BIOLOGICAL EFFECTS OF LOW DOSE RADIATION FROM THE COBALT-60 SOURCE AT ÅS, NORWAY AND OF NATURAL BACKGROUND RADIATION AT THE THORIUM-RICH AREA IN TELEMARK, NORWAY.
STUDIES WITH THE MODEL PLANT ARABIDIOPSIS THALIANA.

BASANTA MALLA

Norwegian University of Life Sciences
Department of Plant and Environmental Sciences
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MASTER THESIS

Biological Effects Of Low Dose Radiation From The Cobalt-60 Source At Ås, Norway And Of Natural Background Radiation At The thorium Rich Area In Telemark, Norway

Studies With The Model Plant *Arabidopsis thaliana*

By

Basanta Malla

Supervisors:

Prof. Dr. John Einset

Co-supervisors:

Prof. Dr. Brit Salbu

Associate Prof. Dr. Lindis Skipperud

Department Of Plant And Environmental Sciences (IPM)

Norwegian University Of Life Sciences

P.O.Box 500, No-1432 Ås, Norway

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Sincerely

BASANTA MALLA

Ås, February 2012

ABSTRACT

Several studies exist on radiation effects on organisms. Although most of the interest has been on humans, during the last few years increasing attention has been directed to other organisms, focusing on an ecosystem perspective with the intention of defining ‘safe’ levels of radiation. Recent nuclear power plant explosions at Fukushima have also heightened public interest in low dose effects. In Norway, interest in developing the thorium resource in Telemark has also been an issue in relation to low dose radiation effects. In this study, we looked at low dose effects of gamma radiation on *Arabidopsis* at the Co-60 source at Ås, analyzing parameters such as seed germination, primary root growth (1 week), seedling mortality and seedling weight. Seed germination was not affected in any of the treatments, including the highest exposure rates of 384 mGy/h. When measured 1 week after sowing seed, we found no effect of continuous gamma exposure as high as dose rates of 270 mGy/h on primary root growth or on seedling weight. Two weeks continuous exposure gave no plant mortality and no obvious growth effects except for a small increase in seedling weight at 0.08 mGy/h (13 mGy total dose). Continuous exposure for 3 weeks resulted in significant effects on plant mortality and seedling weights, even at dose rates as low as 0.01 mGy/h. When seedlings received a 5 or 10 day exposure of radiation followed by several days growth without exposure, we saw effects at the end of a 21-24 day experiment on plant mortality and seedling weights. Both low dose rates (0.01 mGy/h for 10 days, 2.4 mGy total dose) and very high dose rates (270 mGy/h for 5 days, 32 Gy) gave final seedling weights significantly higher than controls. We incubated plants at the Fen site in Telemark for 1 month (dose rate exposure on site of 3.5 mGy/h), then produced seed from 1 plant at Ås. A comparison of seedlings growth from the Fen-exposed plant with control seedlings showed no difference. Similarly, we found no difference in seedling growth with control seedlings grown over soil samples from Fen compared to controls not grown over Fen soil. We conclude that *Arabidopsis* shows effects in response to so-called ‘safe’ doses rates of radiation (e.g. 0.01 mGy/h) and that these effects are not apparent before approximately 3 weeks after the start of exposure. .

INTRODUCTION

The subject of this Master thesis is a topic of broad public interest; namely, the dangers associated with exposure of organisms, including humans, to radioactivity. As recently as 29 December 2011, Norwegian National TV (NRK) had as its first story on the evening news a fire in a Russian atomic submarine off the coast of Murmansk, Russia. The important issue for Norwegian television was whether radioactive pollution would affect areas in northern Norway. Reports in the press the next day cited Russian authorities as saying that there had been no release of radioactive materials. Another example of high public concern about radiation danger, relevant to this thesis, was an article appearing in Aftenposten (Oslo) on 4 January 2010, reporting on high radiation dose rates around mining areas in the Fen area in Ulefoss, Telemark. Without specifying the levels of exposure involved, Aftenposten stated that ‘recent measurements have shown very high radiation levels at the earlier mining project in Telemark that was important for USA’s atomic bomb project’. As will be explained later in the Introduction, relatively high radiation levels have been known from the Fen area for at least the last 30 years.The most recent dramatic example of public concern for radioactive exposure occurred in the aftermath of an earthquake of magnitude 9.0 on the Richter scale which occurred 11 March 2011 off the coast of northern Japan. The subsequent tsunami episode, with ocean waves as high as 15 meters, devastated coastal areas in the Fukushima district of northern Japan, leading to massive property losses and over 15,000 deaths. Loss of electric power in the area resulted in the failure and meltdown of three atomic reactors at the Fukushima nuclear power plant, followed by explosions in these reactors accompanied by release of radioactive materials. As has been seen in many other examples of nuclear accidents, there was widespread concern among public authorities and in the press that release of radioactive materials, independent of the quantities involved, could have serious public health consequences. Reacting to these potential threats, Japanese authorities designated a 20 km radius around Fukushima as an ‘access restricted area’ and evacuated approximately 160,000 people. In the immediate and long-term aftermath of the accident at Fukushima, articles have appeared in national and international press, reporting on the potential dangers of radioactive exposure. As recently as 14October 2011, Aftenposten reported that there was widespread concerns among citizens living in Tokyo, Japan that dangerous levels of radioactivity existed in certain locations in the city. Aftenposten further reported that levels of exposure as high as 3 microsieverts per hour had been measured.

Probably because of the use of atomic bombs during World War I and the Chernobyl nuclear disaster in 1986, there is something special about radioactive exposure as a public health issue. At least two considerations are important:

1. There is generally a poor understanding among the public about what radioactive exposure means and about what levels of radioactive exposure are considered to be dangerous. Moreover, the public often misinterprets events when reports of radioactive are concerned. For example, over 15,000 people died in Japan as a result of the earthquake and subsequent tsunami but there have been no reported deaths attributed to the leakage of radioactive material from the Fukushima facility (B. Salbu, quoted in Teknisk Ukeblad 2011).
2. There still exist gaps in our knowledge about what levels of radioactive exposure can be considered to be ‘safe’, if any levels of exposure can be considered ‘safe’, at all.

Background on Radioactivity and Radiation Exposure

Radioactivity is the act of emitting radiation spontaneously. This is done by an atomic nucleus that for some reason is unstable; it wants to give up some energy in order to shift to a more stable configuration. It is a physical phenomenon and radioactivity of sample is measured by counting how many atoms are spontaneously decaying each second. Radiation is form of energy which travels in the form of waves or high speed particles. Radioactivity is the phenomenon of giving off energy as particles or rays spontaneously. Radioactive atom emits ionizing radiation when they decay. Non-ionizing radiation has enough energy to vibrate atoms in a molecule or to move atoms in a molecules but not enough energy to remove electrons. For example a sound wave, visible light, micro-waves whereas ionizing radiation has enough energy to remove electrons from atoms creating ions. All ionizing radiation removes electrons from most molecules directly or indirectly. There are three main kinds of ionizing radiation:

1) Alpha particles: which include two photons and two neutrons
2) Beta particles: which are essentially electrons
3) Gamma rays: which are pure energy photons.

Although radiation was discovered in the late 19th century, the dangers of radioactivity and of radiation were not immediately recognized. Acute effects of radiation were observed first with the use of X-rays when the American electric engineer Nikola Tesla intentionally subjected his fingers to X-rays in 1896. He published his observation concerning the burns that developed through he attributed them to ozone rather than X-rays. His injuries healed later. Before the biological effects of radiation were known many physicians and corporation had begun marketing radioactive substance as patent medicine and radioactive quackery. Dangers of radiation were not fully appreciated by Scientists until later (Radiation poisoning from Wikipedia). Nuclear Power Plants (NPPs) release various technogenic radionuclide (^{137}Cs, ^{90}Sr, ^{60}Co, ^{54}Mn, ^{14}C, and Pu isotopes) into the environment during operation. Because radionuclide accumulates in abiotic and biotic components of the environment, ionizing radiation can cause toxic and genotoxic effects on organisms (Poliekarpov 1995). It can directly disturb plant breathing, photosynthesis, growth, active transport as well as ionic balance and enzyme synthesis (Evseeva et al. 2000). It has been determined that ionizing radiation in plants can stop cell division (Sidorov 1990). These changes point to the changes in biochemical processes which can decrease cell vitality. After the Chernobyl accident, it was determined that in acute exposure to ionizing radiation, the impact of radionuclide can be two to four times higher in the cell, due to atom decay than in external irradiation. The biological impact of radionuclide depends on their accumulation level and localization in the organism and cells. Radionuclide may enter the inner cell compartments, and sometimes bind to the DNA molecule. The genetic effects can be induced by ionizing radiation due to the radionuclide decay and by transmutation. Transmutation is a change of the chemical nature of decaying atoms and ionizing energy; it affects the site where radioactive decay takes place (Gracheva et al. 1977). Whether the dose of radiation is of small or large, it causes biological effects. Radiation causes ionization of atoms which may effects molecules, cells, tissues and organs. Even though all subsequent biological effects can be traced back to the interaction of radiation with atoms, there are two mechanisms by which radiation ultimately affects cells. These two mechanisms are commonly called direct and indirect effects (USNRC technical Training Center).“The consensus of workshop participants was that the 0.1 rad/d limit for animals and the 1 rad/d limit for plants recommended by IAEA are adequately supported by the available scientific information”(Oak Ridge National Laboratory 1995).

Table 1: Biological, radiological and environmental factors which contribute to variations in radiobiological responses of plants (modified from Gunkel and Sparrow (1961))

BIOLOGICAL	**RADIOLOGICAL**
A) Cytological and genetic	
1) Chromosome number	1) Kinds of radiation(s)
2) Chromosome volume	2) Energy or LET of radiation
3) DNA content(per-chromosome)	3) Exposure fractionation (and previous exposures)
4) Heterochromatin (amount of)	4) Exposure rate
5) Genotype or taxonomic group	5) Exposure duration
6) Length of mitotic cycle	6) Depth dose
7) Percentage of cell dividing	7) Location of radioisotopes
8) Stage of nuclear cycle)	8) Relative humidity
(Especially in meiosis)	9) Shielding (Various)
B) Morphological organization	10) Moisture content (of soil & plant
And development	11) Density of soil
1) Type of cell or tissue	12) Chemical composition of plants & soil for neutron
2) Stage of differentiation	13) Distance from detonation
(e.g. vegetative or floral))	14) Time of detonation
3) Portion(s) of plant irradiated	**Environmental**
4) Size of plant or depth of	1) Temperature
Sensitive organs	2) Wind velocity
C) **Physiological or biochemical**	3) Dust or fallout (amount of and particle size)
1) Age of plant	4) Moisture content (of air, soil and plants)
2) Metabolic rate	5) Insects or other pests
3) Stage of growth cycle (active	6) Competition (Other plants)
or dormant)	7) Season (day length etc)

4) pH of cells (and soil)
5) Nutritional state
6) Concentration of growth hormones
7) Concentration of protective or sensitizing substance
8) Available sunlight
9) Soil fertility

Effects of Radiation Exposure on Plants

A summary of the relative sensitivity of organisms to radiation exposure, according to older studies by Romann and Spirin (1991) and Alexakhin (1996), is given in Table 2. These guideline values were in line with recommendations of the International Commission of Radiological Protection (ICRP 1991) at that time which stated that “The commission believes that the standard of environment control needed to protect man to the degree currently through desirable will ensure that other species are not put at risk. Occasionally individual members of non-human species must be harmed but not to the extent of endangering whole species or creating imbalance between species”.

Table 2: Comparison of the derived levels of exposure of man and biota recommended by the international agencies with dose rates of chronic radiation producing effects at different levels of biological organization (Romann and Spirin 1991 and Alexakhin 1996)

BIOLOGICAL EFFECT	**DOSE RATE Gy/yr**
Dose limit for human(the ICRP, 1990)	10^{-3}
Natural radiation background	10^{-2}-10^{-3}
Casual detection of genetic effects	0.05
Steady registration of genetic effects in the most radiosensitive species	0.1
Dose limit for deterministic effects(ICRP1990)	0.15
Increase of mean population radio-resistance (radio-adaptation)	0.2
Dose to biota considered by the IAEA as not providing any hazard	0.4
Inhibition of growth and development in radiosensitive species	1-3
Disappearance of sensitive species from a community	4
Radiation damage to ecosystems:	
Conifers forest	10
Deciduous forest	30
Agricultural crops	50
Herbaceous phytocenoses	70

More recently, there has been an increased focus on developing radiation protection strategies that look more closely at non-human components of ecosystems. This has led to renewed interest by international agencies in developing better knowledge about organism sensitivities. Among several organizations involved in this effort, the International Commission on Radiological Protection (ICRP) has introduced the concept of representative species of plants and animals to assess radioactive impacts on the environment (Reports 2003, 2007). ICRP's representative plant species are pine trees as a large terrestrial plant and wild grass as a small terrestrial plant. For its part, the European Union EURATOM program has developed a so-called generic screening value corresponding to 0.01 mGy/h for testing species of interest (Andersson et al. 2008). The rationale of this benchmark radiation dose is that species not showing effects at this level of exposure can be screened out with regard to regulatory concern. A further refinement of the scale designates values for organism types as follows:

vertebrates, 0.002 mGy/h; plants, 0.07 mGy/h and invertebrates, 0.2 mGy/h. All of these guidelines emphasize key species in ecosystems, taking into consideration long-term effects on mortality, morbidity and reproduction based on ecological theory in relation to determinants of population sustainability (Stark et al. 2004).

Historically, effects of ionizing radiation on plants have been studied using external radiation sources like x-rays, gamma rays, mono-energetic neutrons of various energies, heavy particles such as nitrogen ions etc. in the earlier experiments which showed that as compared to animals, some plants are more sensitive to ionizing radiation (Sparrow 1972). Radionuclide effects on plants were initiated broadly after the Chernobyl accident when large areas of arable soil and woods were contaminated with radionuclide (Marclulioniene et al 2005). After the Chernobyl nuclear power plant accident the effect of radionuclide incorporated into the organism can be 2-4 times higher than that of external irradiation because of the atom decay in the cell in the case of acute ionizing radiation dose (Grodsinsky 2001). The stimulating effects of radionuclide can cause morphogenetic changes in plant, which reveals themselves in the early development stages (Mericle and Mericle 1967). Internal exposures doses in plant can increase because of large amount of radionuclide accumulated in their tissue especially those with actively dividing cells like in young as well as in meristematic tissues (Sokolor et al. 2001 and Tyson et al.1999).

Most of the earlier studies of gamma radiation effects on plants have used much higher exposures. Sparrow et al. (1965), for example, examined long-term effects of Co-60 irradiation on pitch pine trees at the Brookhaven National Laboratory using 2 mGy/h and higher dosages and causing tree mortality, inhibition of needle growth and reduced seed production. On the other hand, a study involving Scots pine by Sheppard et al. (1982) examined gamma irradiation effects from a Cs source in one year old plants exposed to lower radiation levels during one growing season. Exposures in the range 0.0025-0.078 mGy/h increased needle lengths while 7 mGy/h resulted in impaired seedling growth. Dugle (1986) exposed balsam fir trees to gamma dosages 0.05-62 mGy/h in an 11 year study, observing effects on lateral bud growth in the range 0.05-0.3 mGy/h and effects on needle length 0.5-5 mGy/h.

Ionizing radiation induces many histological and cytological changes in plants. (Saric 1961). Impaired mitosis during germination of seeds is the most striking effects of seeds irradiated at higher doses levels by virtual elimination of cell division in the meristematic zone of growing seedling without any apparent effect of cell expansion. (Annathaswamy et al 1971). Annathaswamy et al (1971) irradiated wheat seed with ^{60}Co gamma rays at 20-200 krad dose level. They found inhibition in seedling growth by 50 -62 percent at low doses from 20 to 40 krad and almost completely at higher doses. However the relative GA induced increase in seedling height in samples irradiated at 20 Krad (69 percent) and at 40 Krad (60 percent). The increase in seedling was 48 percent at 60-200 Krad range suggesting that at higher dose levels GA promoted lesser growth stimulation than at lower doses. Tobacco tissue culture when grown on MS medium readily develops leafy buds in light and not in darkness. When gamma irradiated, the cultures differentiated in darkness too. Furthermore the stimulus for darkness could be transmitted to non-irradiated cultures by the irradiated medium with radiation dose 5krad/min (Degani and Pickholz 1973). Miller and Sparrow (1965) irradiated thalli of *Marchantia polymorpha* with ^{60} Co gamma rays at exposure upto 55 KR and scored for survival after 4 weeks of growth . They found the number of thalli in *M. polymorpha* (n=9) with functioning apical notch decreased with increased in exposure to Co^{60} gamma rays. The greatest number of non apical outgrowth occurred between the 15 and 20 KR exposures. Morgen and Strom Johanson (1964) exposed seeds of *Pinus rigida* (Mill) while they were still in tree and germinated under controlled condition after getting seeds. Up to an exposure rate of 130 r/day and for a total exposure of 16,000 r radiation did not affect germination. However at an exposure rate of 295 r/day germination was reduced after an exposure of 8,000 r. There was temporary stimulation of root growth and increase in fresh weight of the seedling at an exposure of 6,000 to 8,000 r and a temporary retardation in overall growth at exposures above 8,000 r. Gamma radiations generally causes a reduction in stem growth. There was no significant stimulation at any of the dose rate used. There was 50% reduction in stem growth as compared with that of the control when the dose rate of about 16 Gy was used (Amiro1985).

Kurimato et al. (2010) found that the age at the time of radiation exposures plays an important role in integrating radiation effects and the irradiated *A. thaliana* indicated greater divergences in terms of physical growth compared to the internal physiological reactions. The given dose rates were 0.5 Gy, 5 Gy, 50 Gy and 150 Gy and irradiation at one of three different life stages.(15 days, 20 days and 25 days old). Marciulioniene et al. (2005) found that in *Lepidium sativum* L. seed both internal (0.6-600µSv) and external (40-5500µSv) exposure did not have any influence upon seed germination but there was effect on root growth which was 12 and 33% stimulation respectively.To date most studies on radiation effects in plants are based on high dose exposures in the range 100-3000 Gy (Culligan et al. 2006) although effects are reported for lower doses on *Tradescantia* and *Arabidopsis* in the Chernobyl zone (Ambramov et al. 2005).

In the year 1951 Brookhaven national laboratory was established for irradiating cultivated plants with gamma radiation and the native vegetation to the surroundings were also exposed to low levels of radiation. The prospects of injury was not significant as the level of radiation in that area never exceeded 12 r/day and such low exposure rates were not known to produce serious effects in most plant species. However after 7years in 1958 a daily exposure of as low as 5 r/day showed a significant injury in closer examination in *Pinus rigida* (Sparrow et al. 1966). A novel screen was described for mutants hypersensitive to gamma (g) radiation. Of approximately 5000 EMS mutagenized families screened; three were homozygous for recessive mutations that produced both a gamma-hypersensitive but fertile, phenotype. Two of these mutants are both UV and gamma-sensitive and due to defects in each component of the *Arabidopsis* homologues of the human endonuclease complex, ERCC1/ XPF (a defect which does not result in gamma-sensitivity in mammalian cells). The third mutant is gamma-sensitive, but not UV sensitive; its map position was described (Helfner E et al 2003). Seeds of three chicken pea genotypes were irradiated with gamma rays at 40, 50 and 60 Kr separately and post mutagenically with gibberellic acid and plant height, number of primary and secondary branches, pods per plant, seeds per pod and grain yield in M2 generation were noted down and were significantly affected due to genotypes, treatments and also by their interaction (Khan et al. 2005). Taking two varieties of *Arabidopsis* one with having ATM kinase and one without ATM kinase gene, end points based on root growth, petiole elongation and root hair development were used to compare sensitivities of genotypes to gamma exposures 0.125-20 mGy/h from a Co-60 source (Einset and Salbu 2008)

Ecological Consequences of Radiation Exposure

Radiation induced replicate instability of genome. Threshold character of genetic instability induction may be a reflection of the first stage of cytogenetic adaptation that is chronic low dose irradiation appears to be an ecological factor creating a background for alternation of the genetic structure of a population. Chronic exposure at doses above certain value can be ecological factor altering the genetic structure of a population (Graskin et al). The International Conference on the protection of the Environment from the effects of ionizing radiation took place in Stockholm in October 2003(IAEA 2003). It was organized by IAEA in co-operation with the UNSCEAR and EU and IUR. The aims of the work of ICRP’S new task group on Reference Plants and animals are to select and define reference plants and animals to be recommended by ICRP and to define end-points for assessing radiation effects in non-human species (IAEA 2003).

The purpose of developing a reference animals and plants is to derive a reasonably complete set of related information for a few types of organisms that are typical of the major environment.(Clarke and Holm 2003, ICRP 2003). In order to calculate radiation dose a set of reference values is required to describe the anatomical and physiological characteristic of an exposed individual. Such reference values have since long been used for dose assessments in humans (ICRP2002). Each reference organisms serve as a primary point of reference for assessing risks to organisms with similar life cycle and exposure characteristics. “Radionuclide accumulates in abiotic and biotic components of the environment, ionising radiation can cause toxic and genotoxic effects on organisms. It can directly disturb plant breathing, photosynthesis, growth, active transport as well as ionic balance and enzyme synthesis . It has been determined that ionizing radiation in plants can stop cell division. These changes point to the changes in biochemical processes which can decrease cell vitality (Marciulioniene et al. 2006). “Internal exposure in plants can increase with radionuclide accumulated in their tissues, especially in tissues with active cell division.”(Shershunova et al. 2001).

Hypothesis of the Research

- Our first hypothesis is that the recommended 'safe' screening level of 0.01 mGy/h for exposure of plants is correct. We expected to see effects on *Arabidopsis thialiana* only at dose rates >0.01 mGy/h.
- Our second hypothesis is that *Arabidopsis* plants exposed to the Thorium rich area in natural site (Fens Area Telemark Norway) will not show effects of this exposure directly or in progeny (F2 generation)

Objectives of the Research

- Conduct controlled gamma dose experiments, starting from seed, with *Aradiopsis thaliana*, measuring its response to gamma radiation in relation % seed germination, primary root growth, seedling mortality and final seedling weight.
- Look at dose – effects from the gamma dose experiment in the context of possible doses from areas with natural high radiation (ie. Fen area in Telemark.
- Analyze the growth of seedlings produced from plants exposed to the Fen area in Telemark in comparison to seedlings produced from non-exposed plants.
- Analyze the growth response of *Arabidopsis* plants growing on top of soil samples collected from Fen, Telemark area

Why *Arabidopsis*

Arabidiopsis has been used by half of all plant scientists in the world as model system because of its characteristics like small size of genome which is completely sequenced. Life cycle of this plant is short and easy to grow. This plant has mutants and discoveries made may be easily applied to other plant species as well as to animals**.**

MATERIALS AND METHODS

Co-60 Exposure at the Kilden Ås Lab

The Co-60 source at Ås, protected in a lead casing. When the door on the front of the lead chamber is lifted, we can obtain controlled gamma exposures of plants by placing them at appropriate distances from the source.

Here are the distances we used for controlled exposures at the Kilden:

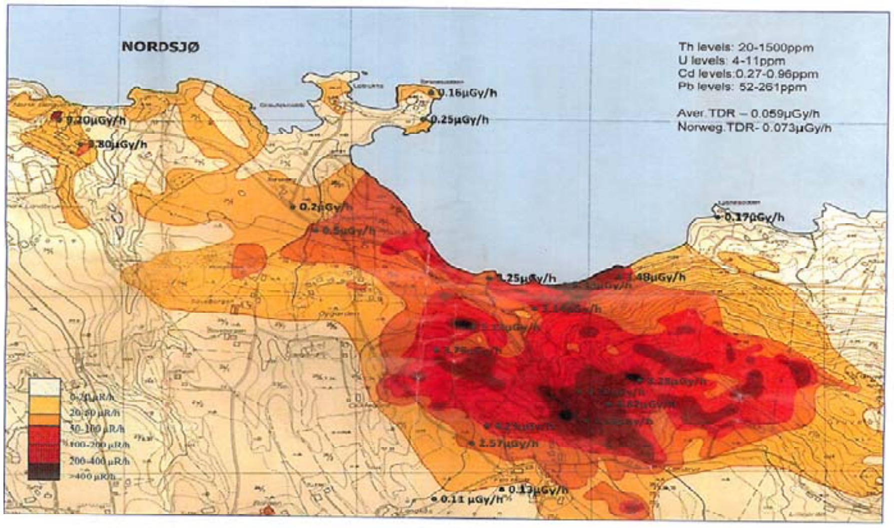
DOSE RATE	DISTANCE FROM SOURCE
270 mGy/h	43 cm
30 mGy/h	75 cm
1 m Gy/h	3.81 cm
0.01 mGy/h	3,81 cm + 2 lead blocks shielding
0.00017 mGy/h	5 m + 3 lead blocks shielding

Study area

The Fen complex (Telemark, Norway) is the type area for carbonatitis (carbonate rock of volcanic origin) and was described by Brogger in 1921. It is located approximately 120 km southwest of Oslo. This area contains rocks of volcanic eruption which was active 600 years ago (Saether 1957, Baerth and Ramberg (1966). Of particular interest for us, the Fen area contains high levels of Th and U as well as high levels of As, Cr, Pb and Cd. Extensive mining activity has occurred in this area during the 1800s and all the way up to the 1960s.

There has been interest in Norway to exploit the Th resources for future use in nuclear power (Thorium Report 2008). In this regard, research is being conducted to asses the potential dangers to workers who might be involved in these mining activities. It has already been determined that the annual total effective radiation dose to the population in the Fen area is as high as 4X the average estimated radiation dose (2.9 mSv/y) for the Norwegian population (Stranden 1982, Standen and Strand 1986, Sundal and Strand 2004). Stranden (1982) calculated that workers involved directly in previous mining work in the Fen area received annual radiation doses equivalent to 150 mSv/y which is much higher than recommended occupational dose limits for radiation workers (20 mSv/y) and 3 times higher than the maximum dose allowed in special situations (50 mSv/y).

For our experiment, we choosed three different site at Fen. They were Sove(site 1), Fengrove (site 2) and Gravahaugen (site 3) and average dose of radiation was 2.30 µGy/h, 3.50 µGy/h and 5.75 µGy/h respectively



(map S. Dahlgren, 1983)

Gamma dose rate (µGy/h), Fen, Ulefoss, Norway (2009)

Fig. 1: Map of the Fen area in Ulefoss, Telemark, Norway. Radiation levels measured 1 m above soil levels are indicated.

Plant Materials and Growing Conditions

Seeds of wild type Columbia variety of *Arabidopsis thaliana* were sterilized with 80% ethanol and then germinated in plastic petri plates of 5.0 cm or 8.5 cm diameter and containing either 10 ml or 20 ml, respectively, of Murashige and Skoog medium (1962) plus 30 g/l sucrose and 8 g/l agar. The plates were incubated for designated time periods at the Co-60 source at the Norwegian University of Life Sciences under controlled environment conditions at 20 C and continuous light with 80 M/m^2/sec intensity, after which time plants were photographed and % germination, primary root growth (1 week) and seedling weights were determined (Einset et al. 2007, 2008). All data were subjected to analysis-of-variance (ANOVA) in groups. Significant differences between treatments were evaluated at the 95% confidence interval.

One Month Exposure of *Arabidopsis* Plants at Fen

Three different sites were selected for this experiment in Fen area having high, middle and low natural doses of radiation; i.e. the Sove, Fengrove and Gravahaugen areas where the average dose rate were 2.30µGy/h, 3.50µGy/h and 5.75 µGy/h, respectively, measured during the experiment using a dosimeter. One week old seedlings of *Arabidopsis thaliana* grown in plastic petri dishes were used. At each site, two petri plates were placed: one shielded with lead while other was without lead (Fig. 2).

Fig 2: Petri plates with 1 week old plantlets of *Arabidopsis* (one unprotected and the other shielded with lead) at the Fen area.

Collection of Soil Samples at Fen and Growing *Arabidopsis* Above Soil Under Controlled Conditions

About 25 gm of soil samples from each site were collected in the plastic bags and transported to the laboratory. Dry soil sample were placed in the lid of a 8.5 cm plastic petri dish which was then covered with white filter paper. Next, we placed fresh plates with medium and newly sown seed on top of the soil treatments plus white paper control. Plants were grown for three weeks at 24 C, 16-h photoperiod and an 80 µM/m^2/sec light intensity in the culture room, after which time seedlings weights were determined.

Incubating *Arabidopsis* Seed in Soil for One Year

About 100 dry seeds per vial of *Arabidopsis thialiana* in plastic vials were buried about 30cm deep in the ground with the help of hand shovel. At each site one vial was shielded with lead while other was kept free. The buried seeds were collected after 1 year for further experiment.

Fig 3: Burying seeds of *Arabidopsis* in soil at Fen.

RESULTS

Overview of Experiments at Co-60 Source, Ås

During the course of this work, we conducted 20 controlled experiments at the Co-60 source with durations varying between 1, 2 and 3 weeks and measuring several different endpoints such as % germination, primary root growth, seedling weights and seedling mortality. We tested exposure rates as high as 384 mGy/h and as low as 0.00017 mGy/h (background). Exposure rates were confirmed on site using a hand held automess. Several of the results we obtained are reported below.

One Week Continuous Exposure

Figs. 4, 5 and 6 summarize the results of a one week experiment, starting from seed of *Arabidopsis*, and measuring seed germination, primary root growth, seedling weight and seedling mortality. There was no effect of any of the exposures compared to controls on seed germination % or one seedling mortality measured after one week. Figs. 7 and 8 show the results of a repeat experiment, scoring primary root growth (Fig. 7) and seedling weights (Fig. 8) after I week continuous expoosure to radiation. The different treatments gave no significant effect on root growth comparaed to controls. As far as seedling weights were concerned, only the 0.08 mGy/h (13.4 mGy total dose) gave a significantly higher seedling weight compared to controls.

As can be seen from the Figures, there appeared to be no effects measurable after one week on primary root growth or final seedling weights exposed for one week to a range of radiation doses, varying from 0.00017-384 mGy/h which corresponds to 0.02 mG-64 Gy, respectively. By comparison, a total dose corresponding to 64 Gy in humans would be expected to result in death within 1-5 days and the LD_{50} range for acute exposure with medical intervention is 5-10 Gy, resulting in probable death within 1-3 weeks (U. S. Department of Energy, 2005).

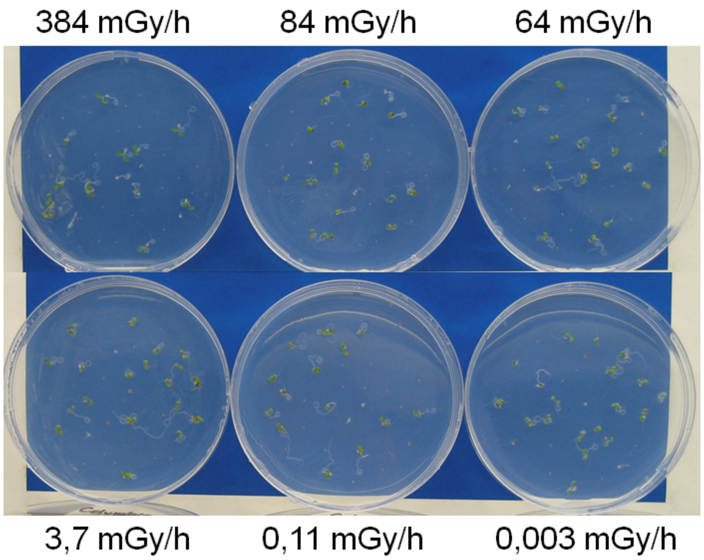


Fig. 4: Plant growth in response to different continuous radiation dose rates during 1 week.

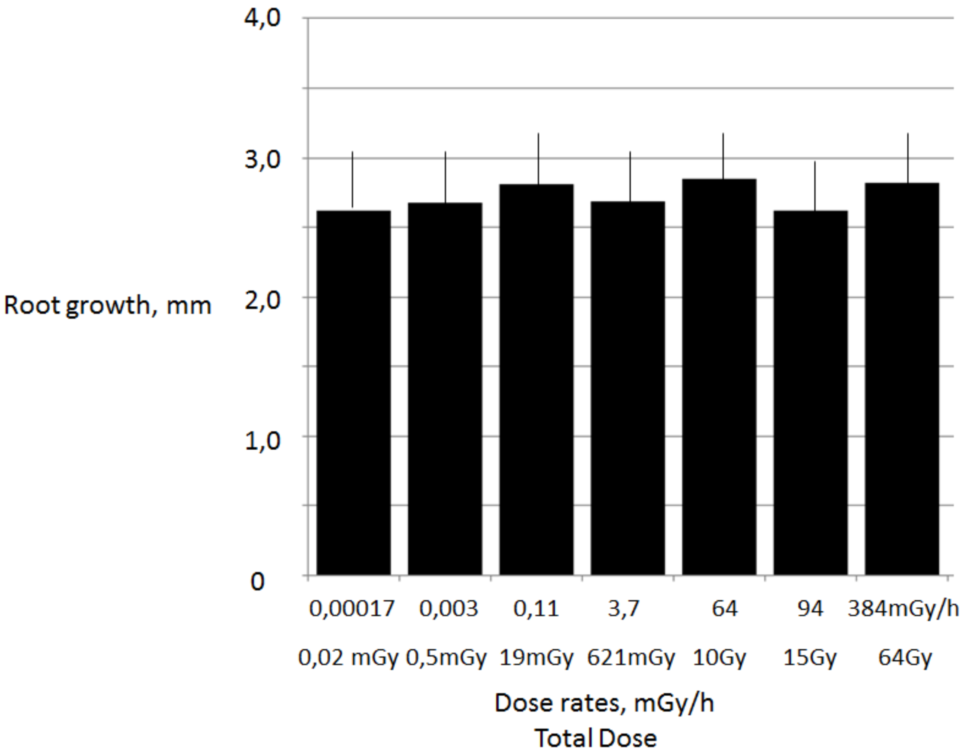


Fig 5: Primary root growth in response to different radiation dose rates during 1 week. For each treatment, 8-10 primary roots were measured. Bars indicate 95% confidence levels based on ANOVA.

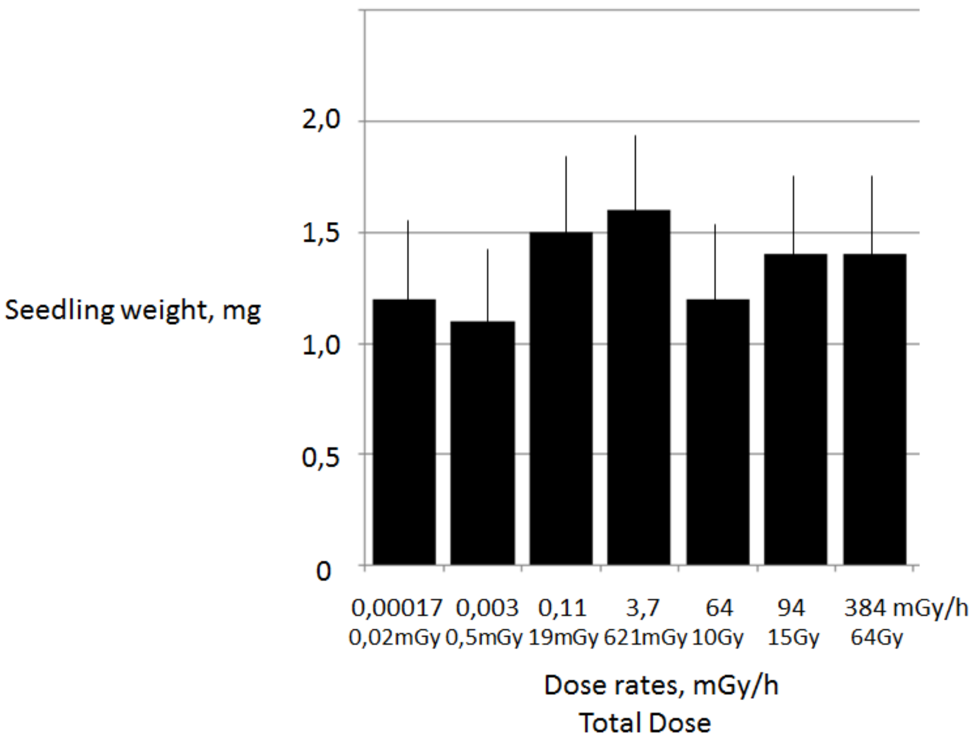


Fig. 6: Seedling weights in response to different radiation dose rates during 1 week. For each treatment, seedling weights of 8-10 plants were determined. Bars indicate 95% confidence levels based on ANOVA.

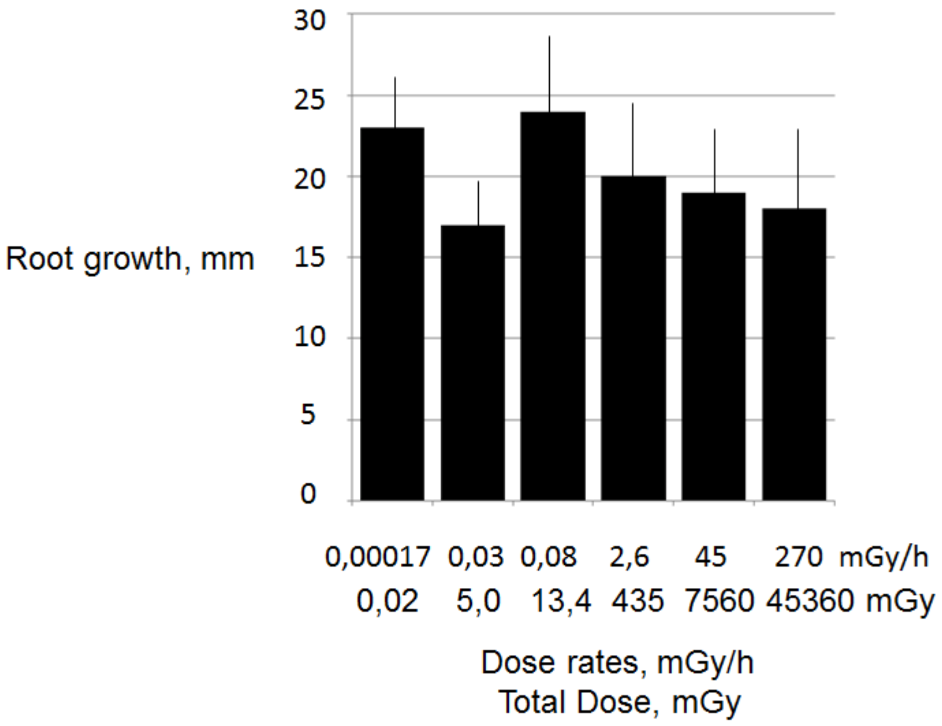


Fig 7: Root growth in response to radiation dose for 1 week

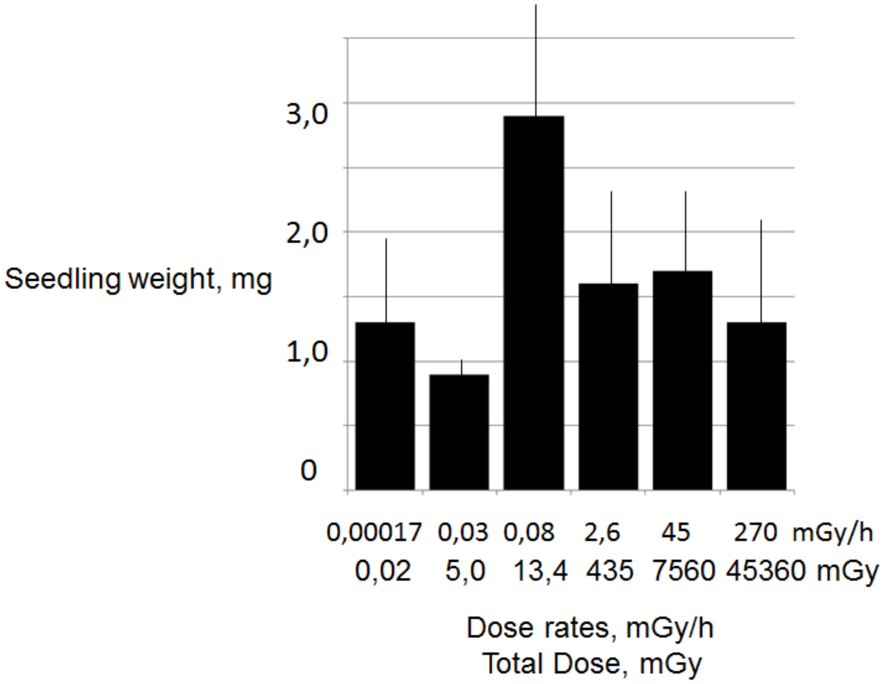


Fig 8: Seedling weight in response to radiation dose for 1 week

Two Weeks Continuous Exposure

Figs. 9 and 10 summarize the results of a two week experiment with continuous exposure, measuring seed germination, seedling weight and seedling mortality. There was no effect of any of the exposures compared to controls on seed germination % or on seedling mortality.

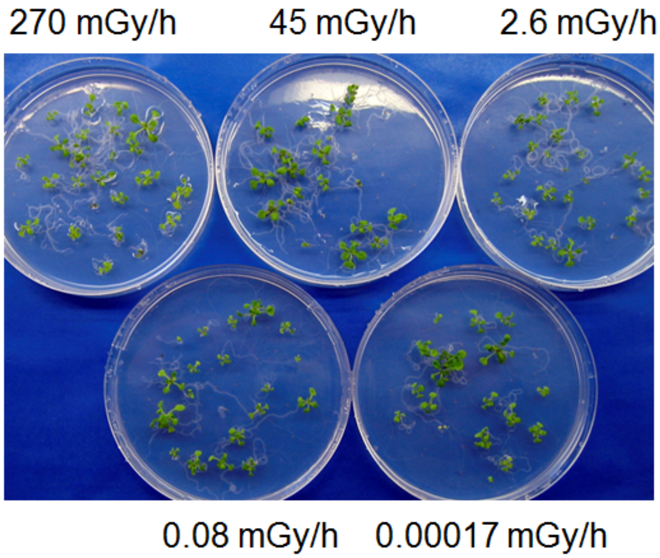


Fig 9: Plant growth in response to continuous radiation doses for 2 weeks.

As can be seen from the Fig. 8, final seedling weights were not significantly different except for the weights of seedling exposed to 0.08 mGy/h (13.4 mGy) during the two week growth period.

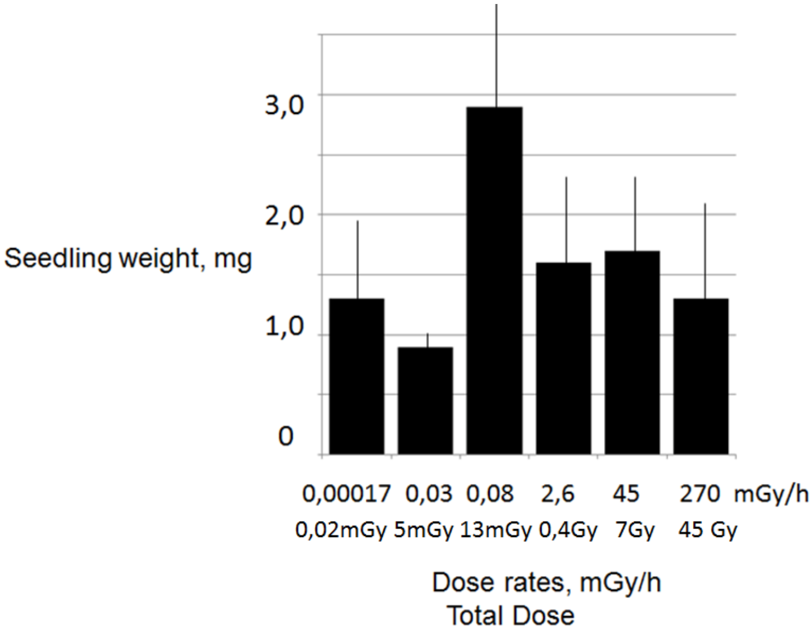


Fig. 10: Seedling weights in response to different radiation dose rates during 2 weeks. For each treatment, seedling weights of 8-10 plants were determined. Bars indicate 95% confidence levels based on ANOVA.

Three Weeks Continuous Exposure

Figures 11 and 12 shows the effects of different radiation exposures during three continuous weeks. As can be seen in Fig. 11, dose rates as low as 0.01 mGy/h (the so-called 'safe' exposure rate, according to international agencies) gave very clear effects compared to control exposures in relation to plant form, color and in relation to mortality. Starting with 15 seeds per 5 cm petri-plate, controls had 100% germination and 100% plant survival during three weeks while plant mortality in plants exposed to 0.01 mGy/h, 30 mGy/ and 270 mGy/h was 35%, 40% and 60%, respectively. The most striking, unexpected result was that 0.01 mGy/h (5 mGy total dose) gave significant effects.

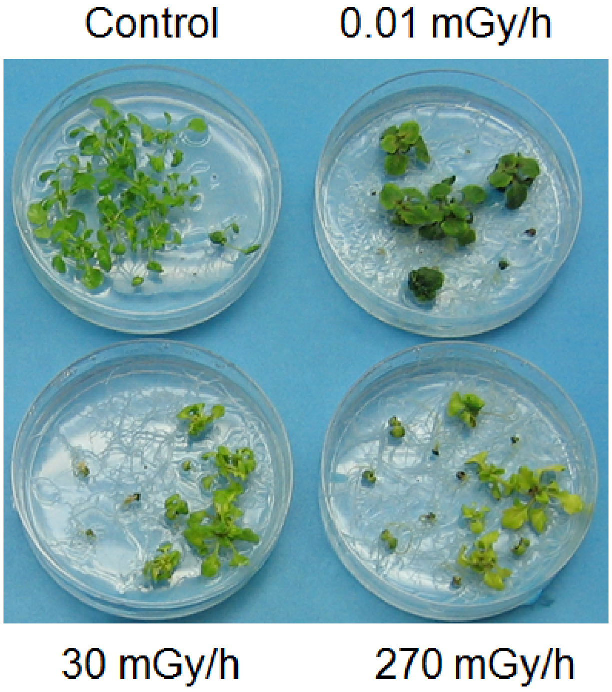


Figure 11: Plant growth responses after three weeks continuous exposure.

Fig. 12 shows average weights of the surviving plants after 3 weeks exposure to different dose rates of gamma radiation at the Co-60 source. As expected from the photos in Fig. 11, the 0.01 mGy/h dose rate exposure resulted in seedling weights significantly greater than the weights of control plants. Dose rates >0.01 mGy/h gave progressively lower average seedling weights.

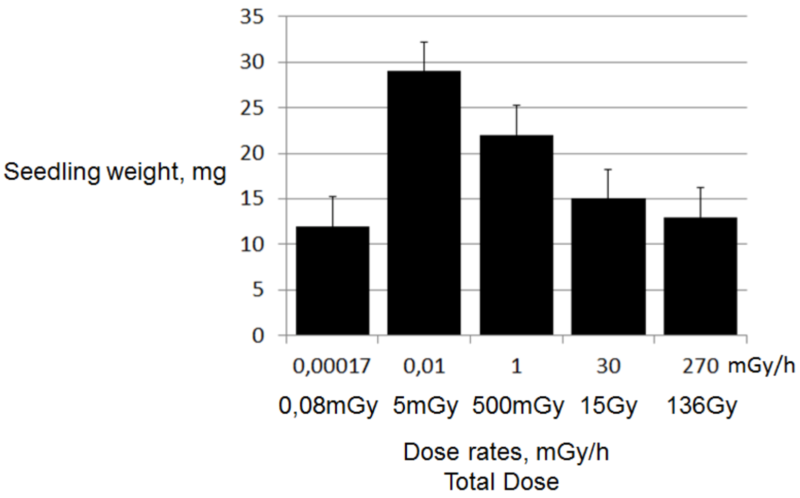


Fig 12: Seedling weights in response to different radiation dose rates during 3 weeks. For each treatment, seedling weights of 8-10 plants were determined. Bars indicate 95% confidence levels based on ANOVA.

Five or Ten Days Exposure, Three Weeks Total Growth Period

A dose rate of 0.01 mGy/h for 3 weeks corresponds to a total dose of 5 mGy. When we exposed plants to a total dose of 5mGy or greater during one week, there were no measurable effects (Figs 4, 5 6, 7 and 8). This led us to suspect that it takes more than one week for effects to be seen. The results shown in Figs. 13 and 14 involve a 5-10 day exposure followed by a period of growth without radiation exposure. As can be seen in Fig. 13, 10 days exposure to 0.01 mGy/h (2.4 mGy total dose) followed by 11 days growth without radiation exposure resulted in significant plant mortality compared to controls. Similarly, 5 days exposure to 270 mGy/h (32 Gy) followed by 16 days growth without radiation exposure resulted in even greater plant mortality and deformed plant morphology.

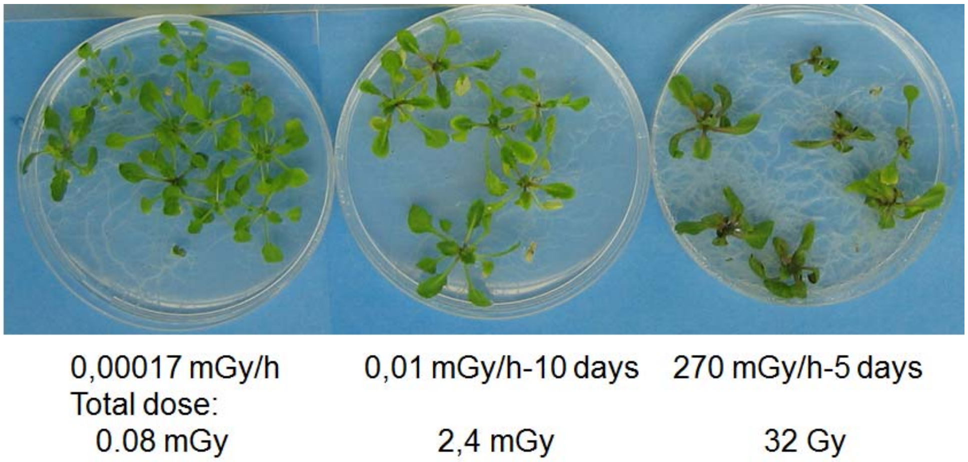


Fig. 13: Plant growth responses after initial radiation exposure followed by growth in the absence of exposure.

Fig. 14 shows the weights of the surviving seedlings at the end of the growth periods. As was seen in Fig. 13, a low dose exposure (2.4 mGy) resulted in seedlings that were significantly larger than control plants. The other treatment giving significantly larger plants was the 270 mGy/h for 5 day treatment (32 Gy dose).

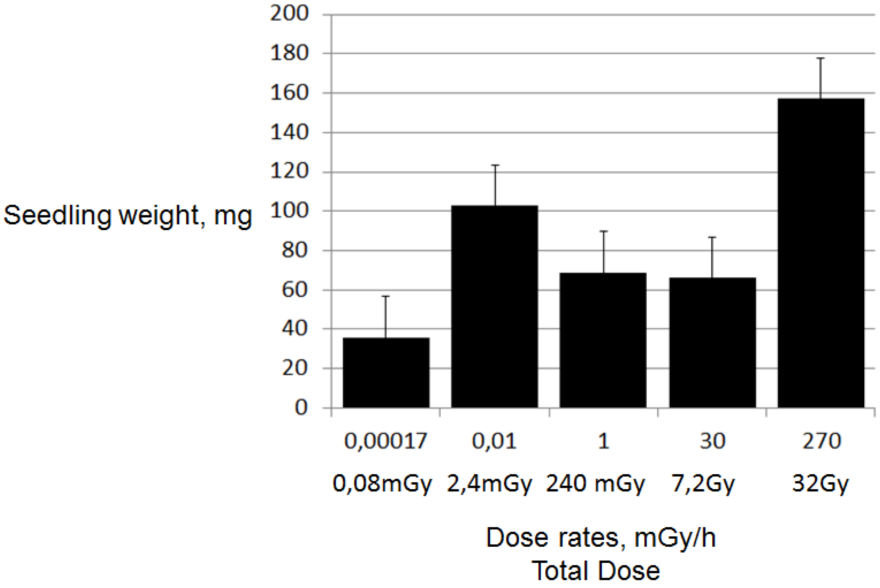
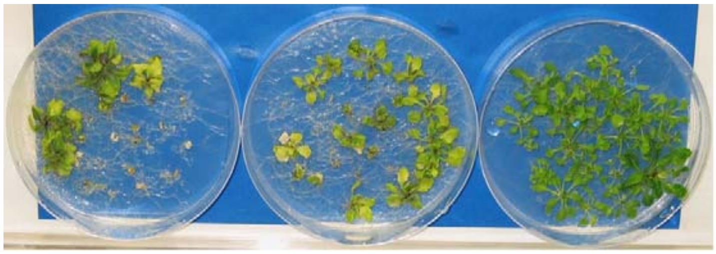


Fig. 14: Seedling weights in response to different radiation doses. For each treatment, seedling weights of 8-10 plants were determined. Bars indicate 95% confidence levels based on ANOVA.

Ten Days Exposure followed by Fourteen Days Without Exposure

Figs. 15 and 16 show results from another experiment involving exposure followed by a non-exposure growth period that was conducted summer 2010. Outside temperatures at this time sometimes exceeded 20 C, so it is possible we were not able to maintain growth conditions at the Co-60 source at a constant 20 C. Nevertheless, the overall results of the experiment were very similar to the experiment reported in Figs. 15 and 16.

As Fig. 15 shows, 10 days exposure to 0.01 mGy/h (2.4 mGy total dose) followed by 14 days growth without radiation exposure resulted in significant plant mortality compared to controls. Similarly, 10 days exposure to 270 mGy/h (64 Gy) followed by 16 days growth without radiation exposure resulted in even greater plant mortality.



Dose rate (10 days):		
270 mGy/h	0,01 mGy/h	0,00017 mGy/h
Total dose:		
64 Gy	2.4 mGy	0.04 mGy
Mortality:		
74%	30%	0%

Fig. 15: Plant growth responses after initial radiation exposure followed by growth in the absence of exposure.

Fig. 16 again shows two peaks of larger seedling weights compared to controls; i.e. one peak at low dose (0.01 mGy/h, 2.4 mGy) and another peak at high dose (270 mGy/h, 64 Gy).

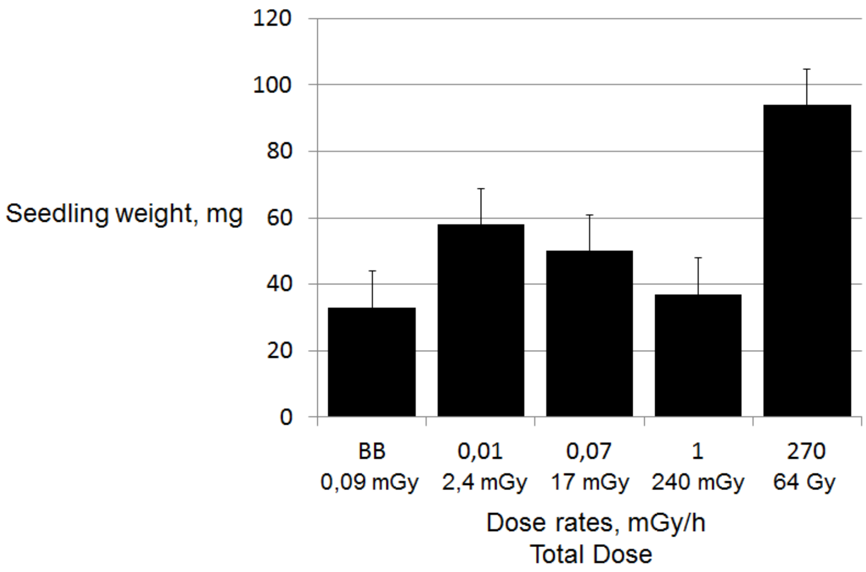


Fig. 16: Seedling weights in response to different radiation doses. For each treatment, seedling weights of 8-10 plants were determined. Bars indicate 95% confidence levels based on ANOVA.

Fen Experiment – Seedlings from Plants Grown One Month at the Fen Area

Because of fungal contamination of the plants we incubated at the Fen area, we were not able to obtain seeds from most of the plants we grew there. The plant incubated at site 2, however, survived and we were able to grow it to seed at the greenhouse at UMB. Site 2 is the Fengrove site where the average dose rate of radiation was 3.5 µGy/h. Seeds from this plant were germinated on petri plates and seedlings were grown in the culture room for 3 weeks. For comparison, a parallel series of plants were grown from seed from our non-exposed Columbia strain. The % germination of the seeds from the two treatments was the same. Fig. 17 shows the average seedling weights plants from the two treatments. The fact that the two types of seedlings are identical in weight indicates that Fen exposure had no effect on germination and seedling growth of progeny.

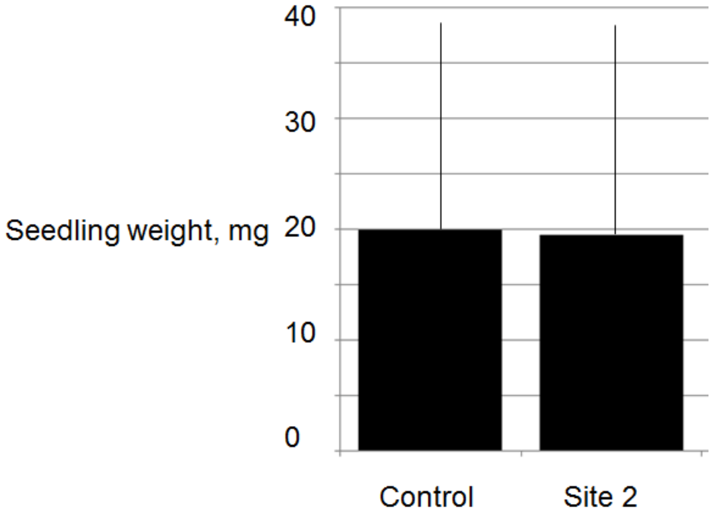


Fig 17: Seedling weights after three weeks growth, beginning with seed of non-exposed *Arabidopsis* plants (control) or with seed from plants incubated 1 month at Fen.

Fen Experiment - Seedling Grown Above Soils from 3 Different Fen Sites

We conducted two experiments of 3 weeks duration with Columbia seed, germinating seeds and growing seedlings above soil collected at Fens. Using a hand used automess we could not detect radiation levels above background in the soil samples. This must mean that the radiation detected on site at Fen is because of underlying rock formations. Fig. 18 shows, there was no significant effect of growing plants above Fen soils, even though control seedling in experiment 2 gave unusually high weights.

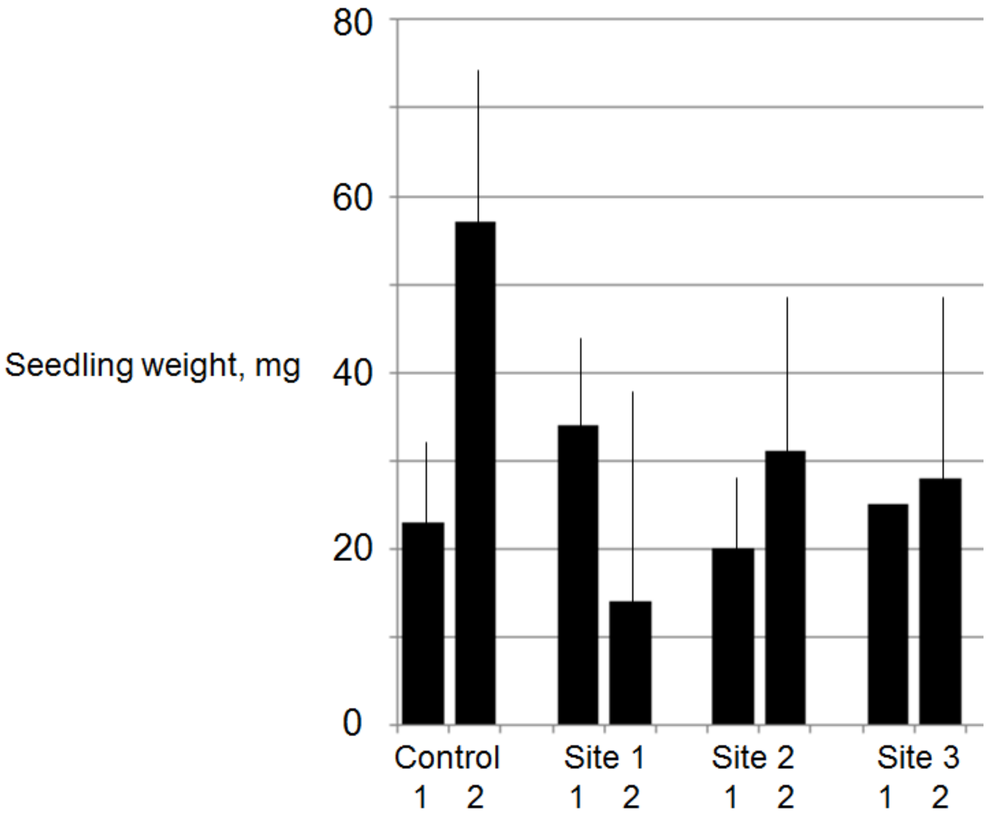


Fig. 18: Growing *Arabidopsis* seedlings above soil collected from Fen.

Seeds Buried at Fen for 1 Year

The seeds that were buried for one year in Fen area at three different locations failed to germinate in the laboratory. All the seeds lost their viability, probably due to severe cold during winter 2010-2011.

DISCUSSION

In relation to the experiments at Fen, we exposed plants to dose rates that were close to 0.01 mGy/h which gave effects at the Co-60 source. At Fen, the rates varied between 0.002-0.0005 mGy/h where we did experiments. On the other hand, the experiments at Fen were different from the experiments at the Co-60 source. In retro-respect, it would have been interesting to give *Arabidopsis* from seed for 3 weeks at Fen.

An important technical detail about this work is the question of whether we are certain that the 0.01 mGy/h exposures were correctly done. To begin our experiments, we consulted with Dr. Ole Christian Lind at Isotopen who helped us to set up the correct distances at the Co-60 source. He also confirmed the correctness of these distances using a dosimeter at the Co-60 source. After obtaining effects in our initial experiments at 0.01 mGy/h dose rates, we independently rechecked the 0.01 mGy/h station using the dosimeter. So, it seems very likely that the dose rates are correct.

The most interesting finding of this work is that so-called ‘safe’ gamma radiation dose rates (0.01 mG/h) gave significant effects on seedling weights and on plant mortality using *Arabidopsis*. In comparison with most other studies, in our study the dose of radiation used was relatively low. The maximum dose that we used was 384 mGy/hr and we used the safe dose rates as low as 0.01 mGy/h (the so-called ‘safe’ exposure rate, according to international agencies). There was no significant effect of radiation exposure on % seed germination, even at the highest exposure that we used in our experiment. There was no effect on seed germination from seed obtained from plants kept in Fengrove for 1 month where the average dose of radiation was 3.50µGy/h. Kim et al(2004) found no effect of acute gamma doses (2, 4, 8 and 16 Gy) on seed germination of two pepper cultivars(*Capsicum annuum*). Morgen and Johanson (1964), while doing experiments with *Pinus rigida*, found that up-to an exposure rate of 130 r/day (approx. 1.3 Gy/day), radiation did not affect germination. However at an exposure of 295r/d germination was reduced after an exposure of 8000r.

Morgen and Johansen also found that temporary stimulation of root growth and seedling weight occurred at exposures of 6000-8000 r. Vandenhove et al (2010) also did not find the effect on germination of seeds from gamma irradiated plants. Similarly, root fresh weight was significantly reduced after gamma exposure compared to controls but, surprisingly, there was no significant difference in root fresh weight for the different doses applied. They exposed *Arabidopsis* plants during a full life cycle ranging from 2.3mGy/h to 0.081mGy/h. Regarding the seedling weight with continuous gamma exposure for 1 to 3 weeks there was slightly increased in seedling fresh weight as compared to control. The effect was seen on so-called 'safe' dose rate according to international agencies. The seedling weight was found to be greater when the *Arabidopsis* plants were exposed to radiation for few days and then grown without gamma exposure. Wi *et al* (2007) found seedling growth of *Arabidopsis* exposed to low-dose gamma rays (1-2Gy) was even slightly increased compared to that of control.

Zaka et al (2004) exposed 5 day old *Pisum sativum* seedling to acute gamma dose from 0.4-60 Gy and studied plant growth and development on 96 day old plants over two generations and found pronounced effects on G2 plants.

CONCLUSIONS

From the result we found we can conclude that:

- In experiment lasting 3 weeks from seed germination, we saw significant effects on plant mortality and seedling weights at dose rates as 270 mGy/h (64- 136 Gy total dose).and as low as 0.01 mGy/h (2.4-5mGy total dose). No effects were seen on seed germination root growth or seedling weights when we measured these parameters after 1 week. We conclude that these effects are apparent only after 3 weeks.
- Attempts to demonstrate radiation effects with *Arabidopsis* exposure for 1 month to 1 year at Fen (2.3- 5.7 µGy/h) showed no effects on seedling weight of progeny plants (mother plant exposed 1 month at Fens) or on control plants grown above soil samples from Fen in the lab. Seeds (including control seeds shielded with lead) incubated 1 year at Fen failed to germinate. We conclude that other types of experiments need to be done to determine whether Fen dose rates affect *Arabidopsis.*

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