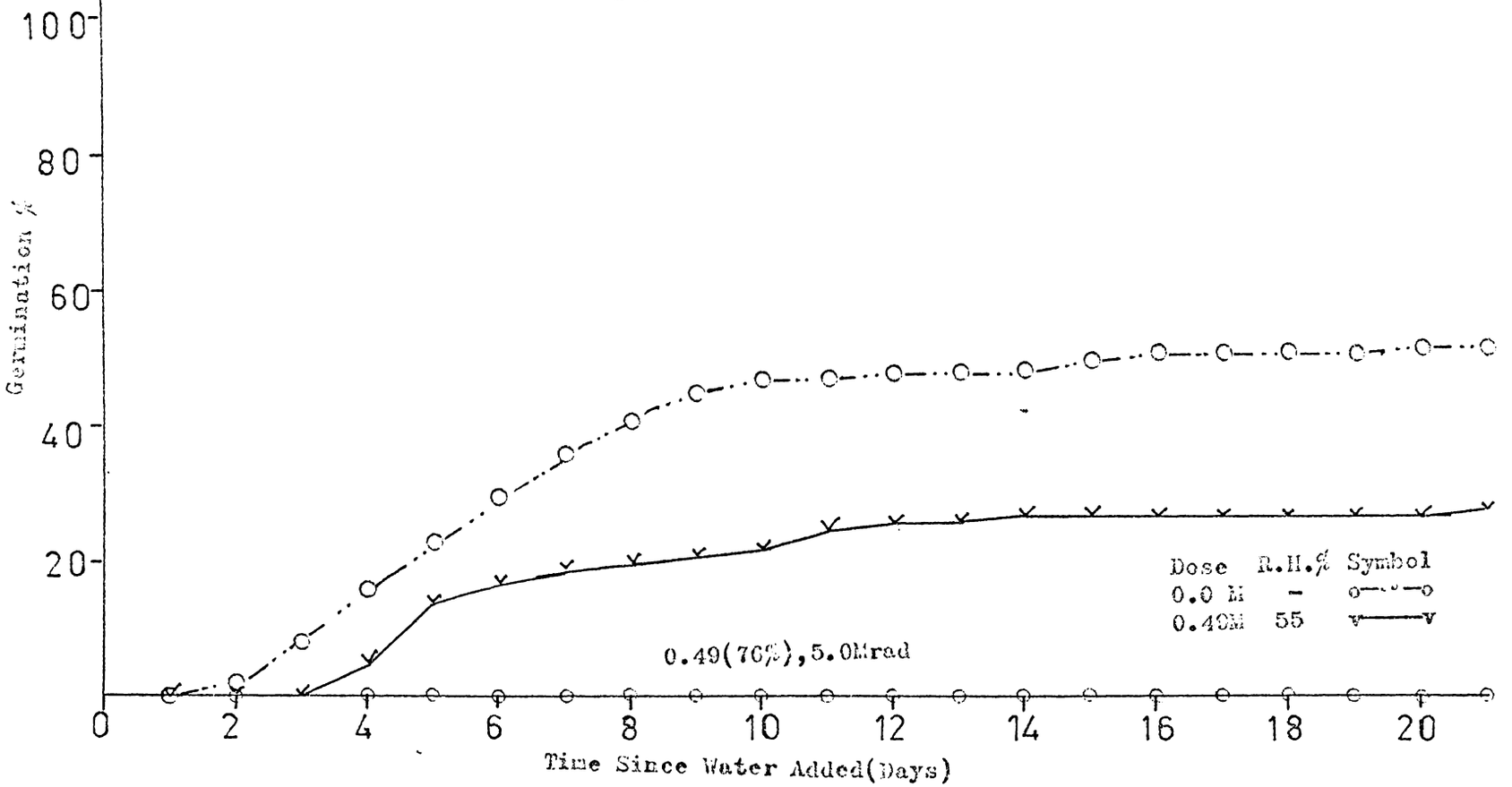


Table 7-3.51

The Effects of Storage at Various Relative Humidities for 14 Months on the Germination of Irradiated Onion Seeds.

Seeds	Onion											
Dose (Mrad)	0.0		32.3		32.3		32.3		32.3			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		14		14		14		14			
R.H.	-		23		45		66		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	2	2	0	0	0	0	0	0	0	0		
3	6	8	0	0	0	0	0	0	0	0		
4	8	16	0	0	0	0	0	0	0	0		
5	7	23	0	0	0	0	0	0	0	0		
6	7	30	0	0	0	0	0	0	0	0		
7	6	36	0	0	0	0	0	0	0	0		
8	5	41	0	0	0	0	0	0	0	0		
9	4	45	0	0	0	0	0	0	0	0		
10	2	47	0	0	0	0	0	0	0	0		
11	0	47	0	0	0	0	0	0	0	0		
12	1	48	0	0	0	0	0	0	0	0		
13	0	48	0	0	0	0	0	0	0	0		
14	0	48	0	0	0	0	0	0	0	0		
15	2	50	0	0	0	0	0	0	0	0		
16	1	51	0	0	0	0	0	0	0	0		
17	0	51	0	0	0	0	0	0	0	0		
18	0	51	0	0	0	0	0	0	0	0		
19	0	51	0	0	0	0	0	0	0	0		
20	1	52	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.52

The Effects of Storage at Room Relative Humidities for 15 Months on the Germination of Irradiated Onion Seeds.

Seeds	Onion											
Dose (Mrad)	0.0		0.49		0.672		2.0		5.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		15		15		15		15			
R.H.	Room humidities											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	11	11	2	2	2	2	0	0	0	0		
4	11	22	8	10	4	6	0	0	0	0		
5	18	40	12	22	7	13	0	0	0	0		
6	4	44	3	25	15	28	0	0	0	0		
7	5	49	3	28	4	32	0	0	0	0		
8	5	54	6	34	3	35	0	0	0	0		
9	1	55	1	35	2	37	0	0	0	0		
10	1	56	2	37	3	40	0	0	0	0		
11	0	56	1	38	2	42	0	0	0	0		
12	1	57	0	38	1	43	0	0	0	0		
13	0	57	1	39	1	44	0	0	0	0		
14	1	58	1	40	0	44	0	0	0	0		
15	1	59	1	41	0	44	0	0	0	0		
16	0	59	0	41	1	45	0	0	0	0		
17	0	59	1	42	1	46	0	0	0	0		
18	1	60	1	43	0	46	0	0	0	0		
19	0	60	1	44	0	46	0	0	0	0		
20	0	60	0	44	0	46	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 7-3. 52
The Effects of Storage at Room Humidities for 15 Months on the Germination % of Irradiated Onion Seeds.

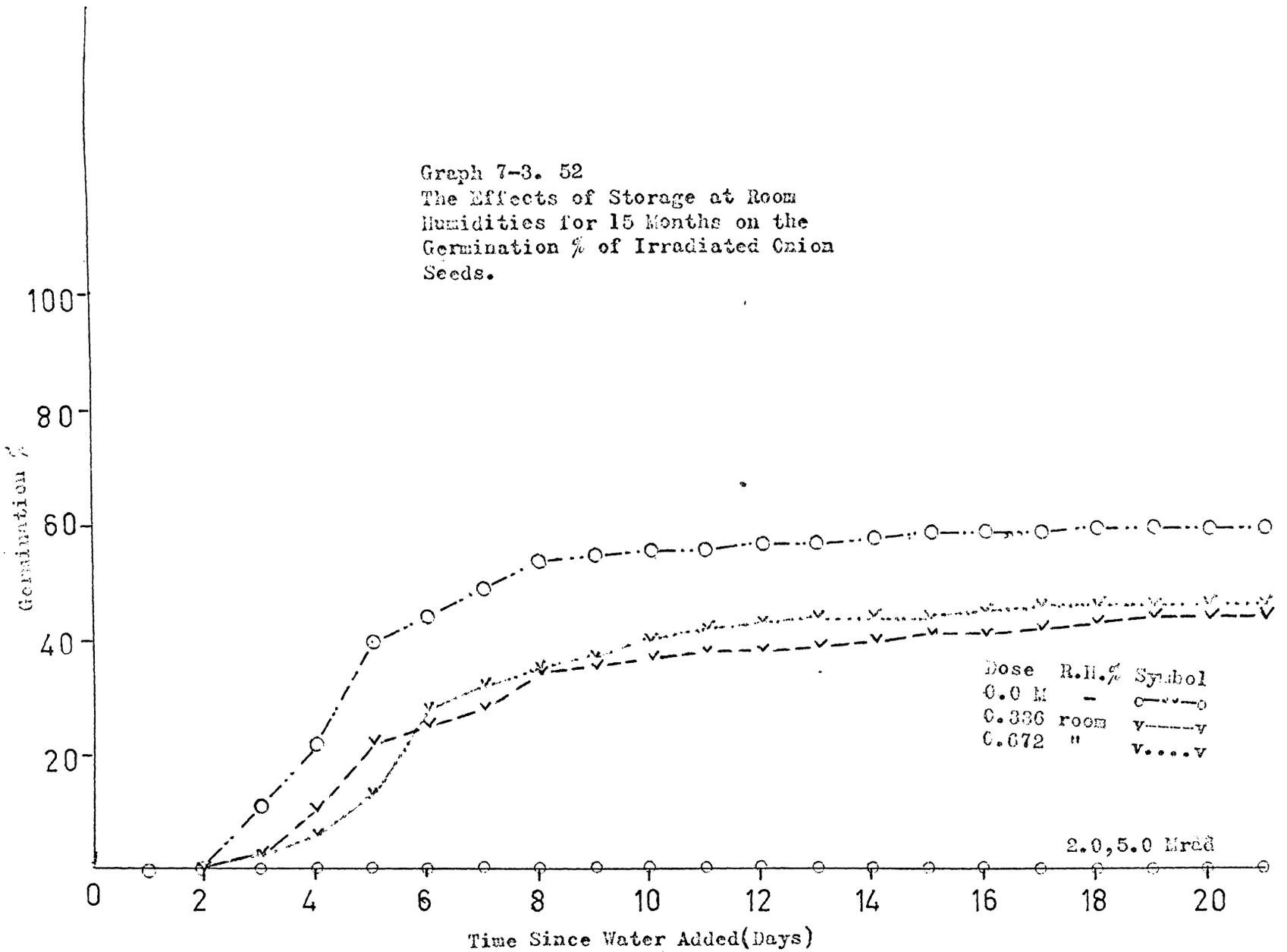


Table 7-3.53

The Effects of Storage at Room Relative Humidities for 24 Months on the Germination of Irradiated Onion Seeds.

Seeds	Onion											
Dose (Mrad)	0.0		2.0		5.0		32.3					
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		24		24		24					
R.H.	-		Room humidities									
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0				
2	1	1	0	0	0	0	0	0				
3	9	10	0	0	0	0	0	0				
4	6	16	0	0	0	0	0	0				
5	2	18	0	0	0	0	0	0				
6	2	20	0	0	0	0	0	0				
7	1	21	0	0	0	0	0	0				
8	0	21	0	0	0	0	0	0				
9	0	21	0	0	0	0	0	0				
10	2	23	0	0	0	0	0	0				
11	1	24	0	0	0	0	0	0				
12	2	26	0	0	0	0	0	0				
13	1	27	0	0	0	0	0	0				
14	0	27	0	0	0	0	0	0				
15	0	27	0	0	0	0	0	0				
16	0	27	0	0	0	0	0	0				
17	1	28	0	0	0	0	0	0				
18	0	28	0	0	0	0	0	0				
19	0	28	0	0	0	0	0	0				
20	0	28	0	0	0	0	0	0				

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.54

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Parsnip Seeds.

Seed	Parsnip											
Dose (Mrad)	0.0		0.51		0.51		5.1		5.1			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2		2		2		2			
R.H.	-		9		93		9		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	0	0	0	0	0	0	0	0	0	0		
4	0	0	0	0	0	0	0	0	0	0		
5	0	0	0	0	0	0	0	0	0	0		
6	0	0	0	0	0	0	0	0	0	0		
7	1	1	0	0	0	0	0	0	0	0		
8	4	5	0	0	0	0	0	0	0	0		
9	6	11	0	0	0	0	0	0	0	0		
10	9	20	0	0	0	0	0	0	0	0		
11	14	34	0	0	0	0	0	0	0	0		
12	7	41	0	0	0	0	0	0	0	0		
13	7	48	0	0	0	0	0	0	0	0		
14	4	52	0	0	0	0	0	0	0	0		
15	5	57	0	0	0	0	0	0	0	0		
16	2	59	0	0	0	0	0	0	0	0		
17	6	65	0	0	0	0	0	0	0	0		
18	1	66	0	0	0	0	0	0	0	0		
19	3	69	0	0	0	0	0	0	0	0		
20	2	71	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.55

The Effects of Storage at Various Relative Humidities for 8 Months on the Germination of Irradiated Parsnip Seeds.

Seeds	Parsnip											
Dose (Mrad)	0.0		0.50		0.50		5.10		5.10			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		8		8		8		8			
R.H.	-		9		93		9		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	0	0	0	0	0	0	0	0	0	0		
4	0	0	0	0	0	0	0	0	0	0		
5	0	0	0	0	0	0	0	0	0	0		
6	1	1	0	0	0	0	0	0	0	0		
7	4	5	0	0	0	0	0	0	0	0		
8	10	15	0	0	0	0	0	0	0	0		
9	11	26	0	0	0	0	0	0	0	0		
10	19	45	0	0	0	0	0	0	0	0		
11	9	54	0	0	0	0	0	0	0	0		
12	12	66	0	0	0	0	0	0	0	0		
13	2	68	0	0	0	0	0	0	0	0		
14	2	70	0	0	0	0	0	0	0	0		
15	3	73	0	0	0	0	0	0	0	0		
16	1	74	0	0	0	0	0	0	0	0		
17	1	75	0	0	0	0	0	0	0	0		
18	0	75	0	0	0	0	0	0	0	0		
19	0	75	0	0	0	0	0	0	0	0		
20	1	76	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.56

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Parsnip Seeds.

Seeds	Parsnip											
Dose (Mrad)	0.0		0.51		0.51		5.1		5.1			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12		12		12		12			
R.H.	-		9		93		9		93			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	0	0	0	0	0	0	0	0	0	0		
4	0	0	0	0	0	0	0	0	0	0		
5	3	3	0	0	0	0	0	0	0	0		
6	1	4	0	0	0	0	0	0	0	0		
7	5	9	0	0	0	0	0	0	0	0		
8	16	25	0	0	0	0	0	0	0	0		
9	15	40	0	0	0	0	0	0	0	0		
10	2	42	0	0	0	0	0	0	0	0		
11	4	46	0	0	0	0	0	0	0	0		
12	2	48	0	0	0	0	0	0	0	0		
13	4	52	0	0	0	0	0	0	0	0		
14	3	55	0	0	0	0	0	0	0	0		
15	3	58	0	0	0	0	0	0	0	0		
16	4	62	0	0	0	0	0	0	0	0		
17	3	65	0	0	0	0	0	0	0	0		
18	2	67	0	0	0	0	0	0	0	0		
19	1	68	0	0	0	0	0	0	0	0		
20	2	70	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.57

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Peas (Pisum sativum) Seeds.

Seeds	Pea (Pisum sativum)											
Dose (Mrad)	0.0		0.51		5.1							
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2		2							
R.H.	-		97		97							
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0						
2	0	0	0	0	0	0						
3	40	40	0	0	0	0						
4	45	85	0	0	0	0						
5	5	90	0	0	0	0						
6	0	90	0	0	0	0						
7	5	95	0	0	0	0						
8	5	100	0	0	0	0						
9	-	100	0	0	0	0						
10	-	100	0	0	0	0						
11	-	100	0	0	0	0						
12	-	100	0	0	0	0						
13	-	100	0	0	0	0						
14	-	100	0	0	0	0						
15	-	100	0	0	0	0						
16	-	100	0	0	0	0						
17	-	100	0	0	0	0						
18	-	100	0	0	0	0						
19	-	100	0	0	0	0						
20	-	100	0	0	0	0						

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.58

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Pea (Pisum sativum) Seeds.

Seeds	Pea (Pisum sativum)											
Dose (Mrad)	0.0		0.51		5.1							
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12		12							
R.H.	-		97		97							
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0						
2	0	0	0	0	0	0						
3	35	35	0	0	0	0						
4	45	80	0	0	0	0						
5	10	90	0	0	0	0						
6	0	90	0	0	0	0						
7	0	90	0	0	0	0						
8	0	90	0	0	0	0						
9	0	90	0	0	0	0						
10	0	90	0	0	0	0						
11	0	90	0	0	0	0						
12	0	90	0	0	0	0						
13	0	90	0	0	0	0						
14	0	90	0	0	0	0						
15	0	90	0	0	0	0						
16	0	90	0	0	0	0						
17	0	90	0	0	0	0						
18	0	90	0	0	0	0						
19	0	90	0	0	0	0						
20	0	90	0	0	0	0						

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.59

The Effects of Storage at Room Relative Humidities for 15 Months on the Germination of Irradiated Pea Seeds.

Seeds	Pea (Pisum sativum)											
Dose (Mrad)	0.0		0.1		0.33		0.51		1.0		3.3	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		15		15		15		15		15	
R.H.	-		Room Humidities									
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	24	24	0	0	0	0	20	20	0	0	0	0
4	16	40	4	4	4	4	20	40	0	0	0	0
5	32	72	4	8	2	6	28	68	0	0	0	0
6	4	76	0	8	2	8	8	76	0	0	0	0
7	2	78	0	8	0	8	0	76	0	0	0	0
8	0	78	0	8	0	8	2	78	0	0	0	0
9	0	78	0	8	0	8	0	78	0	0	0	0
10	4	82	0	8	0	8	0	78	0	0	0	0
11	0	82	0	8	0	8	0	78	0	0	0	0
12	0	82	0	8	0	8	0	78	0	0	0	0
13	0	82	0	8	0	8	0	78	0	0	0	0
14	0	82	0	8	0	8	0	78	0	0	0	0
15	0	82	0	8	0	8	0	78	0	0	0	0
16	0	82	0	8	0	8	0	78	0	0	0	0
17	0	82	0	8	0	8	0	78	0	0	0	0
18	0	82	0	8	0	8	0	78	0	0	0	0
19	0	82	0	8	0	8	0	78	0	0	0	0
20	0	82	0	8	0	8	0	78	0	0	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.60

The Effects of Storage at Room Relative Humidities for 18 Months on the Germination of Irradiated Pea Seeds.

Seeds	Pea											
Dose (Mrad)	0.0		1.0		3.3							
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		18		18							
R.H.	Room Humidities											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0						
2	0	0	0	0	0	0						
3	0	0	0	0	0	0						
4	10	10	0	0	0	0						
5	15	25	0	0	0	0						
6	30	55	0	0	0	0						
7	0	55	0	0	0	0						
8	5	60	0	0	0	0						
9	0	60	0	0	0	0						
10	0	60	0	0	0	0						
11	0	60	0	0	0	0						
12	0	60	0	0	0	0						
13	0	60	0	0	0	0						
14	0	60	0	0	0	0						
15	0	60	0	0	0	0						
16	0	60	0	0	0	0						
17	0	60	0	0	0	0						
18	0	60	0	0	0	0						
19	0	60	0	0	0	0						
20	0	60	0	0	0	0						

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.61

The Effects of Storage at Room Relative Humidities for 8 Months on the Germination of Irradiated Ryegrass Seeds.

Seeds	Ryegrass											
Dose (Mrad)	0.0		0.52		2.0		5.0		10.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		8		8		8		8			
R.H.			Room Humidities									
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	39	39	0	0	0	0	0	0	0	0		
4	35	74	0	0	0	0	0	0	0	0		
5	5	79	0	0	0	0	0	0	0	0		
6	1	80	11	11	0	0	0	0	0	0		
7	1	81	10	21	0	0	0	0	0	0		
8	1	82	15	36	0	0	0	0	0	0		
9	1	83	6	42	0	0	0	0	0	0		
10	0	83	5	47	0	0	0	0	0	0		
11	0	83	9	56	0	0	0	0	0	0		
12	1	84	8	64	0	0	0	0	0	0		
13	0	84	0	64	0	0	0	0	0	0		
14	0	84	0	64	0	0	0	0	0	0		
15	0	84	0	64	0	0	0	0	0	0		
16	0	84	0	64	0	0	0	0	0	0		
17	0	84	0	64	0	0	0	0	0	0		
18	0	84	0	64	0	0	0	0	0	0		
19	0	84	0	64	0	0	0	0	0	0		
20	0	84	0	64	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.62

The Effects of Storage at Room Relative Humidities for 14 Months on the Germination of Irradiated Ryegrass Seeds.

Seeds	Ryegrass											
Dose (Mrad)	0.0		0.52		2.0		5.0		10.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		14		14		14		14			
R.H.			Room humidities									
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	6	6	0	0	0	0	0	0	0	0		
3	45	51	13	13	0	0	0	0	0	0		
4	9	60	10	23	0	0	0	0	0	0		
5	7	67	5	28	0	0	0	0	0	0		
6	8	75	4	32	0	0	0	0	0	0		
7	0	75	4	36	0	0	0	0	0	0		
8	0	75	5	41	0	0	0	0	0	0		
9	2	77	5	46	0	0	0	0	0	0		
10	1	78	4	50	0	0	0	0	0	0		
11	1	79	2	52	0	0	0	0	0	0		
12	0	79	2	54	0	0	0	0	0	0		
13	0	79	1	55	0	0	0	0	0	0		
14	2	81	0	55	0	0	0	0	0	0		
15	0	81	1	56	0	0	0	0	0	0		
16	0	81	0	56	0	0	0	0	0	0		
17	0	81	0	56	0	0	0	0	0	0		
18	0	81	1	57	0	0	0	0	0	0		
19	1	82	0	57	0	0	0	0	0	0		
20	1	83	0	57	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.63

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Spinach Seeds.

Seeds	Spinach											
Dose (Mrad)	0.0		0.51		0.51		5.0		5.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2		2		2		2			
R.H.	-		33		86		33		86			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	1	1	0	0	0	0	0	0	0	0		
3	0	1	1	1	0	0	0	0	0	0		
4	1	2	0	1	0	0	0	0	0	0		
5	0	2	0	1	0	0	0	0	0	0		
6	2	4	0	1	0	0	0	0	0	0		
7	0	4	0	1	0	0	0	0	0	0		
8	1	5	0	1	0	0	0	0	0	0		
9	0	5	0	1	0	0	0	0	0	0		
10	0	5	0	1	0	0	0	0	0	0		
11	0	5	0	1	0	0	0	0	0	0		
12	0	5	0	1	0	0	0	0	0	0		
13	0	5	2	3	0	0	0	0	0	0		
14	0	5	0	3	0	0	0	0	0	0		
15	0	5	0	3	0	0	0	0	0	0		
16	1	6	1	4	0	0	0	0	0	0		
17	0	6	0	4	0	0	0	0	0	0		
18	0	6	0	4	0	0	0	0	0	0		
19	0	6	0	4	0	0	0	0	0	0		
20	0	6	0	4	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.64

The Effects of Storage at Various Relative Humidities for 8 Months on the Germination of Irradiated Spinach Seeds.

Seeds	Spinach											
Dose (Mrad)	0.0		0.51		0.51		5.0		5.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		8		8		8		8			
R.H.	-		33		86		33		86			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	0	0	0	0	0	0	0	0	0	0		
4	0	0	0	0	0	0	0	0	0	0		
5	3	3	0	0	0	0	0	0	0	0		
6	0	3	0	0	0	0	0	0	0	0		
7	1	4	0	0	0	0	0	0	0	0		
8	1	5	1	1	0	0	0	0	0	0		
9	1	6	0	1	0	0	0	0	0	0		
10	1	7	1	2	0	0	0	0	0	0		
11	2	9	0	2	0	0	0	0	0	0		
12	0	9	0	2	0	0	0	0	0	0		
13	2	11	0	2	0	0	0	0	0	0		
14	0	11	0	2	0	0	0	0	0	0		
15	0	11	0	2	0	0	0	0	0	0		
16	1	12	0	2	0	0	0	0	0	0		
17	1	13	0	2	0	0	0	0	0	0		
18	0	13	0	2	0	0	0	0	0	0		
19	1	14	0	2	0	0	0	0	0	0		
20	1	15	0	2	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours
* (b) Total % germination.

Table 7-3.65

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Spinach Seeds.

Seeds	Spinach											
Dose (Mrad)	0.0		0.51		0.51		5.0		5.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12		12		12		12			
R.H.	-		33		86		33		86			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	0	0	0	0		
3	1	1	0	0	0	0	0	0	0	0		
4	0	1	0	0	0	0	0	0	0	0		
5	0	1	0	0	0	0	0	0	0	0		
6	1	2	0	0	0	0	0	0	0	0		
7	0	2	0	0	0	0	0	0	0	0		
8	0	2	0	0	0	0	0	0	0	0		
9	0	2	0	0	0	0	0	0	0	0		
10	0	2	0	0	0	0	0	0	0	0		
11	0	2	0	0	0	0	0	0	0	0		
12	0	2	0	0	0	0	0	0	0	0		
13	1	3	0	0	0	0	0	0	0	0		
14	0	3	0	0	0	0	0	0	0	0		
15	0	3	0	0	0	0	0	0	0	0		
16	0	3	0	0	0	0	0	0	0	0		
17	0	3	0	0	0	0	0	0	0	0		
18	0	3	0	0	0	0	0	0	0	0		
19	0	3	0	0	0	0	0	0	0	0		
20	0	3	0	0	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.66

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Squash Seeds.

Seeds	Squash											
Dose (Mrad)	0.0		0.51		5.1							
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12		12							
R.H.	-		86		86							
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0						
2	5	5	0	0	0	0						
3	20	25	0	0	0	0						
4	0	25	0	0	0	0						
5	5	30	0	0	0	0						
6	0	30	0	0	0	0						
7	0	30	0	0	0	0						
8	5	35	0	0	0	0						
9	10	45	0	0	0	0						
10	15	60	0	0	0	0						
11	5	65	0	0	0	0						
12	0	65	0	0	0	0						
13	5	70	0	0	0	0						
14	0	70	0	0	0	0						
15	0	70	0	0	0	0						
16	0	70	0	0	0	0						
17	0	70	0	0	0	0						
18	0	70	0	0	0	0						
19	0	70	0	0	0	0						
20	0	70	0	0	0	0						

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.67

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Tomato Seeds, Varitties "Frost Resistant" and "Beef Steak".

Seeds	Tomato "Frost Resistant"						Tomato "Beef Steak"					
Dose (Mrad)	0.0		0.51		5.0		0.0		0.51		5.0	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12		12		-		12		12	
R.H.	-		86		86		-		86		86	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	4	4	0	0	0	0
2	8	8	0	0	0	0	16	20	0	0	0	0
3	36	44	0	0	0	0	32	52	0	0	0	0
4	52	96	0	0	0	0	4	56	0	0	0	0
5	0	96	0	0	0	0	4	60	0	0	0	0
6	0	96	0	0	0	0	0	60	0	0	0	0
7	2	98	0	0	0	0	0	60	0	0	0	0
8	2	100	0	0	0	0	0	60	0	0	0	0
9	-	100	0	0	0	0	0	60	0	0	0	0
10	-	100	0	0	0	0	0	60	0	0	0	0
11	-	100	0	0	0	0	0	60	0	0	0	0
12	-	100	0	0	0	0	0	60	0	0	0	0
13	-	100	0	0	0	0	0	60	0	0	0	0
14	-	100	0	0	0	0	0	60	0	0	0	0
15	-	100	0	0	0	0	2	62	0	0	0	0
16	-	100	0	0	0	0	1	63	0	0	0	0
17	-	100	0	0	0	0	0	63	0	0	0	0
18	-	100	0	0	0	0	0	63	0	0	0	0
19	-	100	0	0	0	0	0	63	0	0	0	0
20	-	100	0	0	0	0	0	64	0	0	0	0

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.68

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Water Melon.

Seed	Water Melon											
Dose (Mrad)	0.0		0.51		5.0							
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2		2							
R.H.	-		86		86							
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0						
2	0	0	0	0	0	0						
3	45	45	0	0	0	0						
4	40	85	0	0	0	0						
5	5	90	0	0	0	0						
6	0	90	0	0	0	0						
7	0	90	20	20	0	0						
8	0	90	25	45	0	0						
9	0	90	5	50	0	0						
10	0	90	10	60	0	0						
11	0	90	0	60	0	0						
12	0	90	0	60	0	0						
13	0	90	0	60	0	0						
14	0	90	0	60	0	0						
15	0	90	0	60	0	0						
16	0	90	0	60	0	0						
17	0	90	5	65	0	0						
18	0	90	0	65	0	0						
19	0	90	0	65	0	0						
20	0	90	0	65	0	0						

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.69

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Water Melon Seeds.

Seeds	Water Melon											
Dose (Mrad)	0.0		0.51		0.51		5.0		5.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12		12		12		12			
R.H.	-		55		86		55		86			
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0		
2	12	12	0	0	0	0	0	0	0	0		
3	24	36	0	0	0	0	0	0	0	0		
4	24	60	0	0	0	0	0	0	0	0		
5	8	68	0	0	0	0	0	0	0	0		
6	4	72	0	0	0	0	0	0	0	0		
7	4	76	4	4	0	0	0	0	0	0		
8	4	80	8	12	0	0	0	0	0	0		
9	8	88	24	36	0	0	0	0	0	0		
10	8	96	28	64	0	0	0	0	0	0		
11	2	98	8	72	0	0	0	0	0	0		
12	0	98	8	80	0	0	0	0	0	0		
13	0	98	0	80	0	0	0	0	0	0		
14	0	98	0	80	0	0	0	0	0	0		
15	0	98	0	80	0	0	0	0	0	0		
16	0	98	0	80	0	0	0	0	0	0		
17	0	98	0	80	0	0	0	0	0	0		
18	0	98	0	80	0	0	0	0	0	0		
19	0	98	0	80	0	0	0	0	0	0		
20	0	98	0	80	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7.3.70

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Wheat Seeds.

Seed	Wheat											
Dose (Mrad)	0.0		0.51		0.51		0.51		0.51		0.51	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2		2		2		2		2	
R.H.	-		9		23		45		66		76	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	66	66	60	60	69	69	66	66	55	55	59	59
3	22	88	26	86	11	80	19	85	15	70	17	76
4	4	92	11	97	4	84	0	85	7	77	1	77
5	1	93	0	97	0	84	4	89	0	77	1	78
6	1	94	2	99	3	87	2	91	2	79	1	79
7	2	96	0	99	0	87	0	91	0	79	0	79
8	0	96	0	99	0	87	0	91	2	81	2	81
9	0	96	0	99	1	88	0	91	1	82	0	81
10	0	96	0	99	0	88	0	91	1	83	1	82
11	0	96	0	99	0	88	0	91	0	83	1	83
12	0	96	0	99	0	88	0	91	0	83	0	83
13	0	96	0	99	2	90	0	91	0	83	0	83
14	0	96	0	99	1	91	0	91	0	83	0	83
15	0	96	0	99	0	91	0	91	0	83	0	83
16	0	96	0	99	0	91	0	91	0	83	0	83
17	0	96	0	99	0	91	0	91	0	83	0	83
18	0	96	0	99	0	91	1	92	0	83	1	84
19	0	96	0	99	0	91	0	92	0	83	0	84
20	0	96	0	99	0	91	0	92	0	83	0	84

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

Table 7-3.70 continued

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Wheat Seeds.

Seed	Wheat											
Dose (Mrad)	0.51		0.51									
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	2		2									
R.H.	86		97									
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0								
2	32	32	20	20								
3	8	40	12	32								
4	5	45	12	44								
5	6	51	4	48								
6	0	51	0	48								
7	1	52	0	48								
8	0	52	1	49								
9	0	52	0	49								
10	1	53	1	50								
11	0	53	0	50								
12	1	54	0	50								
13	0	54	0	50								
14	0	54	0	50								
15	0	54	0	50								
16	0	54	0	50								
17	0	54	0	50								
18	0	54	0	50								
19	0	54	0	50								
20	0	54	0	50								

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 7-3.70
The Effects of Storage at Various Relative Humidities for 2 Months on the Germination % of Irradiated Wheat Seeds.

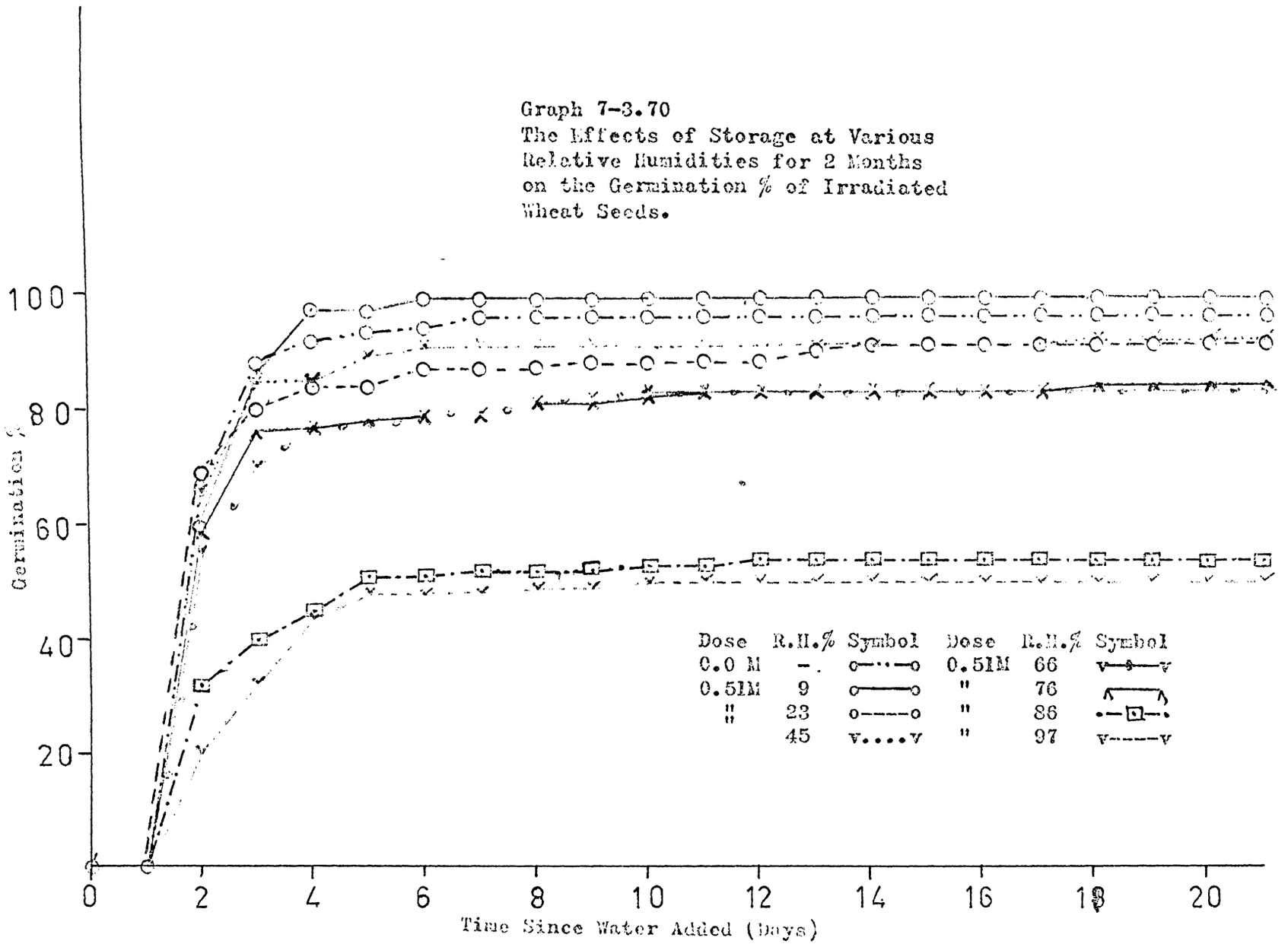


Table 7-3.71

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Wheat Seeds.

Seeds: Wheat

Dose (Mrad)	0.0		5.14		5.14		5.14		5.14		5.14	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		2		2		2		2		2	
R.H.	-		9		23		45		66		86	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	66	66	0	0	0	0	0	0	0	0	0	0
3	22	88	0	0	0	0	0	0	0	0	0	0
4	4	92	0	0	0	0	0	0	0	0	0	0
5	1	93	0	0	0	0	0	0	0	0	0	0
6	1	94	0	0	0	0	0	0	0	0	0	0
7	2	96	0	0	0	0	0	0	0	0	0	0
8	0	96	0	0	0	0	0	0	0	0	0	0
9	0	96	0	0	0	0	0	0	0	0	0	0
10	0	96	0	0	0	0	0	0	0	0	0	0
11	0	96	0	0	0	0	0	0	0	0	0	0
12	0	96	0	0	0	0	0	0	0	0	0	0
13	0	96	0	0	0	0	0	0	0	0	0	0
14	0	96	0	0	0	0	0	0	0	0	0	0
15	0	96	0	0	0	0	0	0	0	0	0	0
16	0	96	0	0	0	0	0	0	0	0	0	0
17	0	96	0	0	0	0	0	0	0	0	0	0
18	0	96	0	0	0	0	0	0	0	0	0	0
19	0	96	0	0	0	0	0	0	0	0	0	0
20	0	96	0	0	0	0	0	0	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued....

Table 7-3.71 continued

The Effects of Storage at Various Relative Humidities for 2 Months on the Germination of Irradiated Wheat Seeds.

Seeds	Wheat											
Dose (Mrad)	5.14											
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	2											
R.H.	97											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0										
2	0	0										
3	0	0										
4	0	0										
5	0	0										
6	0	0										
7	0	0										
8	0	0										
9	0	0										
10	0	0										
11	0	0										
12	0	0										
13	0	0										
14	0	0										
15	0	0										
16	0	0										
17	0	0										
18	0	0										
19	0	0										
20	0	0										

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.72

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Wheat Seeds.

Seeds: Wheat

Dose (Mrad)	0.0		0.51		0.51		0.51		0.51		0.51	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12		12		12		12		12	
R.H.	-		9		23		45		66		76	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	23	23	0	0	0	0	2	2	0	0	0	0
3	15	38	39	39	40	40	36	38	0	0	0	0
4	5	43	4	43	3	43	1	39	0	0	0	0
5	0	43	2	45	1	44	0	39	0	0	0	0
6	0	43	0	45	0	44	1	40	0	0	0	0
7	1	44	0	45	1	45	2	42	0	0	0	0
8	0	44	0	45	0	45	0	42	0	0	0	0
9	0	44	0	45	0	45	0	42	0	0	0	0
10	0	44	0	45	0	45	0	42	0	0	0	0
11	0	44	0	45	0	45	0	42	0	0	0	0
12	0	44	0	45	0	45	0	42	0	0	0	0
13	1	45	0	45	0	45	0	42	0	0	0	0
14	0	45	0	45	0	45	0	42	0	0	0	0
15	0	45	0	45	0	45	0	42	0	0	0	0
16	0	45	0	45	0	45	0	42	0	0	0	0
17	0	45	0	45	0	45	0	42	0	0	0	0
18	0	45	0	45	0	45	0	42	0	0	0	0
19	0	45	0	45	0	45	0	42	0	0	0	0
20	0	45	0	45	0	45	0	42	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

Table 7-3.72 continued

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Wheat Seeds.

Seeds	Wheat											
Dose (Mrad)	0.51		0.51									
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	12		12									
R.H.	86		97									
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0								
2	0	0	0	0								
3	0	0	0	0								
4	0	0	0	0								
5	0	0	0	0								
6	0	0	0	0								
7	0	0	0	0								
8	0	0	0	0								
9	0	0	0	0								
10	0	0	0	0								
11	0	0	0	0								
12	0	0	0	0								
13	0	0	0	0								
14	0	0	0	0								
15	0	0	0	0								
16	0	0	0	0								
17	0	0	0	0								
18	0	0	0	0								
19	0	0	0	0								
20	0	0	0	0								

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Graph 7-3.72
The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Wheat Seeds.

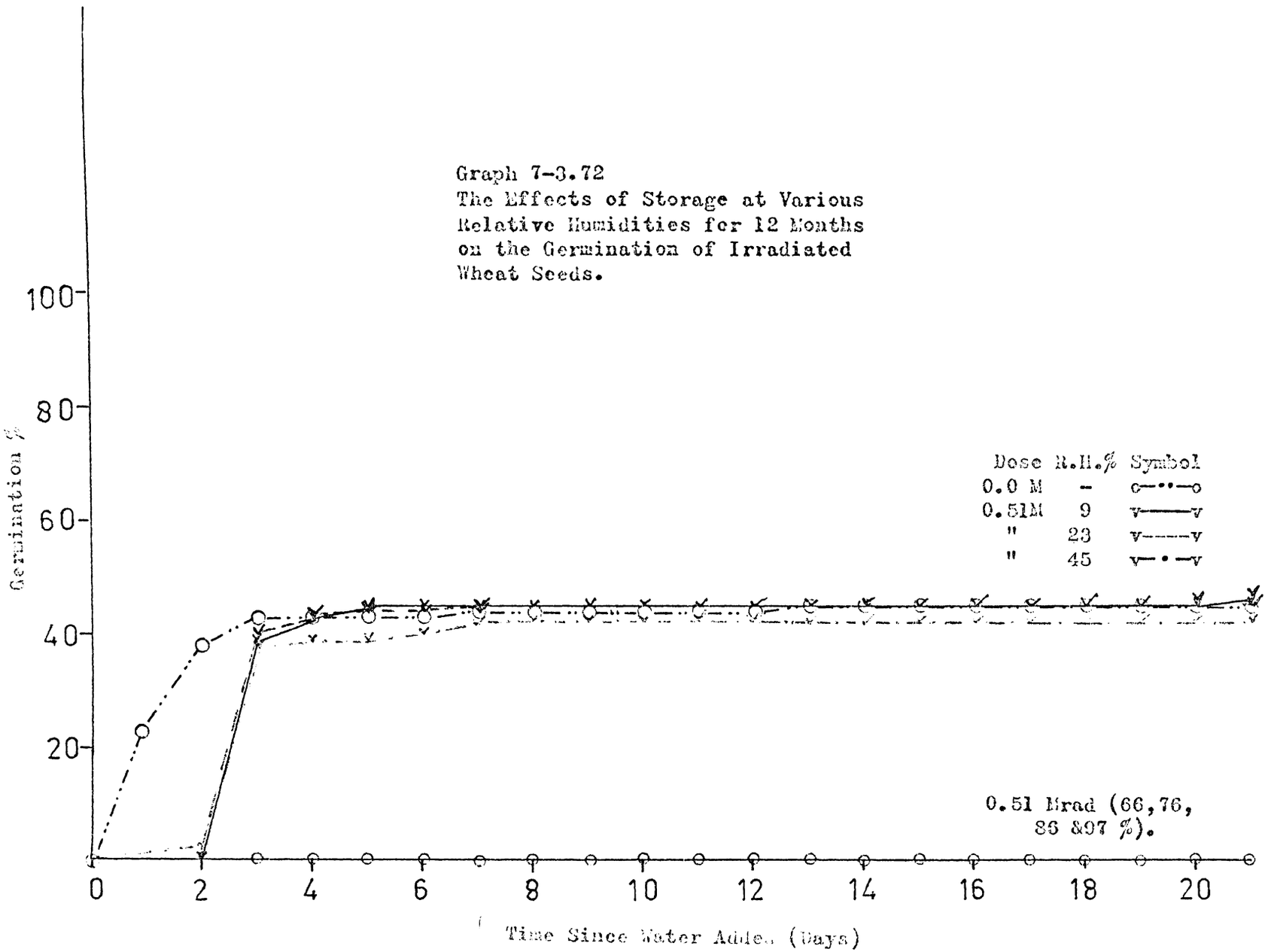


Table 7-3.73

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Wheat Seeds.

Seed	Wheat											
Dose (Mrad)	0.0		5.14		5.14		5.14		5.14		5.14	
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		12		12		12		12		12	
R.H.	-		9		23		45		66		86	
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0	0	0	0	0	0	0	0	0	0	0
2	23	23	0	0	0	0	0	0	0	0	0	0
3	15	38	0	0	0	0	0	0	0	0	0	0
4	5	43	0	0	0	0	0	0	0	0	0	0
5	0	43	0	0	0	0	0	0	0	0	0	0
6	0	43	0	0	0	0	0	0	0	0	0	0
7	1	44	0	0	0	0	0	0	0	0	0	0
8	0	44	0	0	0	0	0	0	0	0	0	0
9	0	44	0	0	0	0	0	0	0	0	0	0
10	0	44	0	0	0	0	0	0	0	0	0	0
11	0	44	0	0	0	0	0	0	0	0	0	0
12	0	44	0	0	0	0	0	0	0	0	0	0
13	1	45	0	0	0	0	0	0	0	0	0	0
14	0	45	0	0	0	0	0	0	0	0	0	0
15	0	45	0	0	0	0	0	0	0	0	0	0
16	0	45	0	0	0	0	0	0	0	0	0	0
17	0	45	0	0	0	0	0	0	0	0	0	0
18	0	45	0	0	0	0	0	0	0	0	0	0
19	0	45	0	0	0	0	0	0	0	0	0	0
20	0	45	0	0	0	0	0	0	0	0	0	0

* (a) % Germinated previous 24 hours
* (b) Total % germination.

/continued...

Table 7-3.73 continued

The Effects of Storage at Various Relative Humidities for 12 Months on the Germination of Irradiated Wheat Seeds.

Seeds	Wheat											
Dose (Mrad)	5.14											
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	12											
R.H.	97											
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	0	0										
2	0	0										
3	0	0										
4	0	0										
5	0	0										
6	0	0										
7	0	0										
8	0	0										
9	0	0										
10	0	0										
11	0	0										
12	0	0										
13	0	0										
14	0	0										
15	0	0										
16	0	0										
17	0	0										
18	0	0										
19	0	0										
20	0	0										

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.74

The Effects of Storage at Room Relative Humidities for 8 Months on the Germination of Irradiated White Clover Seeds.

Seeds	White Clover											
Dose (Mrad)	0.0		0.52		2.0		5.0		10.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		8		8		8		8			
R.H.	-		Room Humidities									
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	75	75	28	28	0	0	0	0	0	0		
2	20	95	20	48	0	0	0	0	0	0		
3	4	99	34	82	0	0	0	0	0	0		
4	1	100	9	91	3	3	0	0	0	0		
5	-	100	1	92	1	4	0	0	0	0		
6	-	100	0	92	5	9	0	0	0	0		
7	-	100	0	92	0	9	0	0	0	0		
8	-	100	0	92	1	10	0	0	0	0		
9	-	100	0	92	0	10	0	0	0	0		
10	-	100	1	93	0	10	0	0	0	0		
11	-	100	0	93	0	10	0	0	0	0		
12	-	100	0	93	0	10	0	0	0	0		
13	-	100	0	93	0	10	0	0	0	0		
14	-	100	0	93	0	10	0	0	0	0		
15	-	100	1	94	0	10	0	0	0	0		
16	-	100	0	94	0	10	0	0	0	0		
17	-	100	0	94	0	10	0	0	0	0		
18	-	100	0	94	0	10	0	0	0	0		
19	-	100	0	94	0	10	0	0	0	0		
20	-	100	0	94	0	10	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

Table 7-3.75

The Effects of Storage at Room Relative Humidities for 14 Months on the Germination of Irradiated White Clover Seeds.

Seeds	White Clover											
Dose (Mrad)	0.0		0.52		2.0		5.0		10.0			
Dose Rate Mrad/hr												
Time Exposed (hrs)												
Time Stored Mths	-		14		14		14		14			
R.H.			Room Humidities									
Conc. Soln-M												
Days After Wetting	a*	b*	a*	b*	a	b	a	b	a	b	a	b
1	83	83	18	18	0	0	0	0	0	0		
2	9	92	34	52	0	0	0	0	0	0		
3	2	94	18	70	0	0	0	0	0	0		
4	0	94	6	76	0	0	0	0	0	0		
5	1	95	4	80	0	0	0	0	0	0		
6	0	95	3	83	0	0	0	0	0	0		
7	0	95	2	85	0	0	0	0	0	0		
8	0	95	2	87	0	0	0	0	0	0		
9	0	95	2	89	0	0	0	0	0	0		
10	0	95	1	90	0	0	0	0	0	0		
11	0	95	0	90	0	0	0	0	0	0		
12	0	95	0	90	0	0	0	0	0	0		
13	0	95	0	90	0	0	0	0	0	0		
14	0	95	1	91	0	0	0	0	0	0		
15	0	95	1	92	0	0	0	0	0	0		
16	0	95	0	92	0	0	0	0	0	0		
17	0	95	0	92	0	0	0	0	0	0		
18	0	95	0	92	0	0	0	0	0	0		
19	0	95	0	92	0	0	0	0	0	0		
20	0	95	0	92	0	0	0	0	0	0		

* (a) % Germinated previous 24 hours

* (b) Total % germination.

REFERENCES

1. Bowen, H., "Introduction to Botany" Newnes (1965).
2. Percival, J. "Agricultural Botany", Duckworth (1910).
3. Hartmann, H. and D. Kester, "Plant Propagation", Prentice-Hall (1959).
4. Mayer, A. and A. Poljackoff-Mayber, "The Germination of Seeds", Pergamon (1963).
5. Hawkes, J. "The Origins of Agriculture", Econ. Bot. 24 (2) 131-133 (1970).
6. Beals, R. and H. Hoijer, "An Introduction to Anthropology", 3rd Edit. MacMillan (1965).
7. "Lawson' Textbook of Botany". Revised by E. Simon, K. Dormer and J. Hartshorne. 14th ed. University Tutorial Press (1966).
8. Toole, E., S. Hendricks, H. Borthwick and V. Toole, Ann. Rev. Plant Physiol. 7, 299 (1956).
9. Brown, R. Ann. Bot., 4, 379 (1940).
10. Abott, D. Rep. 14th Intern. Hort. Congr. 1, 746 (1956).
11. Crocker, W. and L. Barton, Contrib. Boyce Thompson Instit. 3, 385 (1931).
12. Crocker, W. Am. J. Botany, 3, 99 (1916).
13. Joseph, H., Bot. Gaz., 87, 127 (1929).
14. Weiss, F., Am. J. Botany, 13, 737 (1926).
15. Lauer, E. Flora, 40, 551 (1953).
16. Vegis, A., Ann. Rev. Plant Physiol. 15, 185 (1964).
17. Thompson, R., Nature, 33, 610 (1936).
18. Evenari, M. and G. Neumann, Bull. Res. Council Israel, 2, 75 (1952).
19. Kugler, I., Bietr. Biol. Bflanz., 28, 211 (1951), as reported in ref. 8.
20. Black, M. Sci. Prog., 58 (231), 379 (1970).
21. Kahn, A., A. Goss. and D. Smith, Science, 125, 645 (1957).
22. Rollin, P., Can. J. Bot., 42, 463 (1964).
23. MacMillan, J., J. Seaton and P. Suter, Tetrahedron, 18, 349 (1962).
24. Thompson, R., and W. Kosar, Plant Physiol. 14, 567 (1939).

25. Ogawara, K. and K. Ono, Bull. School of Education Okayama University, No.1, 97 (1955) as reported in ref. 8.

26. Roberts, E., Symp. Soc. Exptl. Biol., 23, 161 (1969).

27. Black, M. and M. Richardson, Planta, 73, 344 (1967).

28. Sondheimer, E., D. Tzou and E. Galson, Plant Physiol. 43, 1443 (1968).

29. Irving, R., Plant Physiol. Suppl., 43, 9 (1968).

30. Evenari, M., Bot. Rev., 15, 153 (1949).

31. Kaufmann, E., Planta, 33, 516 (1943).

32. Stout, M., and B. Tolmann, J. Agr. Res. 63, 687 (1941).

33. Thimann, K. V. and W. Bonner, Proc. Natl. Acad. Sci. U.S. 35, 272 (1949).

34. Morse, W., Soybeans and Soybean Products, 1, 135, Interscience (1950).

35. Radley, J., "Starch and its Derivatives ", 4th Ed., Chapman Hall (1968).

36. Gascoigne, J. and M. Gascoigne, "Biological Degradation of Cellulose", Butterworths (1960).

37. Gould, S., and D. Rees, J. Sci. Fd. Agric., 16, 702 (1965).

38. Street, H., "Plant Metabolism", Pergammon (1963).

39. Hilditch, T., and P. Williams, "The Chemical Constitution of Natural Fats", 4th ed. Chapman and Hall (1964).

40. Stumpf, P. K. in "Control of Lipid Metabolism", J. Grand (edit.) Academic Press (1963).

41. Linko, P. and M. Milner, Cereal Chem., 36, 280 (1959).

42. Steward, F., and J. Pollard in "Amino Acid Pools, Distribution, Formation and Function of Free Amino Acids", J. Holden (edit.) Elsevier (1962).

43. Bell, E. In "Comparative Biochemistry of Arginine and Derivatives" Churchill (1965).

44. Arnoff, S., In "Amino Acid Pools", J. Holden (edit.) Elsevier (1962).

45. Noe, F. and L. Fowden, Biochem. J., 77, 543 (1960).

46. Steward, F., R. Zacharius, and J. Pollard, Ann. Acad. Sci. Fennicae, 60, 321 (1955).

47. Davies, D., J. Biovanelli, T. Ap Rees, "Plant Biochemistry", Blackwell (1964).

48. Fowden, L. and D. Gray, In "Amino Acid Pools", J. Holden (edit.) Elsevier (1962).

49. White, A., P. Handler and E. Smith, "Principles of Biochemistry", 3rd ed. McGraw Hill (1964).

50. Thompson, J., C. Morris, W. Arnold, and D. Turner, In "Amino Acid Pools", J. Holden (edit.) Elsevier (1962).

51. Yemm, E., New Phytol., 48, 315 (1949).

52. Linko, P. and M. Milner, Plant Physiol. 34, 392 (1959).

53. Firenzuoli, A., P. Vanni, G. Ramponi and V. Baccari, Plant Physiol. 43, 260 (1968).

54. Vaughan, J. and A. Waite, J. Exp. Bot., 18, 100 (1967).

55. Dure, L., Plant Physiol. 35, 925 (1960).

56. Bianchetti, R., and M. Sartivana, Biochim. Biophys. Acta, 145, 485 (1967).

57. Sartivana, M. and R. Bianchetti, Physiol. Plantar., 20, 1066 (1967).

58. Sturani, E., Life Sci. 7 (II), 527 (1963).

59. Allende, J., and M. Bravo, J. Biol. Chem. 241, 5813 (1966).

60. Ozaki, K. and L. R. Wetter, Can. J. Biochem. and Physiol. 39, 843 (1961).

61. Ozaki, K. and L. Wetter, Can. J. Biochem. and Physiol., 38, 929 (1960).

62. Nezgovorava, L., and N. Borisova, Fiziol. Rast., 14 (1) 75 (1967) from Biol. Abstr. 49 (6), 31105 (1968).

63. Schramm, R., Acta Soc. Bot. Polon, 36, 39 (1967).

64. Gobis, L., Ann. Sperim. Agric., 5, 571 (1951).

65. Schramm, R., T. Mossor and C. Gierczak, Acta Biochim. Polon, 14 (3) 303 (1967).

66. Griffiths, L. A., Biochem. J., 70, 120 (1958).

67. Steward, F. and J. Thompson, Ann. Rev. Plant Physiol., 1, 233 (1950).

68. Sturani, E., S. Cocucci and E. Marre, Plant and Cell Physiol., 9, 783 (1968).

69. Sturani, E. and S. Cocucci, Life Sci., 4, 1937 (1965).

70. Marre, E. in "Current Topics in Developmental Biology", Vol. 2, 0.75, Acad. Press (1967).

71. Brown, E., Biochem. J. 88, 498 (1963).

72. Brown, E., Biochem. J., 85, 633 (1962).

73. Valikhanov, M., Nauch Tr. Tashkent, Gos. Univ. 334, 15 (1968) from CA 74 (11), 50609D (1971).

74. Robinson, M. E., in Chemical Embryology, p.1711. J. Needham (edit.) Hafner (1963).

75. Jones, D., Biochem. J., 101, 153 (1966).

76. Dey, B., and S. Sircar, Ind. J. Agr. Sci., 38 (3), 477 (1968).

77. Winter, A., Arch. Biochem. Biophys., 106, 131 (1964).

78. Addicott, F., and J. Lyon, Ann. Rev. Plant Physiol., 20, 139 (1969).

79. Reinhard, E., and W. Konopka, Planta, 77 (1) 58 (1967).

80. Pryce, R. and J. MacMillan, Tetrahed. Lett., 42, 4173 (1967).

81. Takahash, N., N. Morofushi and T. Yokota, Tetrahed. Lett., 48, 4861 (1967).

82. Handbook of Biochemistry and Biophysics,World Publ. Co. (1966).

83. Marcus, A., and J. Feeley, Proc. Natl. Acad. Sci., 51, 1075 (1964).

84. Malysheva, A. Reported in Biochem. Sections, 63 (2), 2133c (1966).

85. Spragg and Yemen, J. Exptl. Bot., 10, 409 (1959).

86. Mann, L. R., M.Sc. Thesis, V.U.W. (1964).

87. Mayer, A., Sci. News, 29, 84 (1953).

88. Toole, E., Am. J. Bot., 11, 325 (1924).

89. Middendorf, F., Bot. Baz., 100, 485 (1939).

90. Berjak, P. and T. Villiers, News Phytol., 69 (4), 929 (1970).

91. Treffy, T., S. Klein and M. Abrahamsen, Aust. J. Biol. Sci., 20, 859 (1967).

92. Haber, A., and N. Brassington, Nature, 183, 619 (1959).

93. Chen, Y., P. Linko and M. Milner, Plant Physiol., 35, 68 (1960).

94. Dechary, J. M., Econ. Bot., 24, (2), 113 (1970).

95. Pyko, V. and A. Mironenko, Vest. Akad. Navuk. Belarus SSR Ser. Biyal Navuk, 5, 43 (1970) from CA 74 (9) 39268W (1971).

96. Marcus, A. Symp. Soc. Exp. Biol. 23, 143 (1969).

97. Payne, P., and D. Boulter, Planta, 87, 63 (1969).

98. Black, M. and M. Richardson, Bull. Soc. Fr. Physiol. Veg., 14 (1) 73 (1968).

99. Lado, P., Girn. Bot. Ital., 101, 303 (1967).

100. Grellet, F., R. Julien and H. Milhomme, Physiol. Veg. 6 (1) 11 (1968).

101. Wanka, F., and F. Bauer, Z. Pflanzen Physiol. 58 (2), 175 (1967).

102. Lott, J., Can. J. Bot., 48 (12) 2227 (1970).

103. Nezgovorova, L. and N. Borisova, Fiziol. Rast. 14 (1) 75 (1967). from Biol. Abstr. 49 (6), 31105 (1968).

104. Mukherji, S., B. Dey and S. Sircar, Physiol. Plant, 21 (2) 360 (1968).

105. Barker, G., and J. Hollinshead, Biochem. J., 103, 230 (1967).

106. Brown, E., Biochem. J., 95, 509 (1965).

107. Pradet, A., A. Narayanan and J. Vermeersch, Bull. Soc. Fr. Physiol. Veg. 14 (1) 107 (1968).

108. Fowden, L. Ann. Rev. Biochem., 33, 173 (1964).

109. Hopkins, F. and E. Morgan, Nature, 152, 288 (1943).

110. Oaks, A., D. Mitchell, R. Barnard and F. Johnston, Can. J. Bot., 48 (12), 2249 (1970).

111. Webster, G. C., and J. E. Varner, J. Biol. Chem., 215, 91 (1955).

112. Fowden, L. Endeavour, 21, 35 (1962).

113. Whittingham, C. P. "The Chemistry of Plant Processes", Methuen (1964).

114. Johnston, J., Reported in Discussion p.39 in "Comparative Biochemistry of Arginine and Derivatives", Churchill (1965).

115. Walker, J. Reported in Discussion p.40 in "Comparative Biochemistry of Arginine and Derivatives", Churchill (1965).

116. Jones, V.and D. Boulter, New Phytol., 67, 925 (1968).

117. Latzko, E, and J. Kotze, Naturwissen Schaften 52 (5), 111 (1965) from Biochem. Sect., 62 (1), 13524H (1966).

118. Smith, B., and H. Williams, Arch. Biochem. Biophys., 31, 366 (1951).

119. Ohno, M. and K. Okunuki, J. Biochem., 51, 313 (1962).

120. Sivaramakrichan, V, and P. Sarma, Biochem. J., 62, 132 (1956).

121. McKee, H., "Nitrogen Metabolism in Plants", Clarendon Press (1962).

122. Dixon, R, and L. Fowden, Ann. Bot., 25, 513 (1961).

123. Steward, F., R. Bidwell and E. Yemm. Nature, 178, 789 (1956).

124. Stewart, C, and H. Beevers, Plant Physiol. 42, 1587 (1967).

125. Fogg, G. E. "The Growth of Plants", Penguin (1963).

126. Briggs, D. Phytochem., 7 (4), 513 (1968).

127. Duffus, J. Biochem. J., 103, 215 (1967).

128. Hanes, C. S., Proc. Roy. Soc., 128B, 421 (1940).

129. Marve, E., M. Cornaggia, F. Alberghina and R. Bianchetti, Biochem. J. 97 (2) 20P (1965).

130. Cornaggia, M., Giorn. Bot. Ital., 71, 503 (1964).

131. Hanford, J. and D. Davies, Nature, 182, 532 (1958).

132. Chakravorty, M. and D. Burma, Biochem. J., 73, 48 (1959).

133. Gould, S. and D. Rees, J. Sci. Fd Agric., 16, 702 (1965).

134. Veiga, L., Plant Cell Physiol., 9, 1 (1968).

135. Weimberg, R., Plant Physiol., 43, 622 (1968).

136. Alberghina, R., P. Lado, G. Marziani and E. Marre, Giorn. Bot. Ital., 71, 469 (1964).

137. Waygood, E., Can. J. Res., 28, 7 (1950).

138. Lott, J., and P. Castelfranco, Can. J. Bot., 48 (12) 2233 (1970).

139. Yamamoto, Y., and H. Beevers, Plant Physiol., 35, 102 (1960).

140. Longo, C. Plant Physiol., 43 (4), 660 (1968).

141. Lado, P., M. Schwendimann and E. Marre, Biochim. Biophys. Acta 157 (1), 140 (1968).

142. Lado, P., M. Schwendimann and E. Marre, Giorn. Bot. Ital., 73, 313 (1966).

143. Breidenbach, R., and H. Beevers, Biochem. Biophys. Res. Commun., 27, 462 (1967).

144. Breidenbach, R., A. Kohn and H. Beevers, Plant Physiol., 43, 705 (1968).

145. Srere, P., Biochem. Soc. Symp., 27, 11 (1968).

146. Schramm, R., Acta Soc. Botan. Polon, 36 (1), 39 (1967).

147. Evenari, M., Proc. Int. Seed Test Ass., 30, 49 (1965).

148. Neal, G. and H. Beevers, Biochem. J., 74, 409 (1960).

149. Colmann, B. and H. Wager, J. Expt. Bot., 18 (57), 692 (1967).

150. Cossins, E. and S. Sinha, J. Expt. Bot., 18 (55), 215 (1967).

151. Canvin, D., and H. Beevers, J. Biol. Chem., 236 (4), 988 (1961).

152. Singer, T. and B. Hofstee, Arch. Biochem., 18, 229 (1948).

153. Lanzani, F., A. Marchesini, E. Galante, L., Manzocchi and P. Sequi, Enzymol. Acta Biocatal., 33 (6), 361 (1967).

154. Ory, R. and A. St Angelo, Lipids, 6 (1), 54 (1971).

155. Stumpf, P., and C. Bradbeer, Ann. Rev. Plant Physiol., 10, 197 (1959).

156. Stumpf, P., Plant Physiol., 30, 55 (1955).

157. Stumpf, P. and G. Barber, J. Biol. Chem., 227, 407 (1957).

158. Ching, T., Lipids, 3 (6), 482 (1968).

159. Haber, A., and N. Tolbert, Plant Physiol., 34, 376 (1959).

160. Cartwright, P., J. Sykes, and R. Wain. In "The Chemistry and Mode of Action of Plant Growth Substances", R. Wain and F. Wightman (edit.) Butterworths (1956).

161. Skrabka, H., Acta Soc. Botan. Polon., 33 (4) 689 (1964) from Biochem. Sect., 62 (10) 12152D (1966).

* 162. Kende, H., Plant Physiol., 42, 1612-1618 (1967).

*Due to revision of material 10 numbers were left unallocated.

172. Darbre, A. and F. Norris, Biochem. J. 64, 441-446 (1956).

173. Spedding, D. and A. Wilson, Phytochem. 7, 897 (1968).

174. Mann, W. and S. Garfinkel, "Radioactivity and its Measurement", Van Nostrand (1966).

175. Ackerman, E. "Biophysical Science", Prentice-Hall (1962).

176. Putman, J. L. "Isotopes", Penguin (1960).

177. Jefferson, S. "Massive Radiation Techniques", George Newnes (1964).

178. Dale, W. M. and J. V. Davies, Biochem. J., 48, 129-32 (1951).

179. Lea, D., Parasitology, 36, 110 (1944).

180. Guild, W., Phys. Rev., 83, 230 (1951).

181. Ciment, D., and W. Overend, Arch. Biochem. Biophys. 122, 563 (1967).

182. Contreras, G., R. E. Spejo, E. Mery, A. Ohlbaum and J. Toha, Biochim. Biophys. Acta, 61, 718 (1962).

183. Roberts, E. Plant Physiol., 31, xi (1956).

184. Salunkho, D., Nature, 179, 585 (1957).

185. Manant, J. and J. Schaeverbeke-Sacre, Radiat. Bot. 10, 175 (1970).

186. Kavalam, J.and W. Nawar, J. Am. Oil Chem. Soc., 46 (8), 387 (1969).

187. Piraux, M., "Radioisotopes and their Industrial Applications", Phillips (1964).

188. Crippa, P. and A. Vecli, Biophys. J., 10 (3), 269 (1970).

189. S. Chen and J. Varner, Plant Physiol., 44, 770 (1969).

190. Poljakoff-Mayber, A. and M. Evenari, Physiol. Plant, 11, 84 (1958).

191. Bacq, Z. M., and P. Alexander, "Fundamentals of Radiobiology", Pergamon (1961).

192. Luse, L. R., Radiat. Res. Suppl. 4, 192 (1964).

193. Hevesy, H. and L. Forsberg, Proc. 3rd Intern. Congr. Biochem. p.479 (1955).

194. Lett, J. and C. Sun, Radiat. Res., 44 (3), 771-87 (1970).

195. Sutton, H., Personal communication.

196. Romani, R., Radiat. Bot., 6, 87 (1966).

197. Dugsley, A. T., T. H. Oddie and C. Eddy, Proc. Roy. Soc. B118, 276 (1933).

198. Slaughter, J. C. and G. Failla, Radiology, 39, 663 (1942).

199. Edelson, E., New Scientist, 38 (599), 476 (1968).

200. Brinkman, R, and H. Lamberts, Nature, 181, 774 (1958).

201. Brinkman, R., and H. Lamberts, Int. J. Radiobiol. Supple. 1, 167 (1960).

202. Tanada, T., Radiat. Res., 9, 552 (1958).

203. Ahnstrom, G. and T. Sanner, Radiat. Bot., 11, 27 (1971).

204. Mikaelson, K. and H. Halvarsen, Physiol. Plant. 6, 873 (1953).

205. Woodstock, L. and O. Justice, Radiat. Bot., 7, 129 (1967).

206. Romani, R. and L. Miller, Radiat. Res., 25, 234 (1965).

207. Grosch, D. "Biological Effects of Radiation", Blaisdell (1965).

208. Ord, M. and Stocken, L. in "Mechanisms in Radiobiology", M. Erra and A. Fossberg (editors) Vol. I Acad. Press (1961).

209. Farrar, J., M. Small, D. Bullard and F. Ingelfinger, Am. J. Physiol., 186, 549 (1956).

210. Levy, B. and R. Rugh, Proc. Soc. Exptl. Biol. Med., 82, 223 (1953).

211. Maas, H., and G. Schubert, "Proc. 2nd Intern. Conf. on Peaceful Uses Atomic Energy, 1958, p.449, U.N. (1959).

212. Dubouloz, P., J. Dumas and J. Vigne, J. Can. Roy. Soc. Biol. 144, 1080 (1950).

213. Bacq, Z., C. Burg, A. Chevallier and C. Heusghern, J. Physiol. 43, 640 (1951).

214. Hogan, V., and J. Philpot, Brit. J. Radiol. 27, 63 (1954).

215. Dubouloz, P. and J. Fondarai, Bull. Soc. Chim. Biol., 35, 819 (1953).

216. Joshi, V. and B. Gaur, Intern. J. Rad. Biol., 18, 173 (1970).

217. Haber, A., R. Carrier and L. Foard, Am. J. Bot., 48, 431 (1961).

218. Lee, T., W. McGlasson and R. Edwards, Radiat. Bot., 11 (3), 137 (1971).

219. Govindjee, G., Naturwiss., 44, 183 (1957).

220. Sytinskii, I., G. Sazonets and K. Shang, Tr. Vses. Konf. Biokhim. Nerv. Sist. 4th 1966 (Publ. 1969) p.581 reported in Chem. Abstr. 74 (1) 38932w (1971).

221. Barron, E. and V. Flood, J. Gen. Physiol., 33, 229 (1950).

222. Barron, E, S. Dickman, J. Muntz and T. Singer, J. Gen. Physiol., 32, 537 (1949).

223. Haber, A. and H. Luippold, Plant Physiol., 35, 168 (1960).

224. Gordon, S. Quart. Rev. Biol., 32, 3 (1957).

225. Brucs, A. and H. Patt, Physiol. Rev., 33, 85 (1953).

226. Virovets, O. and A. Pasynskii, Biokhimiia, 24 848 (1959) from Biol. Abstr. 35, 49970.

227. Riov, J., S. Monselise and R. Kahan, Radiat. Bot., 8, 463 (1968).

228. Pollard, P. and H. Powell, Radiat. Res., 2, 109 (1955).

229. Ananthaswamy, H., U. Vakil, and A. Sreenivasan, Radiat. Bot., 11, 1 (1971).

230. O'Hara, C. Radiat. Bot., 9, 33 (1969).

231. Aronson, D., M. Fraser and C. Smith, Radiat. Res., 5, 225 (1956).

232. Sydorenko, I. Ukr. Botankhnvi. Zh. 19, 3 (1962).

233. Gunckel, J., Quart. Rev. Biol., 32, 46 (1957).

234. Forssberg, A., "Advances in Radiation Biology", Vol. I, p.117 Acad. Press (1964).

235. Howard, A., and S. Pelc, Heredity Supple. 6, 261 (1953).

236. Evans, H., Radiat. Biol., 5, 171 (1965).

237. Loug, T. and A. Haber, Radiat. Bot., 5, 223 (1965).

238. Roth, J. and H. Eickel, Radiat. Res., 11, 572 (1959).

239. Ledoux, L. p.175 "Biological Effects of Ionizing Radiation at the Molecular Level" IAEA (1962).

240. Ledoux, L., P. Garland and R. Huart, Radiat. Bot., 2, 119 (1962).

241. Van Huystee and J. Cherry, Radiat. Bot., 7, 217 (1967).

242. Sawant, P., T. Ramakrishnan and U. Kumta, Radiat. Bot., 10, 169 (1970).

243. Gailey, F. and N. Tolbert, Arch. Biochem. and Biophys., 76 188 (1958).

244. Seufert, R., Radiat. Bot. 5, 153 (1965).

245. Clark, G., W. Sweaney, W. Bunting and D. Baker, Radiat. Bot., 8 59 (1968).

246. Sax, K., Radiat. Bot., 3, 179 (1963).

247. Guseva, V., Preoposevn. Obluchenie Semyan Sel'skokhoz Kul'tur p.72 (1963) from Chem. Abstr., 61, 8609d (1964).

248. Singleton, W., "Nuclear Radiation in Food and Agriculture", Van Nostrand (1958).

249. Stein, O. and A. Sparrow, Radiat. Bot., 3, 207 (1963).

250. Long, T. and M. Kersten, Plant Physiol., 11, 615 (1936).

251. Bilquez, A., J. Agric. Trop. Bot. Appl. 4-5, 365 (1958).

252. Skok, J., W. Chorney and E. Rakosnik, Radiat. Bot., 5, 281 (1965).

253. Jolles, B., and S. Greening, Nature, 179, 1082 (1957).

254. Wyckoff, R., J. Exptl. Med., 52, 435, 769 (1930).

255. Sawyer, R. andS. Dallyn, Am. Pot. J., 32, 141 (1955).

256. Lawrence, W. "Plant Breeding" Edward Arnold Publ. (1968).

257. Dallyn, S., R. Sawyer and A. Sparrow, Nucleonics 13 (4), 48 (1955).

258. Madsen, K., and D. Salunkho, Radiat. Res., 10, 48 (1959).

259. Cornford, C., and D. Powell, Nature, 180, 966 (1957).

260. Conger, A. and H. Stevenson, Radiat. Bot., 9, 1 (1969).

261. Ali, A. and A. Rafiq, Biologia (Lahore), 12 (2), 55 (1966). from Biol. Abstr., 49 (5), 25917 (1968).

262. Notani, N. and B. Gaur, p.443 "Biological Effects of Ionizing Radiation at the Molecular Level, IAEA (1962).

263. Bowen, H. and S. Smith, Nature, 183, 907 (1959).

264. Sax, K. Nucleonics, 8 (4), 28 (1952).

265. Bajaj, Y., A. Saettler, M. Adams, Radiat. Bot., 10, 119 (1970).

266. Sparrow, A., S. Schwemmer and P. Bottino, Radiat. Bot., 11, 85 (1971).

267. Haber, A. and H. Luippold, Intern. J. Radiat. Biol., 1, 317 (1959).

268. Saric, S. Proc. 2nd U.N. Conf. on the Peaceful Uses of Atomic Energy, 27, 233 (1958).

269. El-Lakeny and O. Sziklai, Radiat. Bot., 10, 411 (1970).

270. Mergen, F., and J. Johansen, Radiat. Bot., 4, 417 (1964).

271. Heaslip, M. Ecology, 40 (3), 383 (1959).

272. Consumer Publication, "Radiation, Part of Life" (1965).

273. Johnson, L. and T. Osborne, Can. J. Bot., 42, 195 (1964).

274. Bacq, Z. Proc. 3rd Intern. Congr. Biochem. p.494 (1955).

275. Pepper, R., N. Buffa and V. Chandler, Appl. Microbiol., 4, 149 (1956).

276. Brewbaker, J. and G.Emergy, Radiat. Bot., 1, 101 (1962).

277. Sparrow, A. and B. Rubin, Survey of Biological Progress, Vol. II G.Avery (edit.) Acad. Press (1952).

278. Hayden, B. and L. Smith, Genetics, 34, 26 (1949).

279. Adams, J. and R. Nilan, Radiat. Res., 8, 111 (1958).

280. Osborne, T. and F. Elliot, Am. J. Bot., 42, 646 (1955).

281. Sire, M. and R. Nilan, Genetics, 44, 124 (1959).

282. Jackson, W. Radiat. Biol. 5, 109 (1958).

283. Yagyu, P. and R. Morris, Genetics, 42, 222 (1957).

284. Neary, G. Nature, 180, 248 (1957).

285. Powers, E. and B. Kaleta, Science, 132, 959 (1960).

286. Wolf, S.and A. Sicard "The Effects of Ionizing Radiations on Seeds", p.172, IAEA (1961).

287. Klingmüller, W. "The Effects of Ionizing Radiations on Seeds", p.68, IAEA (1961).

288. Conger, A. and M. Randolph, Radiat. Res., 11, 54 (1959).

289. Osborne, T. Reported in Discussion p.179 "The Effects of Ionizing Radiations on Seeds", IAEA (1961).

290. Mergen, F. and J. Cummings, Radiat. Bot., 5, 39 (1965).

291. Sparrow, A., R. Cuany, J. Miksche and L. Schairer, Radiat. Bot., 1, 10 (1961).

292. Lunden, A. Radiat. Bot., 4, 429 (1964).

293. Joshi, R., B. Gaur and N. Notani, Radiat. Bot., 9, 141 (1969).

294. Chlichlias, M. Reported in Discussion, p.178 "The Effects of Ionizing Radiations on Seeds", IAEA (1961).

295. Sparrow, A., S. Schwemmer, E. Klug and L. Puglielli, Science, 169 (3950), 1082 (1970).

296. Gaur, B. and N. Notani, Intern. J. Radiat. Biol. 2, 257 (1960).

297. Holmson, T., H. Teas and A. Koch, Radiat. Bot., 4, 413 (1960).

298. Mathur, P., Nature, 190, 547 (1961).

299. Ikenaga, M. and T. Mabuchi, Radiat. Bot., 6, 165 (1966).

300. Zill, L. and N. Tolbert, Arch. Biochem. Biophys., 76, 196 (1958).

301. Hall, E. and L. Lajtha, Radiat. Res., 20, 187 (1963).

302. Hall, E. and J. Bedford, Intern. J. Radiat. Biol., 8, 467 (1964).

303. Pratt, C. Radiat. Bot., 3, 193 (1963).

304. Setlow, R. Prog. Nucl. Acid Res. and Molec. Biol. 8, 257 (1968).

305. Holland, I. Sci. Prog. (Oxford), 58, 71 (1970).

306. Town, C., K. Smith and H. Kaplan, J. Bacteriol. 105 (1) 127 (1971).

307. Elkind, M. and C. Kamper, Biophys. J. 10 (3), 237 (1970).

308. Alexander, P. "Radiation Damage and Sulphydryl Compounds", p.63 IAEA (1969).

309. Hollaender, A., and J. Curtis, Proc. Soc. Exp. Biol., 33, 61 (1935).

310. Hollaender, A. and D. Doherty, "Radiation Damage and Sulphydryl Compounds", p.1 IAEA (1969).

311. Duncan, J. and G. Cook "Isotopes in Chemistry", Clarendon Press (1968).

312. Lewis, G. Science News Lett. 24, 116 (1933).

313. Brun, J., L. Tronstad and K. Norske, Vidensk Selsk Forhandl (Trondheim) 7, 1171 (1935) In Biol. Abstr., 11, 18207 (1937).

314. Melot, G. Proc. Soc. Exp. Biol. Med., 32,79 (1934).

315. Spedding, D. J., M.Sc. Thesis, V.U.W. (1962).

316. Wagner, C. and V. Guinn, J. Am. Chem. Soc., 75, 4861 (1953).

317. Tolbert, G. Nucleonics, 18 (8) 74 (1960).

318. Miller, K. Personal communication.

319. Raaen, V., G. Ropp and H. Raaen, "Carbon-14" McGraw-Hill (1968).

320. Baillie, W.J.H., M.Sc. Thesis, V.U.W. (1966).

321. Wilson, A. T. and M. J. Calvin, J. Amer. Chem. Soc., 77, 5950 (1955).

322. Schmidt, G. C., C. Fisher and J. McOwen, J. Pharm. Sci., 52, 468 (1963).

323. Dawson, R., D. Elliott, W. Elliot and K. Jones (editors) "Data for Biochemical Research", Oxford University Press (1959).

324. Smith, I. "Chromatographic Techniques", Heinemann (1958).

325. Meyer, B. S. and D. B. Anderson, "Laboratory Plant Physiology", 2nd ed. Van Nostrand (1941)

326. Osborne, T. "Mutations in Plant Breeding", p.187, IAEA (1966).

327. Barton, L., Contrib. Boyce Thompson Inst. 12, 85 (1941).

328. Lindley, D. and J. C. Miller "Cambridge Elementary Statistical Tables", Cambridge University Press (1963).

329. Missen, A. M.Sc. Thesis, V.U.W. (1968).

330. Bergmeyer "Methods of Enzymatic Analysis", Academic Press (1963).

331. Smith, I. "Chromatographic Techniques"

332. Dixon, M. and E. Webb "Enzymes", Longmans (1964).

333. Wilson, A. and M. Calvin, J. Amer. Chem. Soc., 77, 5950 (1955).

334. Vickers, M. Personal communication.

335. Bassham, J. and M. Calvin, "The Path of Carbon in Photosynthesis", Prentice Hill (1957).

336. McGeer, E. M. Robertson and P. McGeer, Can. J. Biochem. Physiol., 39, 605 (1961).

337. Meister, A. "Biochemistry of the Amino Acids", Vol. 1, Academic Press (1965).

338. Irwin, G.P. M.Sc. Thesis, V.U.W. 1968

339. Wilson, A.T. Personal communication.

```
      DOC PETE-DATA
            INTEGER  CARD,DAY,DOSE,POT,PRINT
            DIMENSION DRYWT(44,3,4),TTLLG(4),TLDAY(3),TTLLD(4,3),TTLTR(4)
            DIMENSION TTLTL(4,4),TTLTD(4,3),TLTLD(4,4,3),TLPOT(11,4,3)
            DIMENSION TEMP(12),TEMP1(16),TEMP2(12),TEMP3(132),TEMP4(48)
            DIMENSION TEMP5(528)
            EQUIVALENCE (TEMP(1),TTLLD(1)),(TEMP1(1),TTLTL(1))
            EQUIVALENCE (TEMP2(1),TTLTD(1)),(TEMP3(1),TLPOT(1))
            EQUIVALENCE (TEMP4(1),TLTLD(1)),(TEMP5(1),DRYWT(1))
            DATA CARD,PRINT/2,3/
            WRITE(PRINT,20)

      C     READ AND LIST ALL DATA
            DO 1 DOSE=1,4
            DO 1 I=1,44
            READ(CARD,21) (DRYWT(I,DAY,DOSE),DAY=1,3)
          1 WRITE(PRINT,21) (DRYWT(I,DAY,DOSE),DAY=1,3)
      C     CALCULATE TLTLD=TOTAL TREATMENT,LIGHT,DAY
      C     TTLTD=TOTAL TREATMENT DAY,TLDAY=TOTAL DAY,GRTTL=GRAND TOTAL
            GRTTL=0
            DO 5 DAY=1,3
            TLDAY(DAY)=0
            DO 4 DOSE=1,4
            TTLTD(DOSE,DAY)=0
            DO 3 LIGHT=1,4
            TLTLD(DOSE,LIGHT,DAY)=0
            M=11*LIGHT
            N=M-10
            DO 2 I=N,M
          2 TLTLD(DOSE,LIGHT,DAY)=TLTLD(DOSE,LIGHT,DAY)+DRYWT(I,DAY,DOSE)
          3 TTLTD(DOSE,DAY)=TTLTD(DOSE,DAY)+TLTLD(DOSE,LIGHT,DAY)
          4 TLDAY(DAY)=TLDAY(DAY)+TTLTD(DOSE,DAY)
          5 GRTTL=GRTTL+TLDAY(DAY)
      C     CALCULATE TTLLD=TOTAL LIGHT DAY,TTLLG=TOTAL LIGHT
            DO 7 LIGHT=1,4
            TTLLG(LIGHT)=0
            DO 7 DAY=1,3
            TTLLD(LIGHT,DAY)=0
            DO 6 DOSE=1,4
          6 TTLLD(LIGHT,DAY)=TTLLD(LIGHT,DAY)+TLTLD(DOSE,LIGHT,DAY)
          7 TTLLG(LIGHT)=TTLLG(LIGHT)+TTLLD(LIGHT,DAY)
      C     CALCULATE TTLTR=TOTAL TREATMENT,TTLTL=TOTAL TREATMENT LIGHT
            DO 9 DOSE=1,4
            TTLTR(DOSE)=0
            DO 9 LIGHT=1,4
            TTLTL(DOSE,LIGHT)=0
            DO 8 DAY=1,3

          8 TTLTL(DOSE,LIGHT)=TTLTL(DOSE,LIGHT)+       TLTLD(DOSE,LIGHT,DAY)
```

```
      8 TTLTL(DOSE,LIGHT)=TTLTL(DOSE,LIGHT)+      TLTLD(DOSE,LIGHT,DAY)
      9 TTLTR(DOSE)=TTLTR(DOSE)+TTLTL(DOSE,LIGHT)
C       CALCULATE TLPOT=TOTAL POT LIGHT,DAY
        DO 10 LIGHT=1,4
        DO 10 POT=1,11
        DO 10 DAY=1,3
        TLPOT(POT,LIGHT,DAY)=0
        I=11*LIGHT-11
        DO 10 DOSE=1,4
        J=I+POT
     10 TLPOT(POT,LIGHT,DAY)=TLPOT(POT,LIGHT,DAY)+DRYWT(J,DAY,DOSE)
C       CALCULATE SUMLT=SUMSQLIGHT,SUMDY=SUMSQDAY,SUMTR=SUMSQTREAT )TS
        SUMLT=SUMSQ(TTLLG,4)
        SUMDY=SUMSQ(TLDAY,3)
        SUMLD=SUMSQ(TEMP,12)
        SUMTR=SUMSQ(TTLTR,4)
        SUMTL=SUMSQ(TEMP1,16)
        SUMTD=SUMSQ(TEMP2,12)
        SUMPT=SUMSQ(TEMP3,132)
        GTLSQ=GRTTL*GRTTL/528
        SMTLD=SUMSQ(TEMP4,48)
        TOTAL=SUMSQ(TEMP5,528)-GTLSQ
C       DEFINE CONSTANTS FOR SS CALCULATION.
        C132=1/132.0
        C176=1/176.0
        C44=1/44.0
        C33=1/33.0
        C11=1/11.0
C       CALCULATE SSL,SSD,ETC
        SSL=C132*SUMLT-GTLSQ
        SSD=C176*SUMDY-GTLSQ
        SSLD=C44*SUMLD-GTLSQ-SSL-SSD
        SSED=0.25*SUMPT-C44*SUMLD
        SST=C132*SUMTR-GTLSQ
        SSTL=C33*SUMTL-GTLSQ-SST-SSL

        SSTD=C44*SUMTD-GTLSQ-SST-SSD
        SSTLD=C11*SMTLD-GTLSQ-SSTL-SSTD-SSLD-SST-SSL-SSD
        SSET=TOTAL-SSL-SSD-SSLD-SSED-SST-SSTL-SSTD-SSTLD
C       PRINT RESULTS.
        WRITE(PRINT,22) GRTTL
        WRITE (PRINT,23) TTLLG
        WRITE (PRINT,24) TLDAY
        WRITE(PRINT,25)
        DO 11 DAY=1,3
     11 WRITE (PRINT,26) (TTLLD(LIGHT,DAY),LIGHT=1,4)
        WRITE (PRINT,27)
        DO 12 LIGHT=1,4
        WRITE(PRINT,28)
        DO 12 DAY=1,3
```

```
      SSET=TOTAL-SSL-SSD-SSLD-SSED-SST-SSTL-SSTD-SSTLD
C     PRINT RESULTS.
      WRITE(PRINT,22) GRTTL
      WRITE (PRINT,23) TTLLG
      WRITE (PRINT,24) TLDAY
      WRITE(PRINT,25)
      DO 11 DAY=1,3
   11 WRITE (PRINT,26) (TTLLD(LIGHT,DAY),LIGHT=1,4)
      WRITE (PRINT,27)
      DO 12 LIGHT=1,4
      WRITE(PRINT,28)
      DO 12 DAY=1,3
   12 WRITE(PRINT,29) (TLPOT(POT,LIGHT,DAY),POT=1,11)
      WRITE(PRINT,30) TTLTR
      WRITE(PRINT,31)
      DO 13 LIGHT=1,4
   13 WRITE(PRINT,26) (TTLTL (DOSE,LIGHT),DOSE=1,4)
      WRITE(PRINT,33)
      DO 14 DAY=1,3
   14 WRITE(PRINT,26) (TTLTD(DOSE,DAY),DOSE=1,4)
      WRITE(PRINT,35)
      DO 15 DAY=1,3
      WRITE(PRINT,36)
      DO 15 DOSE=1,4
   15 WRITE(PRINT,26) (TLTLD(DOSE,LIGHT,DAY),LIGHT=1,4)
      WRITE(PRINT,38) SSL,SSD,SSLD,SSED,SST,SSTL,SSTD,SSTLD,SSET
      CALL EXIT
C     ALL FORMAT STATEMENTS FOLLOW
   20 FORMAT(//' STATISTICAL ANALYSIS OF EXPERIMENT'///)
   21 FORMAT (19X,3F10.5)
   22 FORMAT (/////' GRAND TOTAL',F10.5)
   23 FORMAT (//' TOTALS FOR LIGHT GROUPS A,B,C,D',4F10.5)
   24 FORMAT (//' TOTALS FOR DAYS 1,2,3',3F10.5)
   25 FORMAT (//' TOTALS LIGHT,DAY.ROWS REPRESENT DAYS'/)
   26 FORMAT (10X,4F10.5)
   27 FORMAT(//' TOTALS POT,LIGHT,DAY.ROWS REPRESENT DAYS'/)
   28 FORMAT(/' LIGHT GROUP')
   29 FORMAT(11F10.5)
   30 FORMAT (//' TOTAL IN TREATMENT',4F10.5)
   31 FORMAT (//' TOTAL DOSE,LIGHT.ROWS REPRESENT LIGHTS'/)
   33 FORMAT (//' TOTAL TREATMENT,DAYS.ROWS REPRESENT DAYS'/)
   35 FORMAT (///' TOTAL DOSE,LIGHT,DAY.ROWS REPRESENT TREATMENTS8',
     1'BLOCKS REPRESENT DAYS')
   36 FORMAT(//)
   38 FORMAT(/////' SSL',F10.5//' SSD',F10.5//' SSLD',F10.5//' SSED',
     1F10.5//' SST',F10.5//' SSTL',F10.5//' SSTD',F10.5//' SSTLD',F10.5/
     2/' SSET',F10.5)
      END
```