THESIS

ORNITHOGALUM VIRENS AS A PLANT ASSAY
FOR BETA AND GAMMA RADIATION EFFECTS

Submitted by Vicki J. Herron

In partial fulfillment of the requirements
for the Degree of Master of Science
Colorado State University
Fort Collins, Colorado
Summer, 1979

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER
OUR SUPERVISION BY VICKI J. HERRON ENTITLED *ORNITHOGALUM*
VIRENS AS A PLANT ASSAY FOR BETA AND GAMMA RADIATION
EFFECTS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work

Co-Adviser   Co-Adviser

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ABSTRACT OF THESIS

ORNITHOGALUM VIRENS AS A PLANT ASSAY FOR

BETA AND GAMMA RADIATION EFFECTS

With the increasing threat of nuclear pollution, bio-
logical assays to monitor and characterize the effects
of radiation are needed. The purpose of this study was
to determine if the monocotyledonous angiosperm, *Ornitho-
galum virens* (Quintanilha and Cabral, 1947), could be
used in such a biological assay system.

The nuclear and interphase chromosome volumes of
pollen grains and root tip cells of *O. virens* were
determined and found to be reliable indicators of
radiosensitivity.

After exposing *O. virens* plants to acute (^{60}Co) and
chronic (^{137}Cs) gamma radiation and internal beta radi-
ation (^{32}P), lethality (LD_{50}, LD_{100}), growth inhibition,
and chromosome aberrations were investigated. To deter-
mine the LD_{50} and LD_{100} for *O. virens*, a procedure in
which the irradiated bulbs were first dried and then
given the opportunity to sprout was found to be more
practical than the classical method of waiting for death
of irradiated plants. Also, root growth was found to be
a better measure of mortality than leaf growth because
leaf growth is variable even for nonirradiated *O. virens*
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plants. Using this method, the LD_{50} and LD_{100} for acute
gamma radiation were estimated to be between 0.91 to
1.8 krad and less than 3.6 krad, respectively. In this
regard, *O. virens* was found to be more radiation sensi-
tive than the majority of the herbaceous plant species
examined by Sparrow and Schwemmer (1974).

Though growth inhibition and abnormal growth were
observed in the acute and chronic gamma radiation studies,
the changes in the growth of the plants were so variable
that these parameters were found to be unreliable measures
of radiation effects. On the other hand, chromosome
aberrations were a more reliable measure of radiation
damage because linear relationships between total aberra-
tions and dose were found for both gamma and beta radia-
tion. The types of aberrations that are the least and
most sensitive indicators of radiation damage appeared
to be pulverized chromosomes and fragments, respectively.

In conclusion, *Ornithogalum virens* appears to be a
useful biological assay system for the effects of beta and
gamma radiation on chromosomes and mortality within the
dose range of this study.

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CHAPTER ONE   INTRODUCTION

With the recent growth of the nuclear industry,
studies of the biological effects of potentially harmful
low level radiation (Eisenbud, 1973) are needed because
of the widespread threat and occasional reality of pollu-
tion. To monitor radioactive pollution and to character-
ize the associated biological effects, biological assays
have been used with varying success in the past (Mericle
and Mericle, 1965; Nayar et al., 1970). An ideal assay
system must minimize or eliminate a variety of short-
comings that have to some extent plagued earlier inves-
tigations. For instance, one of the problems in inves-
tigating the effects of low level radiation is that for
many organisms the damage may occur in so few cases that
it is statistically immeasurable. Thus, a very radio-
sensitive assay system should be used. A second poten-
tial problem is that, since biological damage may accu-
mulate over time (Sparrow et al., 1961), the organism
must remain in the irradiated area for an extended period.
Thus, an organism which is both stationary and long-lived
should be used. A third problem involves obtaining an
adequate measure of radiation effects. Rate of growth,
changes in physiology and induction of somatic mutations
have been studied in the past (Van't Hof and Sparrow,

1965; Beatty and Beatty, 1955; Nishiyama et al., 1964).
However, besides not representing transmissible genetic
damage, these effects are not easily quantified and often
are difficult to compare between species. In contrast,
chromosome aberrations are genetically transmissible and
can be used as a quantitative measure of radiation damage
(Wolff, 1959) for direct comparison between species.
Therefore, a stationary, long-lived organism that is
very radiosensitive and suitable for chromosome studies
should be ideal as an assay system.

In searching for a suitable assay system for low
level radiation, *Ornithogalum virens* was selected because
as a perennial plant, *O. virens* is both stationary and
long-lived. Also, previous work (Sparrow, 1962) has
indicated that plants with a few large telocentric
chromosomes are very sensitive to radiation, and *O. virens*
has a diploid number of six large telo-acrocentric
chromosomes (Godin and Stack, 1976). Additional favor-
able characteristics of *O. virens* include its hardy, fast
growth and resistance to disease. As a member of the
lily family, *O. virens* forms a bulb that can be dried
and stored. Propagation is readily accomplished either
sexually by forming seeds or vegetatively by forming
bulbils (small bulbs off of the main bulb). One detracting
aspect of *O. virens* is that it is susceptible to freezing.

The following is a summary of the objectives of this study to determine whether *O. virens* could be used as a biological assay for the effects of radiation:

I   To determine the radiation sensitivity of *Ornithogalum virens*. This included:

A. Finding the nuclear volume and interphase chromosome volume as the most generally accepted indicators of radiation sensitivity (Sparrow, 1962).

B. Finding the LD_{50} for *O. virens* using acute gamma radiation (^{60}Co).

C. Finding the LD_{50} for *O. virens* using chronic gamma radiation (^{137}Cs).

II To determine which chromosome aberrations are the most and least sensitive indicators of radiation damage by scoring the number and types of chromosome aberrations using acute gamma radiation, chronic gamma radiation and internal beta radiation.

III To determine the effects of acute and chronic gamma radiation on the growth of *O. virens*.

CHAPTER TWO   LITERATURE REVIEW

Introduction

Before investigating the biological effects of gamma
and beta radiation on *Ornithogalum virens*, it was neces-
sary to review previous radiation studies on higher
plants. Included in the review are studies on radiation
induced chromosome aberrations, growth inhibition and
mortality (LD_{50}). Since the present study included a
field experiment, past work on the effects of irradiating
plants in the field is discussed. Also included are
studies on the use of the nuclear and interphase chromo-
some volumes to predict radiation sensitivity of higher
plants.

Chromosome Aberrations

The study of radiation genetics began in 1927 (Crow
and Abrahamson, 1965) when Muller discovered that X-rays
produced mutations in *Drosophila*. In 1928, Stadler, with
his work on corn, confirmed Muller's discovery. The types
of chromosome aberrations induced by radiation were inves-
tigated by Mather (1934) who found that X-rays produced
chromosome fragmentation. Catcheside et al. (1946) noted

that the time of observation after irradiation determined
whether chromosome or chromatid aberrations would be seen.
In 1940, Sax described the types of aberrations produced
by X-rays. He concluded that aberrations requiring a
single hit increased linearly with dose and aberrations
requiring two hits increased as a function of dose
squared. Caldecott and Smith (1952) found anaphase
bridges in barley increased linearly with dose. However,
Wolff (1954), analyzing metaphase dicentric chromosomes
and rings, found a dose squared relationship. In 1957,
Wolff and Luippold showed that metaphase exchanges gave
a dose squared relationship and the anaphase bridges
gave a linear relationship. In a later study, Conger
(1965) agreed with Wolff and Luippold's findings and
stated that anaphase bridges underestimate the amount
of damage. In 1966, Revell found chromatid interchanges
increased with the square of the dose if gaps were ex-
cluded from the analysis. In a recent study, Yamaguchi
(1974) concluded repair of chromosome aberrations and
recombination have a common basis.

LD_{50} and Growth Studies

One of the most common measurements of radiation
effects is growth inhibition (Sparrow, 1962). In 1961,
Sparrow et al. reported studying growth inhibition in

many plant species. They found a correlation between
the acute dose required to stop growth and the chronic
dose necessary for growth inhibition. Stein and Sparrow
(1963) described the effects of chronic irradiation on
the growth of *Kalanchoe*. They reported abnormal growth
of both leaves and stems. In 1965, Van't Hof and Sparrow
studied the changes in the growth rate of roots irradi-
ated when actively growing or dormant. They found
actively growing roots are more radiosensitive. In a
later study, Bass et al. (1975) examined inhibition of
growth and the damage to leaves of tobacco plants when
exposed to irradiation from ^{14}C incorporated as $^{14}CO_2$.
They found abnormal leaf growth, especially in younger
leaves. In 1966, Yamakawa and Sparrow found the expo-
sure necessary to produce 50% pollen abortion varied
from 10.4 to 1650 R/d for several plant species. Pollen
abortion also was used by Underbrink et al. (1973) to
examine the differences between 15 species with varying
ploidy. They found pollen abortion was not correlated
to nuclear volume (see below). In 1974, Sparrow and
Schwemmer determined that GR_{50} values (dose of radiation
required to reduce the wet or dry weight of aboveground
plant material to 50% of that of the control plants)
varied from 6.4 to 23 kR for 32 herbaceous plant species.
Two years later, the D_{50} (dose required to reduce seed-
ling growth to 50% of that of the controls) was deter-
mined by Conger (1976) to be 35 to 40 krad for maize.

In other experiments, both growth inhibition and/or LD_{50} (dose required for 50% lethality) were studied. In 1962, Bowen et al. found reduction in growth of several plant species when high doses of gamma radiation were given. For these species, they also reported the LD_{50}'s varied from 0.75 to 100 krad. Ichikawa and Sparrow (1966) studied growth inhibition and determined the LD_{50}'s of several polyploids of *Rumex*. Later, Sparrow and Schwemmer (1974) determined both LD_{50} and LD_{10} values for 32 herbaceous plant species. They found a correlation between growth inhibition and the LD_{10}. In 1973, Sparrow et al. concluded that to determine the LD_{50} for some species, very long observation times (several months) were needed. A year later, Sparrow and Schwemmer (1974) determined that the average energy absorbed per chromosome at the LD_{50}'s of several herbaceous species was 4.9 MeV. The LD_{50}'s for several woody species were found by Sparrow et al. (1968) to range from 473 to 17,500 R. They found a correlation between the LD_{50} and the interphase chromosome volume (IVC, see below) and used it to predict the LD_{50}'s for many other woody species. Baetcke et al. (1967) found both the ICV and the DNA content per chromosome were inversely proportional to the LD_{50}. They concluded there was a good correlation between radiosensitivity, as measured by LD_{50}, and nuclear parameters.

Studies of Nuclear Volume (NV) and

Interphase Chromosome Volume (ICV)

The question of why some plant species are more sen-
sitive to radiation than other plant species has been
the subject of many studies. Early work indicated that
species with large chromosomes are more radiosensitive
(see Sparrow, 1962 for review). In 1961, Sparrow et al.
concluded the larger the interphase nuclear volume the
more sensitive the nucleus and ultimately the whole plant.
For higher plants, Sparrow and Miksche (1961) concluded
there was a positive relationship between nuclear volume,
nuclear DNA content and radiosensitivity. In a comparison
between active and dormant roots of *Tradescantia*, Van't Hof
and Sparrow (1965) determined the active root had larger
nuclear volumes and were more sensitive to X-rays. They
believed the difference in the volumes explained part of
the differences in the radiosensitivities. In later
studies, Sparrow et al. (1973) found the mean survival
time of irradiated plants was negatively correlated to
the nuclear volumes of the plants. On the other hand,
Conger (1976) concluded there was not a consistent rela-
tionship between the effect of gamma radiation and fission
neutrons on maize seeds and the nuclear volume.

In 1962, Sparrow suggested there was another nuclear
parameter which can be used to measure radiosensitivity.
This parameter was the interphase chromosome volume

(interphase nuclear volume divided by the number of chromosomes, subsequently referred to as ICV). Sparrow et al. (1963) found that dormant white pines had smaller ICV's and were more radioresistant than actively growing pines. Alvarez and Sparrow (1965) compared the ICV's for *Tradescantia* stamen hair cells and root meristem cells. They found the values were not significantly different and concluded the contact between cells (as in roots) did not affect significantly radioresistance. In 1966, Ichikawa and Sparrow determined the ICV's for several species of *Rumex* with various ploidies. They found the ICV to be smaller in the more resistant poly-ploids and concluded the resistance was due to smaller ICV's rather than genetic redundancy. Later, Ichikawa (1970) qualified this conclusion by saying that genetic redundancy did act as a modifier. Underbrink et al. (1973) also found polyploids more resistant, but they concluded that it was a function of the ICV and not of ploidy.

In several studies, the relationships between ICV and parameters of radiosensitivity were investigated. Yamakawa and Sparrow (1966) found ICV to be inversely proportional to the exposure necessary to produce 50% pollen abortion. Ichikawa and Sparrow (1967a, b) found the D_{37} values (the dose required to reduce the popula-tion of plants to 37% survival) for several *Tradescantia* species were inversely proportional to their ICV's. That

same year, Baetcke et al. (1967) determined that ICV
was inversely proportional to LD_{50} for several plant
species. Miller and Colaiace (1971) pointed out that
the survival of a meristem should be compared with the
ICV of that meristem because the ICV's may be different
for different meristems. However, Baetcke et al. (1967)
found the volumes of the shoot and root meristems were
not different for most species. In 1971, Nayer et al.
found a positive correlation between meiotic abnormali-
ties and ICV values for several plant species growing
in a naturally radioactive area. Sparrow and Schwemmer
(1974) reported a correlation between D_{37} values for
whole plants and ICV values. In several studies (Taylor,
1966; Sparrow et al., 1968), the ICV's were used to pre-
dict radiation sensitivities of plant species although
the predictions were not tested experimentally.

Field Studies

Most studies of plants irradiated in the field have
been on communities irradiated by sealed artificial sources.
However, a few studies on communities affected by nuclear
detonations and accidents have been reported. Richard
and Shields (1963) studied a plant community three years
after a detonation at Yucca Flat, Nevada. Their results
indicated that annual plants were dominant and that early

recolonization stages were similar to the ones expected
from conventional burning or blading. In another post-
detonation study, Beatley (1966) observed the winter
annual vegetation. During the second year a large and
vigorous population was found, which Beatley concluded
was due to the "stimulatory" effect of radiation. A
desert shrub community was studied by Ragsdale and Rhoads
(1974) four years after an accidental venting of radio-
active material from Project Pinstripe. They observed
mortality, crown death and regrowth or lack of regrowth
of the plants.

A widely known study using a sealed artificial source
(^{137}Cs) to irradiate a plant community was conducted at
Brookhaven National Laboratory. In 1963, Woodwell des-
cribed the design of this study on an oak-pine forest
community. He commented that the plants were more sen-
sitive than had been predicted. In that same year,
Woodwell and Sparrow (1963) described shoot growth and
tree mortality in the community. They concluded that
growth retardation was due not only to radiation damage
but to natural stresses inherent in the community. In
1966, Wagner studied the herbaceous plants of the com-
munity. He found that at exposures higher than 45 R/d
most higher plants were dead and perennial species were
favored over annuals. In a later study, Flaccus et al.
(1974) found that at lower exposures the herbaceous
community had increased eight-fold in density. Sparrow

(1966) reviewed the uses of the Brookhaven radiation facilities and described some of the studies conducted there.

Studies in the western United States included one in the Mojave Desert and one on the shortgrass plains. In 1971, Kaaz et al. reported the installation of a ^{137}Cs source in the Mojave Desert. They investigated the early effects on the shrubs of the area and found only *Ephedra* had failed to grow vegetatively and to produce flowers. They concluded *Ephedra* was more sensitive because it had a larger ICV. In a later study, Vollmer and Bamberg (1975) observed the shrub *Krameria*. They found a decrease in density and cover and concluded that equilibrium in response to the radiation had not yet been established. Fraley and Whicker used a ^{137}Cs source to irradiate, both chronically (1973a) and seasonally (1973b), a shortgrass plains community. In the seasonal experiment, they found the community was most sensitive in the late fall. Recovery from the 30 d irradiation was similar to secondary succession except for the role played by regenerating perennials. They concluded from the chronic experiment that the shortgrass vegetation as a whole was very resistant. They also found the parameters, diversity, and coefficient of community (comparison of irradiated species to control species), were not sensitive enough adequately to measure the changes and stability of the community.

Other field studies include the irradiation by McCormick (1963) of a herbaceous "island community" growing on a granite outcrop. He found both growth stimulation and inhibition and selection for resistant species. In that same year, Kawara (1963) described a gamma field in Japan used for breeding agricultural plants. Woodwell and Oosting (1965), after chronically irradiating an old field, observed a decrease in density and plant cover. Monk (1966) found a decrease in stem elongation, root biomass, and diversity when he chronically irradiated a field of five year old long leaf pine. In 1972, Fabries et al. reported on the chronic irradiation of Mediterranean type vegetation and found the herbaceous species to be more resistant than the woody species. In the same year, Holt and Bottino (1972) irradiated a winter rye-weed community. Their findings indicated the weeds were more resistant than the rye. The respiration rates of irradiated red pines was the subject of a study in 1974. Jordan found the respiration rate increased at radiation levels of 4 to 6 rad/d and Jordan believed the increase was an indication of repair.

The studies that measured the effects of ionizing radiation on terrestrial plant communities have been reviewed by Whicker and Fraley (1974).

CHAPTER THREE   MATERIALS AND METHODS

Introduction

Three basic studies were conducted on plants of the
monocotyledonous angiosperm, *Ornithogalum virens* (Liliaceae)
(Quintanilha and Cabral, 1947). In the first study, the
plants received acute doses of gamma radiation from a
^{60}Co source. In the second study, plants were exposed
in the field to chronic gamma radiation from a ^{137}Cs
source. The last study involved plants being irradiated
by beta particles from ^{32}P as an internal emitter.

Determination of Nuclear Volume and
Interphase Chromosome Volume

To predict the radiation sensitivity of *Ornithogalum
virens*, the nuclear volumes and the interphase chromosome
volumes of the pollen grains and the root tip meristematic
cells were determined from sectioned material. These two
measurements have been used as indicators of radiation
sensitivity of plants (Sparrow, 1962).

Both pollen grains (microspores) and root tips were
killed and fixed in Navashin's fluid (Johansen, 1940).
The tissues were put through the dehydration-infiltration

sequence using tertiary butyl alcohol described by Conn et al. (1960). The tissues were embedded in rubber Paro-wax and sectioned at 12 μm. The sections were mounted on slides using Haupt's adhesive and stained with iron hematoxylin (Johansen, 1940).

In longitudinal sections of root tips, nuclei were measured that lie between mitotically dividing cells in the same column. This should insure that the measured nuclei were still diploid and capable of division. Using an ocular micrometer and an oil immersion objective (100 X), the diameters of the nuclei were measured perpendicular and parallel to the column of cells. For each nucleus, the average of these two diametric measurements was calculated and the nuclear volume (NV) was found by using the equation,

$$NV = \frac{\pi D^3}{6}$$

where D equals the average diameter (Sparrow et al., 1968). Using this volume, the interphase chromosome volume (ICV) was determined by using the equation,

$$ICV = \frac{NV}{6}$$

(Sparrow and Schwemmer, 1974). The interphase chromosome volume is one sixth of the nuclear volume because the cells of the root tip are diploid (2n = 6).

Using an ocular micrometer and a 100 X oil immersion objective, the diameter of the nuclei of the pollen grains were measured. Two measurements were taken at right angles to each other except where nuclei were not spherical. Then, major and minor axes were measured. The average diameter was found and the nuclear volume calculated using the same equation as for the root tip cells. The interphase chromosome volume was found by dividing the nuclear volume by three because the pollen grain cells are haploid (n = 3).

Acute Gamma Radiation Study

Summary

In the two experiments comprising this study, mature *O. virens* bulbs were grown and irradiated in wax-coated paper cups (15 cm high and 9 cm wide at the top) containing vermiculite. These containers and this growth medium insured minimum absorption of the gamma radiation from the ^{60}Co source by material surrounding the roots and bulbs. In the first experiment, the dose ranged from 0 to 32 krad. The second experiment included control plants and plants exposed to 32 to 96 krad.

First Experiment

Flowering plants were exposed by placing them at varying distances from the source. There were three palnts at each dose level (Table 1).

Table 1. Length of irradiation and the doses received by plant tops and bulbs at each level for the first experiment of the acute gamma radiation study (see Appendix A for determination of bulb doses).

Level Number	Irradiation Length (h)	Plant Top Dose (krad)	Bulb Dose (krad)
8	0	0	0
7	0.23	0.5	0.46
6	1.8	1.0	0.91
5	1.8	2.0	1.8
4	1.8	4.0	3.6
3	14.6	8.0	7.3
2	14.6	16	14
1	14.6	32	29

After irradiation, the plants were grown in the greenhouse for 16 weeks. During this time, they were watered with a nutrient solution (Appendix B). Pollen was collected from the plants two, six and eight days after irradiation and observations on growth and the general conditions of the plants were recorded. The measurements included the length of the flower stalk and of the raceme, number of open flowers, and the conditions of the leaves, buds, flowers, and pollen.

After the period in the greenhouse, the plants were removed from the cups. The leaves and roots were cut and then the bulbs were dried on benches in the laboratory for three weeks. The bulbs were replanted in vermiculite in 10 by 10 cm plastic pots. Over seven months, the regrowth of the roots, leaves, and flower stalks was observed. The number and length of the roots, the time until the shoot appeared and the number and length of the leaves were recorded. Other measurements included the length of the flower stalk, the time until flowering, the length of the flowering raceme and the number of flowers.

Second Experiment

The same general procedure for irradiation was used. Again, there were three plants per dose level, but these plants were not flowering (Table 2).

Table 2. Length of irradiation and the doses received by plant tops and bulbs at each level for the second experiment of the acute gamma radiation study (see Appendix A for determination of bulb doses).

Level Number	Irradiation Length (h)	Plant Top Dose (krad)	Bulb Dose (krad)
6	0	0	0
5	17.3	32	29
4	17.3	48	34
3	17.3	64	58
2	17.3	78	71
1	17.3	96	87

After irradiation, the plants were grown in the greenhouse and watered with the same nutrient solution as used in the first experiment. For nine weeks, data only were collected on the number, length and condition of the leaves because none of the plants were flowering at the time of irradiation. Then the plants were removed from the cups and the leaves and roots cut. The bulbs were dried for one month and then replanted in vermiculite in 10 by 10 cm plastic pots. Growth measurements were taken over four months. They were the same as the ones taken in the first experiment, except that there were no observations on the inflorescences because the plants did not reach flowering stage.

Chronic Gamma Radiation Field Study

Summary

Plants were grown in the field at varying distances from a ^{137}Cs source. They received a chronic dose over a period of 30 days. After irradiation, they were grown in the greenhouse for 14 weeks. During this time, root tops and pollen were collected and observations on the growth of the plants made.

Study Area

The ^{137}Cs source is located on the Central Plains Experimental Range (administrated by the Agricultural Research Service, USDA), approximately 55 km northeast of Fort Collins, Colorado. A detailed description of the site was given by Fraley (1971) and Fraley and Whicker (1973a). The site was a circular area which was divided into six sectors with the source in the center. The plants were grown close to the source in sector two which had been irradiated continuously since April 1969. Control plants were grown in an area between sectors which was shielded from the source (Figure 1).

Experimental Design

Plants were first grown in the greenhouse until they were mature with bulbs at least 3 cm in diameter. Then the plants were transplanted into greenhouse soil (Appendix C) in 18 cm clay pots. The centers of the bulbs were approximately 8 cm below the rims of the pots while the surface of the soil was level with the rims. There were five plants per level of radiation and ten control plants. Each level had two flowering plants, two plants with flower stalks and one plant with no flower stalk, except the control level where plant numbers were doubled.
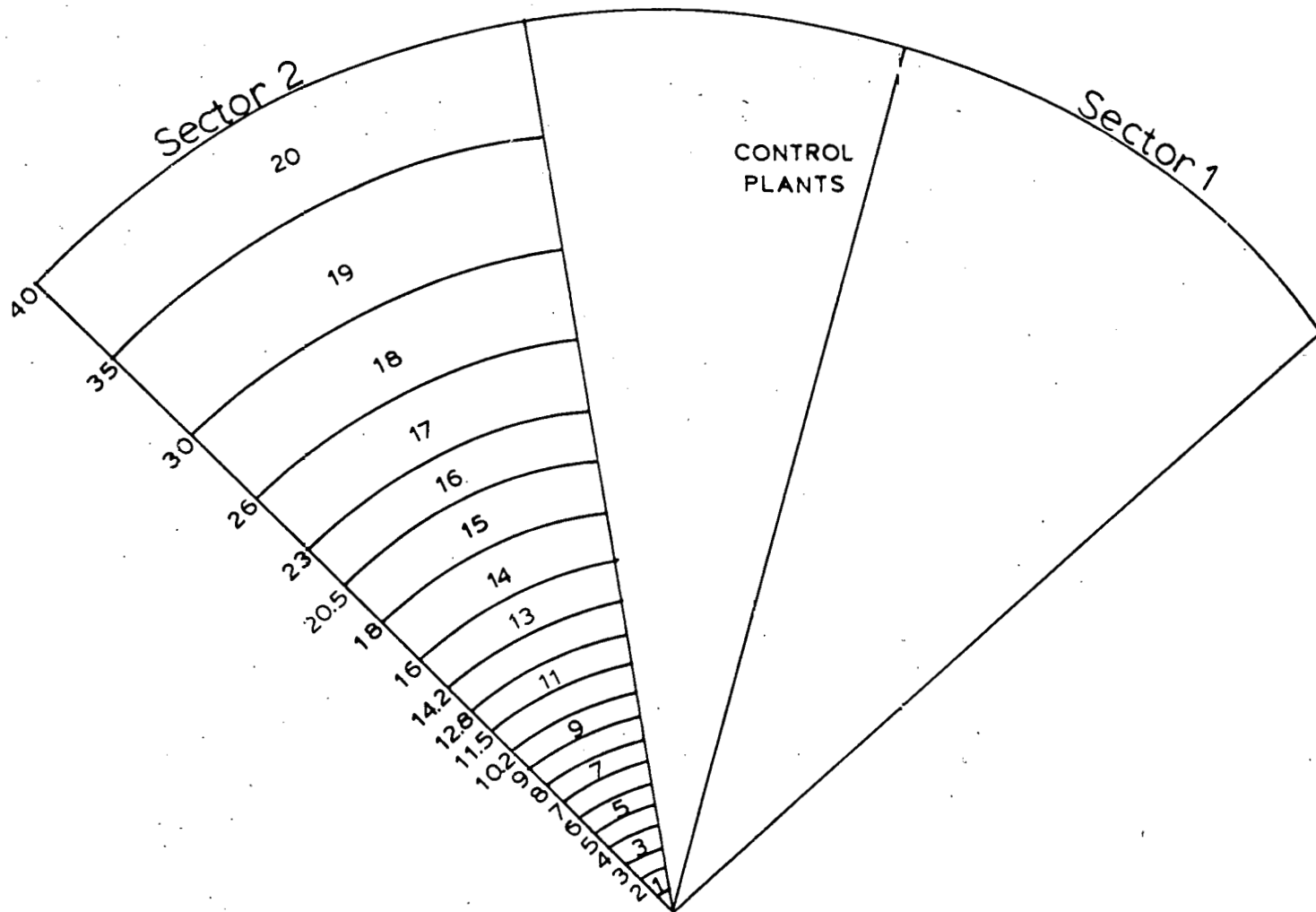
Figure 1. Sectors one and two of the study area of the chronic gamma radiation study, showing the position of the control plants (level 6) in the shielded area between sectors (inside numbers refer to macroplots, outside numbers refer to distance from source, areas are not drawn to scale).

On 26 May 1978 the plants (including pots) were placed in the ground of the study area. For each level of five plants, a trench was dug at the back edge of the macroplot, 15 cm from the side edge of the sector and tangential to the source (Figure 2). The pots were placed in a line in the trench with rims touching. The trench was filled so that the original soil of the study area surrounded the pots and was level with the rims.

The control plants (level six) were placed close to sector one in the shielded area between sectors one and two. Sector one is the control area that has not been irradiated except for scattered radiation. The plants were placed in the ground in two lines with the rims of the two groups of pots touching.

Dose rates were calculated from previously determined rates by correcting for decay of the source (Fraley, 1971). The rates were not the same as the values for the back edge of the macroplots found in Figure 2 because the plants were approximately 9 cm closer to the source than the back edge. The total dose received by the tops of the plants and the bulbs (Table 3) were determined by using the dose rate and a time of 30 days (down time of source not included but was minimal).
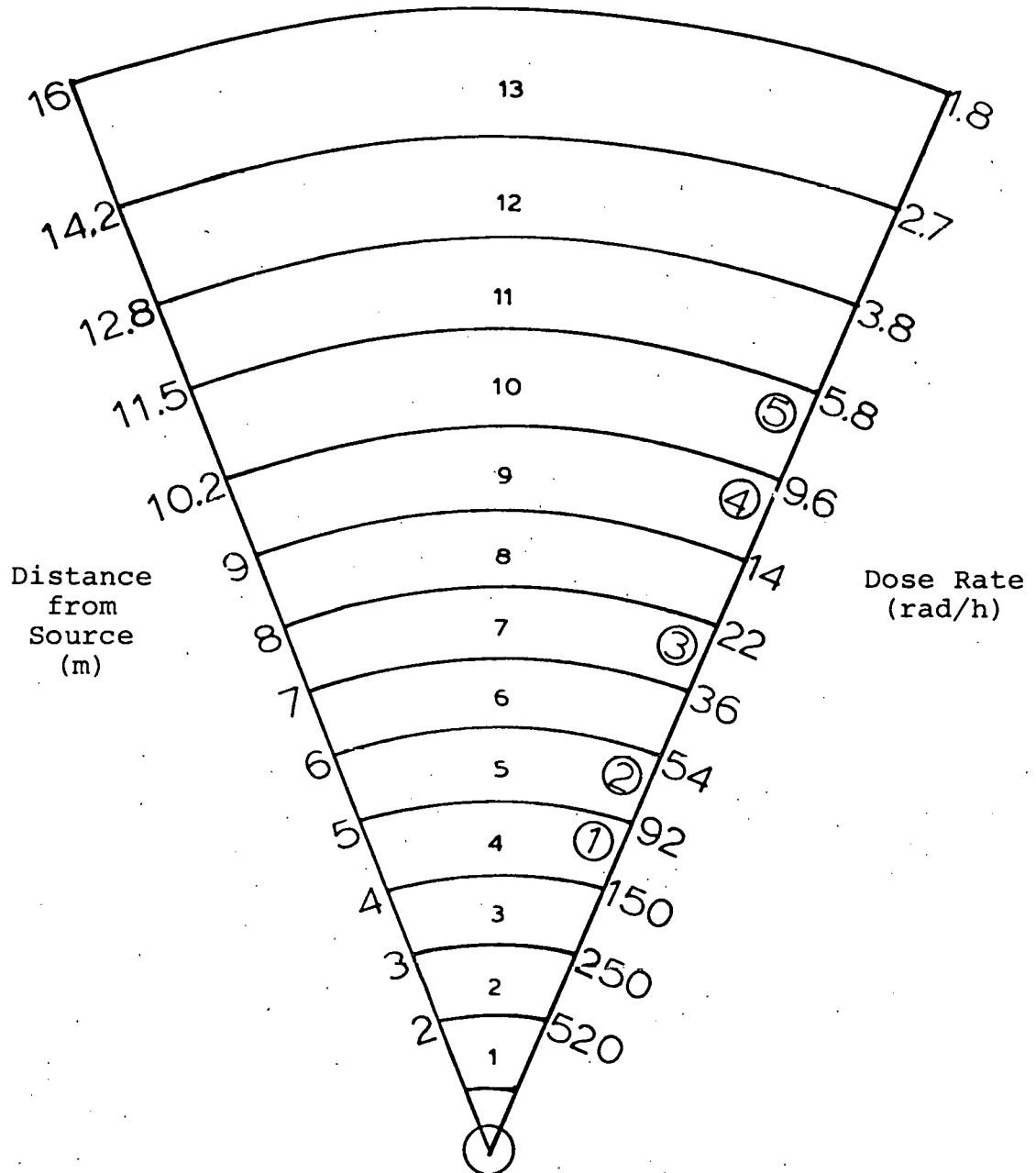
Figure 2. Sector two of the study area of the chronic gamma radiation study, showing the positions (circled numbers) of the levels of the irradiated plants (dose rates for plants differed from rates shown - see text for explanation).

Table 3.   The macroplot number, the dose rate, and total
dose for the tops of the plants and bulbs
for each level in the chronic gamma radiation
study (see Appendix D for determination of bulb
dose).

Level Number	Macroplot Number	Dose Rate (krad/d)		Total Dose (krad)	
		Plant Top	Bulb	Plant Top	Bulb
6	19	2.4×10^{-4}	9.6×10^{-4}	7.2×10^{-3}	2.9×10^{-3}
5	10	0.14	7.1×10^{-3}	4.2	0.21
4	9	0.24	1.4×10^{-2}	7.0	0.41
3	7	0.55	0.05	16	1.5
2	5	1.3	0.19	40	5.6
1	4	2.3	0.42	70	12

Several procedures were followed to insure that the
major stress put on the plants was from the radiation
source. The plants were left in their pots so that
transplanting shock did not occur. Also, they were not
transplanted into the sandy silt soil of the study area,
but were left in their original soil. The plants were
hand watered at the rate of eleven liters per five plants
every two days.

Observations

During the irradiation, the inflorescences of the
plants were examined periodically in reference to the
lengths of the flower stalks and flowering racemes and
the condition of the leaves, flower stalks, and flowers.
Leaf characteristics such as the length and number were
not measured because of some leaf damage by rabbits.

Greenhouse Study

When the 30-day period was over, the potted plants were dug up and placed in the greenhouse. Root tips were collected from two of the five plants of each level and then the plants were repotted in the same pots and soil. The observations described above were continued. In addition, observations on the condition of the anthers and pollen and the appearance of new leaves were recorded, and pollen was collected periodically.

After 14 weeks in the greenhouse, the plants were removed from the pots. The leaves and roots were cut and root tips again were collected. The bulbs were dried for three weeks.

After drying, the bulbs were planted in vermiculite in 10 cm round plastic pots. Growth in the greenhouse continued for seven months. Observations on the plants included the number and length of the roots, the number and length of the leaves, the appearance and length of the flower stalks, the length of the flowering raceme and the number and condition of the flowers.

Internal Beta Radiation Study

Summary

Mature plants were placed in a solution of ^{32}P and allowed to absorb it. After 24 hours (enough time for the mitotic cycle to occur - personal communication from David Brown) they were removed from the solution. The plants were sacrificed over time and the roots and pollen were collected for squashing and staining with aceto-orcein.

To determine the amount of ^{32}P present, samples of the root tips, buds, anthers, and pollen were collected. The samples were counted by the Cerenkov method in a liquid scintillation counter (Ross and Rasmussen, 1974).

^{32}P Solution

The original 5 mCi solution was ordered from New England Nuclear Corporation. The solution was 1 ml of orthophosphoric acid in 0.02 M hydrochloric acid. According to the manufacturer, it was carrier free and had greater than 99% radionuclidic purity. To help alleviate the problem of plating (ions of the solution attaching to the walls of the container), ten times as much nonradioactive phosphoric acid was added to the solution as a carrier. Using secondary standardization (Overman and Clark, 1960), the approximate activity of the solution was determined. For use with the plants,

this solution was diluted to an activity concentration of 0.2 μCi/ml.

Experimental Design

For the ^{32}P experiment, mature flowering *O. virens* plants, grown in vermiculite in 15 by 9 cm waxed paper cups, were used. Ten plants were used as controls and twenty plants were irradiated. For the controls, 250 ml of a nonradioactive phosphoric acid solution of the same concentration as the solution used for incorporation of ^{32}P was put in each of ten 400 ml beakers (subsequently called control beakers). Two hundred fifty milliliters of the 0.2 μCi/ml solution was put into each of twenty 400 ml beakers for uptake by plants (subsequently called uptake beakers). The same solution was put in three 400 ml beakers used to monitor plating (subsequently called plating beakers). Aliquots (1.0 ml) from one control beaker, two uptake beakers, and the three plating beakers were taken and put in standard 20 ml liquid scintillation vials. Sampling of the control beaker, the two uptake beakers and the three plating beakers was done to monitor background, uptake and plating, and plating alone, respectively.

The plants were removed from the paper cups, and the vermiculite was rinsed from the roots. One plant each was placed in the control and uptake beakers so that the bulbs, and roots were immersed in the solution,

but the leaves were above the surface of the liquid.
The plants were left in the beakers in the lighted
and ventilated hood at 22°C.

After 24 hours, the plants were removed from the
beakers. The roots were rinsed in 400 ml of distilled
water in 600 ml beakers (five plants per beaker). Sub-
sequently, the plants were repotted in vermiculite and
paper cups. Then the plants were grown in the labora-
tory under 40 watt fluorescent lights (Sylvania Gro-Lux).

To determine the amount of ^{32}P rinsed from the roots,
1.0 ml aliquots were taken from each rinse beaker. Also,
two plant beakers and the three plating beakers were
sampled. All aliquots were placed in liquid scintilla-
tion vials.

Plant Sacrifice

On the fourth day after removing the plants from
the ^{32}P solution, pollen was collected from several plants.
On the fifth day and every day after that for ten days,
two ^{32}P plants and one control plant were sacrificed,
each in the same way. First, the raceme was cut and
saved. Then the plant was removed from the cup and the
roots rinsed in 600 ml of distilled water in a 1000 ml
beaker (one beaker per two ^{32}P plants). The roots were
cut and saved and the rest of the plant was discarded.
To determine the amount of ^{32}P rinsed from the roots, the
rinse solution was sampled on the eighth and thirteenth days.

The tips of half the roots were collected for squashing while the rest of the roots were used for Cerenkov counting. In reference to counting, the apical one to two millimeters of half the roots from each plant were cut off and placed in a preweighed liquid scintillation vial, i.e., one vial per plant. Then the vials were reweighed and prepared for counting.

Every day the pollen from the saved racemes was collected for squashes. Also, to insure a large enough sample, pollen was collected from nonsacrificed plants.

On the seventh and twelfth day after removing the plants from the ^{32}P solution, anthers and pollen were collected for Cerenkov counting. For each of five flowers, the anthers were removed and put in one preweighed vial. For five additional flowers, the pollen was put in one preweighed vial and the vials reweighed.

On the fifth, ninth, and fourteenth day, buds were collected from one of the radioactive racemes. On these three days, individual buds from the raceme, starting with the largest bud, were removed and placed in preweighed vials until the buds were too small to handle. Then, the rest of the raceme (the tip) was placed in a preweighed vial. Each vial, containing one bud or the tip, was reweighed and prepared for counting.

Preparation of the Samples for Cerenkov Counting

The vials containing tissue were treated to dissolve the tissue (Fric and Palovcikova, 1975). To the vials containing root tips, pollen or anthers, 0.5 ml concentrated H_2SO_4 was added. To the vials containing the buds, 1.0 ml was added. Then the vials were placed in a 60°C water bath for two to three hours. After cooling, 0.5 ml of 30% H_2O was added to each vial to decolorize the solution. After letting them stand at room temperature for one to two days (until clear), 10 ml of distilled water was added to each vial. The samples that contained aliquots and no tissue did not need additional treatment (Ross, 1976) and 10 ml of distilled water was added to them. All samples were counted by the Cerenkov method of counting ^{32}P.

Cerenkov Counting of ^{32}P

In this study, the Cerenkov method of counting the amount of beta radition (^{32}P) in samples was used. Cerenkov radiation, as photons, is produced when beta particles (electrons) are emitted and move faster than the speed of light through the aqueous sample (Gelsema et al., 1975). The photons are detected by the photo-multiplier tube of the counter (Wang et al., 1975). One of the advantages of this method is that it can be used with a standard liquid scintillation counter (Ross and Rasmussen, 1974; Ashcroft, 1969).

Using the Cerenkov method, samples were counted in a Mark II Liquid Scintillation Counter (Model 6847). To reduce the background noise level (Wang et al., 1975), the samples were cooled and counted at 3°C. The optimum spectrum of ^{32}P for the channels ratio method (Fric and Palovcikova, 1975) was greatest when the least amount of attenuation (maximum gain) and range were used (Figure 3). Though the samples were decolorized, the channels ratio method indicated some color quenching was present.

To correct for the color quenching, a standard quench curve was prepared. First, samples containing a known activity of ^{32}P and water were counted. The settings necessary for the channels ratio method of correcting for quench were determined using these unquenched samples. Then, samples of root tips and buds were prepared in the same manner as the samples from the ^{32}P experiment. These samples were spiked with a known amount of ^{32}P. An initial count was made using the settings previously determined. Then, increasing amounts of a quenching agent, made from dissolved plant material, were added to each sample. After each addition, the samples were counted. The count yield and the channels ratio were determined and a standard curve developed (Figure 4).

To find the amount of quench for the samples of the ^{32}P experiment, the channels ratio of each sample was
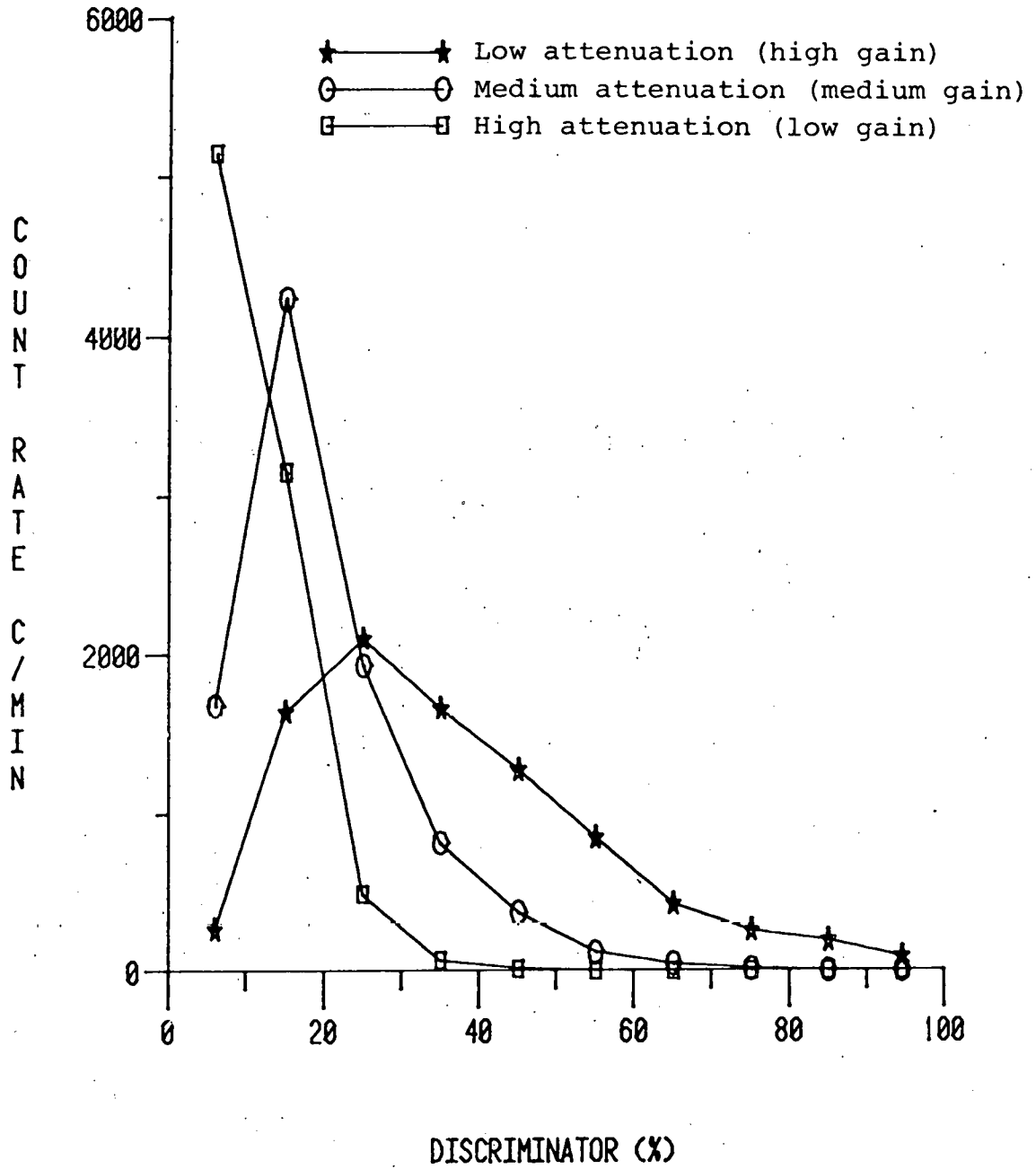
Figure 3.   The counting spectrum of ^{32}P at three levels of attenuation (gain).
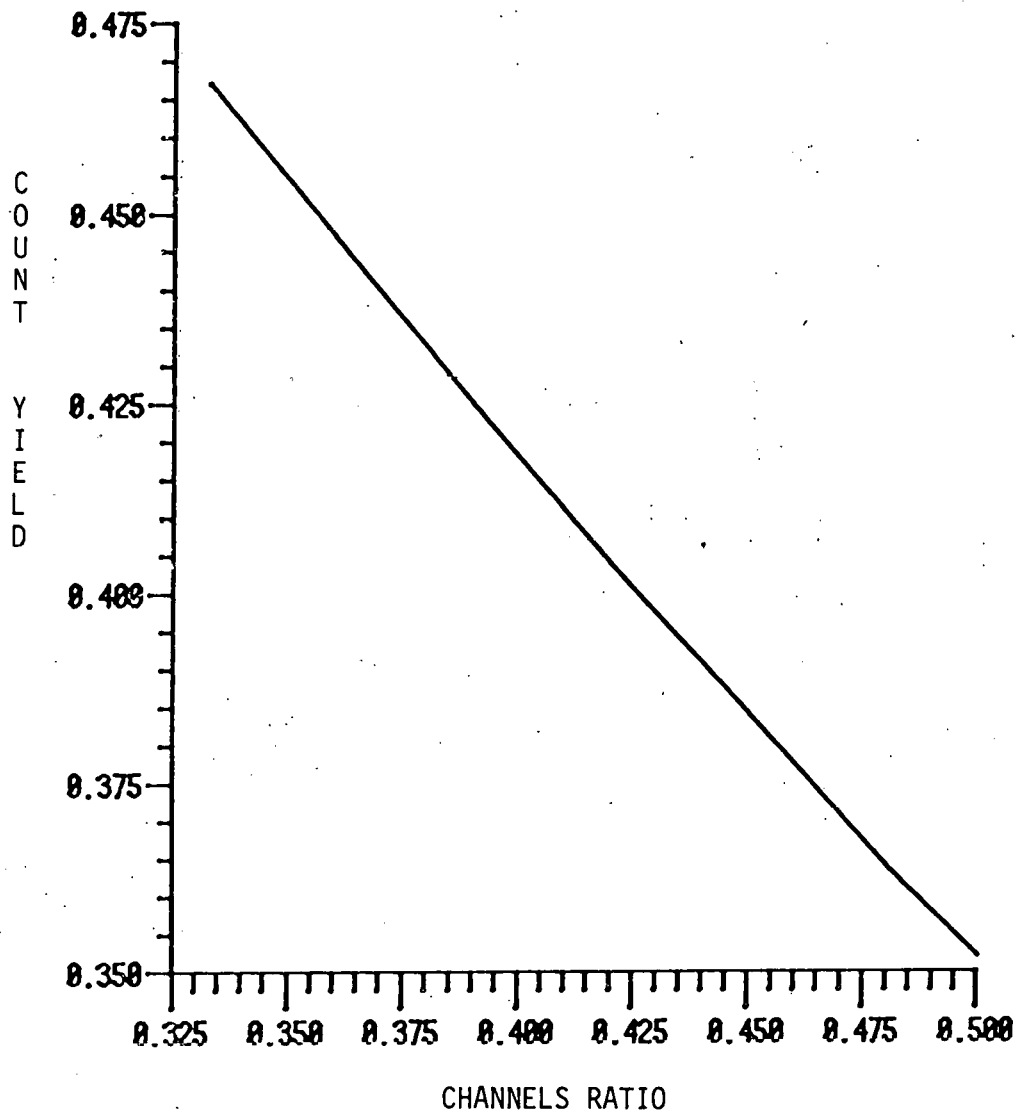
Figure 4. The standard quench curve used to predict the count yield of the samples from the internal beta radiation study based on the amount of quench found by the channels ratio method.

calculated. Then, using the standard curve, the count
yield was determined. By using the count yield (Y)
and the count rate (R) in the equation,

$$A = \frac{R}{Y}$$

the activities (A) of the samples were determined
(Appendix E).

Pollen and Root Tip Collection and Squashes

Pollen was collected by removing the anthers from
each flower. To avoid mixing the pollen from different
flowers, the tweezers were flamed between flowers. The
anthers were placed in 1.5 ml plastic tubes with caps
(all anthers of one flower per tube). The tubes had
been filled with 1.0 ml of a pollen growth medium
(Brewbaker and Kwack, 1964; see Appendix F for formula).
After centrifuging at 700 G for seven minutes to separate
the pollen from the anthers, the tubes were left at room
temperature for 0.5 to 1 hour for the germination and
growth of the pollen. The medium and the anthers were
removed and 1.0 ml of 3:1 acetic ethanol was added as a
fixative (Darlington and La Cour, 1976). The tubes were
refrigerated until the squashes were made.

To make the pollen squashes, the tubes containing
the germinated pollen were centrifuged at 700 G three

to four minutes to form a pellet of the pollen at the bottom of the tube. The fixative was removed and the pollen was placed in 3 µl of 45% acetic acid on a clean slide. Using dissecting needles, the pollen was spread on the slide as evenly as possible before covering the cells with a siliconized cover slip. The slide was heated gently over an alcohol lamp. The cells were thumb squashed, the slide frozen on dry ice (Conger and Fairchild, 1953). A razor blade was used to pry off the cover slip and the slide was air dried. One drop of 2% aceto-orcein was placed on the cells to stain the chromonosomes (Galigher and Kozloff, 1971). After placing another cover slip over the cells, the slide again was heated gently over an alcohol lamp before rinsing the cover slip from the slide with 100% ethanol. After air drying, a cover slip was mounted over the squashes with Permount. The slides were observed with a Leitz Orthoplan microscope using an oil immersion objective, and the chromosome aberrations were tabulated.

The root tips were collected by cutting off the apical 1 to 2 cm of the root. The root tips from each plant were then placed in 1.5 ml of 3:1 acetic ethanol in a 4 ml glass vial with cap. The vials were refrigerated until the squashes were made. To make the squashes, each tip was placed in a drop of 45% acetic acid on a clean slide. The root cap and the nonmeristemic cells were dissected out and removed from the

slide before covering with a siliconized cover slip.
Subsequent treatment and observation was performed as
described above for the pollen.

CHAPTER FOUR   RESULTS AND DISCUSSION

Introduction

Three biological effects of acute and chronic gamma radiation and internal beta radiation on *Ornithogalum virens* were studied. The first of these effects was lethality, measured by determining the dose at which 50% of the plants died (LD_{50}) and the lowest dose at which 100% of the plants died (LD_{100}). The second effect involved changes in the growth of the roots, leaves, and inflorescences. The third effect was chromosome damage observed in pollen tubes and root tips. All three effects were investigated in the acute and chronic gamma radiation studies, but only chromosome aberrations were examined in the internal beta radiation study. In addition, nuclear and interphase chromosome volumes were determined for *O. virens* pollen and root tips to predict radiation sensitivity.

Nuclear Volume and Interphase Chromosome Volume

The nuclear (NV) and the interphase chromosome volume (ICV) have been used as indicators of radiosensitivity in many studies (Van't Hof and Sparrow, 1965; Ichikawa and Sparrow, 1966). These volumes have been found to be

correlated with LD_{50}, growth inhibition, and other measures of radiation damage (Yamakawa and Sparrow, 1966; Baetcke et al., 1967). In this study, the NV and ICV of *O. virens* cells were determined to predict its radiation sensitivity.

NV and ICV values were determined for the root tip cells and the pollen grains (microspores). The NV's of the root cells and pollen were calculated to be 120 $\pm$ 43 μm^3 (one standard deviation) and 78 $\pm$ 17 μm^3, respectively, based on nuclear diameters (see Materials and Methods). The ICV's were 20 $\pm$ 7.2 μm^3 (roots) and 26 $\pm$ 5.7 μm^3 (pollen). Since the ICV for the pollen is not significantly larger than the ICV for roots, the two should have approximately the same radiation sensitivity. In comparison with the ICV's for other herbaceous species, *O. virens* was predicted to be more radiosensitive than the majority of the herbaceous plants so far investigated (Sparrow and Schwemmer, 1974).

The nuclear volume can be used to predict the daily exposure necessary to produce severe growth inhibition. Using a nuclear volume of 120 μm^3 in the equation, log y = 4.07 - 0.73 log x where y equals NV and x equals daily exposure (Sparrow, 1962), the predicted exposure rate necessary for severe growth inhibition of *O. virens* would be 533 R/d. This exposure rate was compared to the dose rate used in the chronic gamma radiation study because 1 R is approximately equal to 1 rad for gamma

radiation (Hall, 1973). Though the bulb dose rates in this study varied from 7.1 to 420 rad/d, severe growth inhibition was not observed (see section on growth observations). However, irradiation only lasted 30 days, and severe growth inhibition may have been seen if the irradiation period was longer.

The ICV can be used to predict the LD_{50} and the acute exposure that would be lethal. Using the ICV of 20 μm^3 in the equation $\log_{10} y = 1.69422 - (0.93025) \log_{10} x$ where y equals acute lethal exposure and x equals ICV (Sparrow et al., 1963), the LD_{100} was predicted to be 3.0 kR. In this study, the acute lethal dose was found to be less than 3.6 krad (see next section). Thus, there is agreement between the results of the acute gamma radiation experiment and the predicted result. Again, if an ICV of 20 μm^3 is used, the LD_{50} was predicted to be approximately 4 kR based on Figure 2 of Sparrow and Schwemmer (1974) and presumably is higher than the LD_{100} found because of a refinement in the prediction relationships. This value was roughly in agreement with the estimated LD_{50} of 0.91 to 1.8 krad found in this study (see next section).

LD_{50} Studies

In general, LD_{50} studies are performed by exposing organisms to different doses of radiation, tabulating the deaths at each dose and determining at which dose 50% of the organisms were killed (Hall, 1973). This procedure was used in attempts to determine the LD_{50}'s of *O. virens* for acute and chronic gamma radiation.

Acute Gamma Radiation Study

In the first acute gamma radiation experiment, three plants at each of eight dose levels were irradiated. After four months, the tops of only five plants, each at different dose levels, had died. Because of these ambiguous results, a second experiment using higher doses was conducted, but again, the results were inconclusive. In this case, the tops of a few plants died within two months, but again at no one dose level did the tops of all three plants die.

To remedy the problem, a different approach was attempted. The irradiated bulbs from both previous experiments were dried and replanted. The results from this method were more interpretable. Table 4 shows the condition of the plants from the first experiment seven months after replanting and the condition of the plants from the second experiment four months after replanting. The dried bulbs exposed to 7.3 krad or more did not sprout. After seven months, the three

Table 4. The condition of the plants in the first and second experiments of the acute gamma radiation study, seven months and four months after replanting, respectively (see Appendix A for determination of bulb doses).

Bulb Dose (krad)	Experiment Number	Condition
0	1	Variable leaf and flower stalk growth and abundant root growth
0	2	Variable leaf growth and abundant root growth
0.46	1	Variable leaf growth and abundant root growth
0.91	1	Variable leaf and flower stalk growth and moderate root growth
1.8	1	Inhibited and isodiametric leaf growth and modest root growth
3.6	1	Bulbs partially rotted, little root growth
7.3	1	Bulbs rotted
14	1	Bulbs rotted
29	1	Bulbs rotted
29	2	Bulbs rotted
34	2	Bulbs rotted
58	2	Bulbs rotted
71	2	Bulbs rotted
87	2	Bulbs rotted

dried bulbs exposed to 3.6 krad produced a total of only four roots. It was highly unlikely that these plants would have survived. Two of the bulbs exposed to 1.8 krad produced an average of 17 roots. Also at this level, though no new leaves were produced, there was some growth of the leaves that were cut when the bulbs were dried (see section on growth observations). Even with this amount of growth, these plants probably would not have survived. The control bulbs and the bulbs exposed to 0.46 and 0.91 krad produced roots, leaves, and flower stalks. Based on this growth information it would appear that the LD_{50} for *O. virens* was between 0.91 and 1.8 krad and the LD_{100} was less than 3.6 krad. These LD_{50} and LD_{100} values indicated that *O. virens* was more radiosensitive than the major of the herbaceous plant species so far investigated (Sparrow and Schwemmer, 1974).

Though some researchers have stressed that long observation times may be necessary to find the LD_{50} of some plant species (Sparrow et al., 1973), the drying procedure used here appears to be a better approach for *O. virens* and possibly any plant with a bulb. In this regard, some of the plants whose leaves died after irradiation were actually still alive while the tops of some nonirradiated *O. virens* plants died periodically (not necessarily in response to season) only to regrow later. However, if after irradiation and drying, roots and leaves are produced, this indicates the meristems are still alive and the cells capable

of successful division. In addition, because leaf
growth is somewhat variable for *O. virens* plants, root
growth may be the more adequate measure of survival
because it is more regular.

Chronic Gamma Radiation Field Study

In this study, thirty-five plants were exposed to
varying doses of chronic gamma radiation. After 30 days
of irradiation in the field, none of these plants had
died. Therefore, the plants were grown in the green-
house for the subsequent 14 weeks, but still after this
time all the plants were living. At this point, the
bulbs were dried for three weeks. Seven months after
replanting the dried bulbs, bulbs at all dose levels
had produced roots, leaves, and flowers. Presumably,
the soil in and around the buried pots had shielded the
bulbs sufficiently to prevent lethal irradiation (Appen-
dix D). Because of this the LD_{50} could not be deter-
mined.

Growth Observations

Acute Gamma Radiation Study

In both experiments of this study, growth inhibition
of irradiated plants was observed before and after drying
the bulbs. During the observation period between

irradiation and drying, the plants of the first experiment were measured for leaf and inflorescence growth. However, for this same period, the plants of the second experiment were not flowering and only leaf growth was measured.

In the first experiment, the number of leaves was used as an indicator of the changes in growth due to radiation damage. Other researchers (Gomez-Campo and Delgado, 1964) have used this measurement in the past. However, in the present study, the variation in the number of leaves of the plants at each dose level was so large that the different levels could not be compared.

The data on the changes in the growth of the flower stalks and the inflorescences also was inconclusive except for the changes in the lengths of the racemes. *O. virens* plants have indeterminate racemes, which means new buds are produced at the top of the inflorescence as the raceme grows. However, if the apical meristem dies the length of the raceme that consists of unopened flower buds becomes shorter over time as the buds open. The racemes of all irradiated plants were killed while those of the control plants continued to grow. Additionally, at higher doses it appeared that the diameters of the flowers were smaller and the only flowers with pollen were on plants that had received 4 krad or less. Eight weeks after irradiation, only plants that had received less than 3 krad had inflorescences. Similar observations

have been made in the past for other plants (Brittingham, 1931). Although these observations indicated growth inhibition of the acutely irradiated plants, the observations were difficult to quantify.

The plants of the second experiment also showed variable leaf growth. During the two month observation period, the number of leaves decreased on plants at all dose levels and the controls. The control plants did show an increase in the length of the leaves for the first five weeks after the irradiation. However, after the fifth week, the total length of the leaves decreased. All levels of irradiated plants showed decreases in the total length of the leaves over the two-month period due to death of cells at the distal ends with some compensating growth proximally. However, after two months the reduction in the length of leaves for the control plants was less than the reduction in the length of leaves for the irradiated plants. Although not statistically significant, the reduction perhaps indicated growth inhibition of the irradiated plants.

After drying and replanting, the bulbs of both experiments were observed for root, leaf, and flower stalk growth. The bulbs that received a dose of 7.8 krad or more did not growth roots or leaves. After seven months, the bulbs exposed to 3.6 krad produced normal numbers of roots. As seen in Table 4, the control bulbs of the first and second experiments had

produced abundant roots at the end of seven and four months of growth, respectively. The bulbs receiving doses of 0.46 and 0.91 krad also produced abundant roots after seven months. Two of the bulbs at the 1.8 krad level produced roots, but the third did not, and this may have indicated growth inhibition or even mortality.

Normal leaves were produced only by bulbs at dose levels less than 1.8 krad. However, at 1.8 krad there was some growth of the leaves that were cut before drying. These leaves never exceeded 1.5 cm in length, but grew to be 1.0 cm thick, whereas normal leaves are approximately 1 to 2 mm thick. This phenomenon occurred in all three plants at the 1.8 krad dose level and was probably only due to abnormal (isodiametric) cell enlargement without cell division (probable death of the meristem). A similar phenomenon was observed by Stein and Sparrow (1963) in *Kalanchoe*.

In the second experiment, only one of the control plants had produced leaves within four months after replanting the dried bulbs. However, this was consistent with the control plants of the first experiment. In that experiment, for two of the control plants, leaves were not observed until seventeen weeks after replanting. None of the control plants of the second experiment produced flower stalks within the four months. Again, this was consistent with the first experiment because flower stalks were not observed until six and a half months after replanting.

The three control bulbs and two of the bulbs that had been exposed to 0.91 krad in the first experiment grew inflorescences. One plant from each of these two groups had begun anthesis by the end of seven months. Abnormalities such as fused flower parts and abnormal pollen were not observed in these two plants. However, the inflorescence of the other irradiated plant was abnormal because there were two racemes on one stalk.

In the two experiments of the acute gamma radiation study, death, growth inhibition and abnormal growth were observed. The changes in the inflorescences seemed to be a more adequate measure of radiation damage than the changes in the leaves.

Chronic Gamma Radiation Field Study

Observations on the growth of inflorescences and leaves were made during the irradiation period in the field, during the postirradiation period in the greenhouse, and during the regrowth period in the greenhouse after drying the bulbs. Root growth from the repotted dried bulbs also was investigated.

The conditions in the field had some effects on the growth of the plants, especially grazing by rabbits in the study area. Though the rabbits damaged both leaves and inflorescences, they did not appear seriously to harm the plants. After removal to the greenhouse, the plants at all dose levels produced new leaves and

flower stalks. Figure 5 shows the total number of leaves produced 6, 12, and 19 weeks after irradiation began. Though the differences in leaf growth are not statistically significant, there appears to be some evidence of growth inhibition of the plants receiving higher doses.

Although abnormalities such as too few or too many petals, stamens or anthers and the fusion of petals, stamens and anthers were observed in the flowers of some irradiated and control plants from the time of irradiation until the bulbs were dried, there was no interpretable pattern in the occurrence of the abnormal flowers. However, one possible indication of growth inhibition was that by the nineteenth week after the start of irradiation, the only two growing racemes were on control plants.

After drying and replanting the bulbs, observations on the growth of the roots, leaves, and flower stalks were made. Root production on all bulbs occurred within two weeks after replanting with no indication of inhibition. After seven months, all plants had produced abundant roots. The plants at all dose levels produced leaves with no indication of growth inhibition. Four of the control plants and one plant from the 7.0 krad dose level had flower stalks within six months after replanting. However, three weeks later, there was an approximate average of one flower stalk per plant for
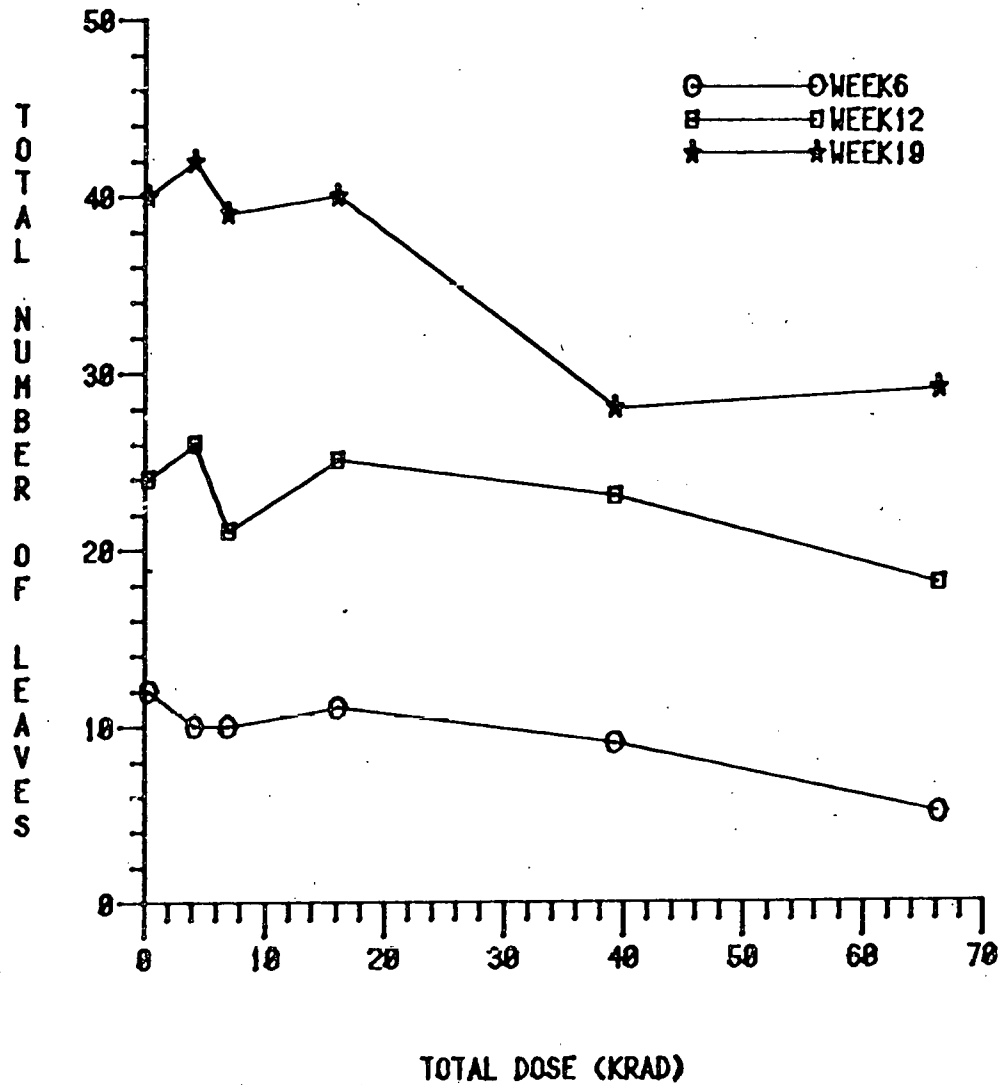
Figure 5. The total number of leaves produced by the plants at each dose level of the chronic gamma radiation study, six, twelve, and nineteen weeks after the initiation of irradiation.

all dose levels. The abnormal flowers seen before drying

were not observed afterwards. The flower and pollen of

the plants from all dose levels appeared normal. Even

so, the retardation of inflorescence growth may indicate

some inhibition due to irradiation.

Chromosome Aberrations in Pollen and Root Tips

Types of Chromosome Aberrations

In all three studies, chromosome aberrations were

examined at all stages of mitosis. The types of

aberrations scored were the same for both root tip

and pollen cells and included gaps, rough surfaces,

bridges, fragments, abnormal connectives and pulverized

chromosomes (extreme fragmentation). In addition to

these types of aberrations, the chromosomes observed in

some cells were too clumped to distinguish whether they

were damaged or not.

The haploid cells of pollen tubes and diploid

cells of root tips have three and six chromosomes,

respectively. Figure 6a shows an example of the non-

irradiated chromosomes of a pollen cell and Figure 6b

shows an example of the nonirradiated chromosomes of a

root tip cell.

In some irradiated cells, gaps were observed

(Figure 6c). Gaps have been defined by Revell (1966)

as short achromatic regions that do not yield fragments.

Figure 6. Photographs of the types of chromosome
            aberrations observed in these studies
            (magnified approximately 2000 X).

a. Nonirradiated chromosomes of a pollen cell (internal
    beta radiation study, control level).

b. Nonirradiated chromosomes of a root tip cell (chronic
    gamma radiation study, control level).

c. Chromosomes with gaps (acute gamma radiation study,
    1.0 krad).

d. Chromosomes with rough surfaces (chronic gamma
    radiation study, 40 krad).

e. Chromosome bridge (acute gamma radiation study,
    1.0 krad).

f. Chromosome fragment (acute gamma radiation study,
    0.5 krad).

g. Pulverized chromosomes (acute gamma radiation study,
    32 krad).

h. Chromatin connective (arrow) between nonirradiated
    chromosomes (acute gamma radiation study, control
    level).

i. Abnormal chromatin connectives between irradiated
    chromosomes (chronic gamma radiation study, 70 krad).

52
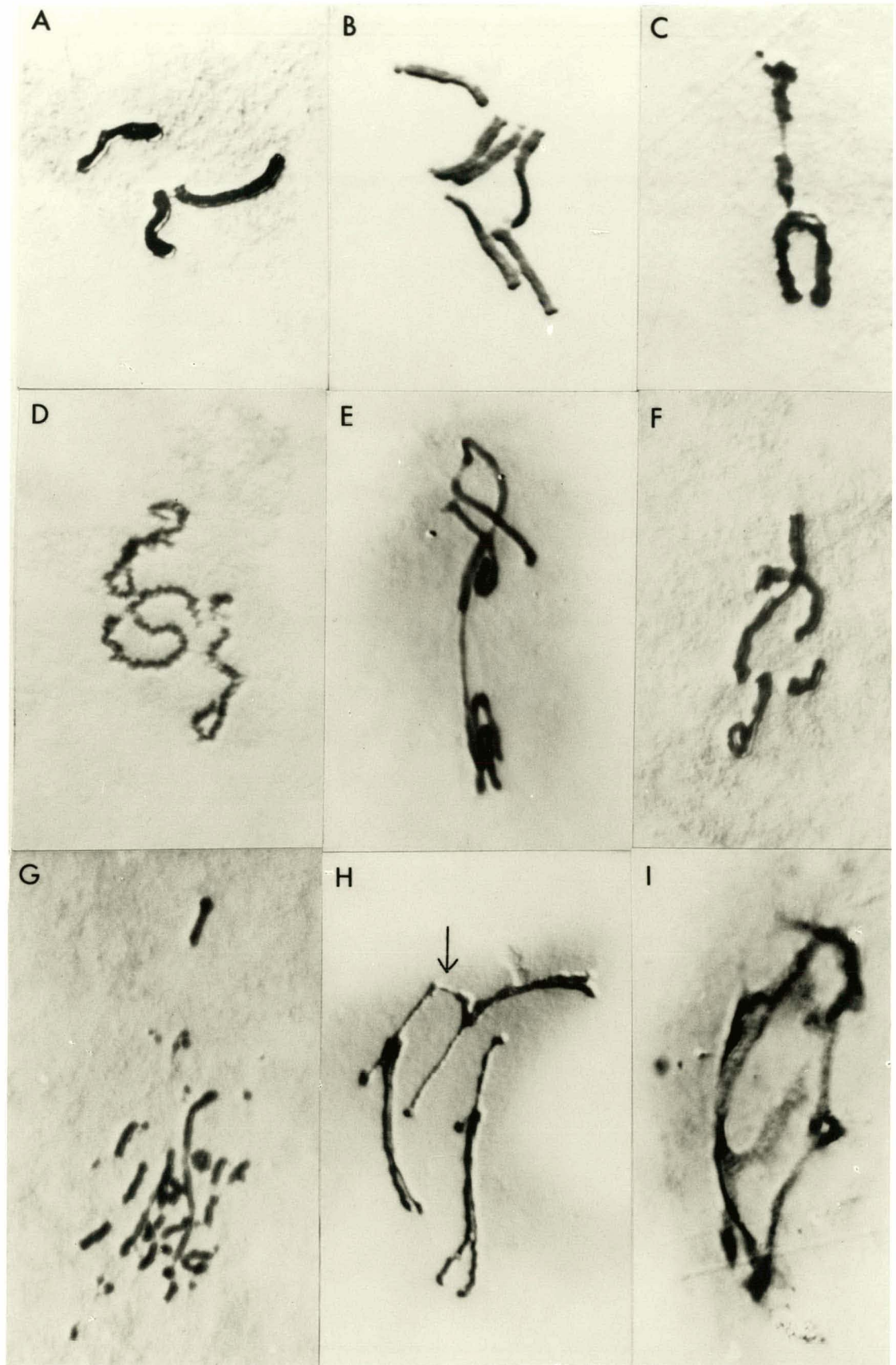
Brewen (1964) called them achromatic lesions. Sax (1940) believed they are due to partial breakage or imperfect reunion of a fragment.

In rare cases, chromosomes with rough surfaces were observed (Figure 6d). Although these chromosomes tended to occur in cells exposed to higher doses of radiation, the nature of the damage is unclear.

Historically, bridges frequently have been scored as a type of chromosome aberration (Sax and Brumfield, 1943; Wolff and Luippold, 1957). After a dicentric chromosome is formed following the breakage and reunion of chromatids, a bridge occurs when the chromatids separate at anaphase (Figure 6e). These bridges can occur with or without fragments (see below). Sax (1973) found that more than half the bridges did not have fragments. In 1965, Conger found anaphase bridges underestimate radiation damage. However, in the present study, since bridges were scored along with other aberrations, it is believed that most of the damage was assessed.

Another type of aberration was fragments. These are acentric pieces which have been produced by breaks in the chromosomes. Although most cells with fragments had either one or two (Figure 6f), in some cases the number of fragments was very large (Figure 6g). These cells were scored as having pulverized chromosomes, indicating more damage than a few fragments.

In nonirradiated *O. virens* plants, chromatin connections between chromosomes can be observed in some cells. The frequency of connectives in the pollen grains of the control plants was 6.9% ± 2.3% (one standard deviation). This frequency agrees closely with the value of 7.1% found by Godin and Stack (1976) for mitotic cells of *O. virens* plants. Usually, when connectives are observed, there are only one or two per cell (Figure 6h). However, in some cells from irradiated plants, ten times this number were seen (Figure 6i). Such cells were scored as having abnormal connectives.

Because the number of cells with clumped chromosomes increased with the dose of acute gamma radiation (Figure 7), it seems likely clumped chromosomes represent a final class of radiation damage.

Acute Gamma Radiation Study

In the first experiment of the acute gamma radiation study, all of the previously described chromosome aberrations were observed in dividing generative cells in pollen tubes. Though a poor relationship between the percent of pollen grains with any form of aberration (total aberrations) and dose was found for pollen collected six days after irradiation, a linear relationship was found for pollen collected eight days after irradiation (Figure 8). The relationship was
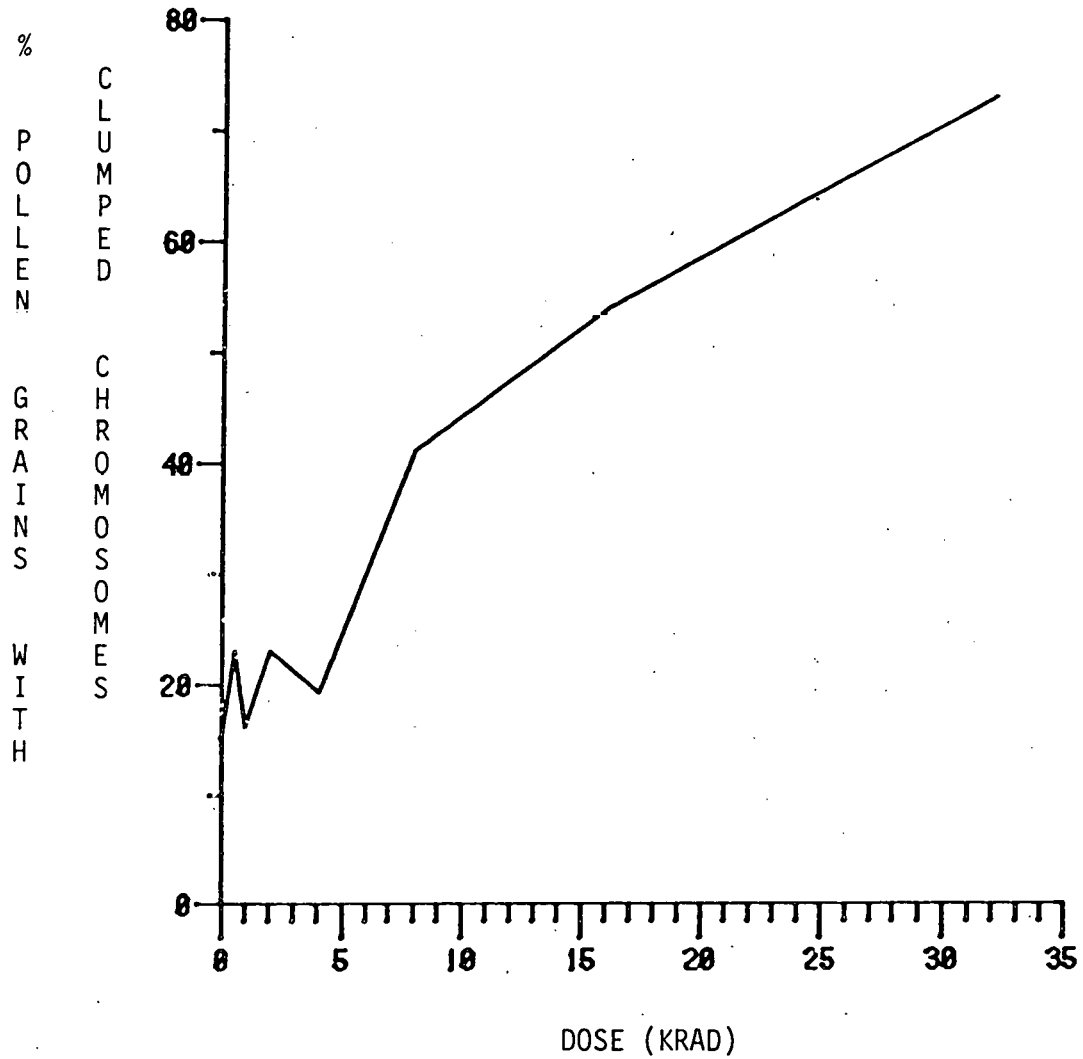
Figure 7. The percent of pollen grains with clumped chromosomes from plants at different dose levels in the acute gamma radiation study six days after irradiation (for data see Appendix G).
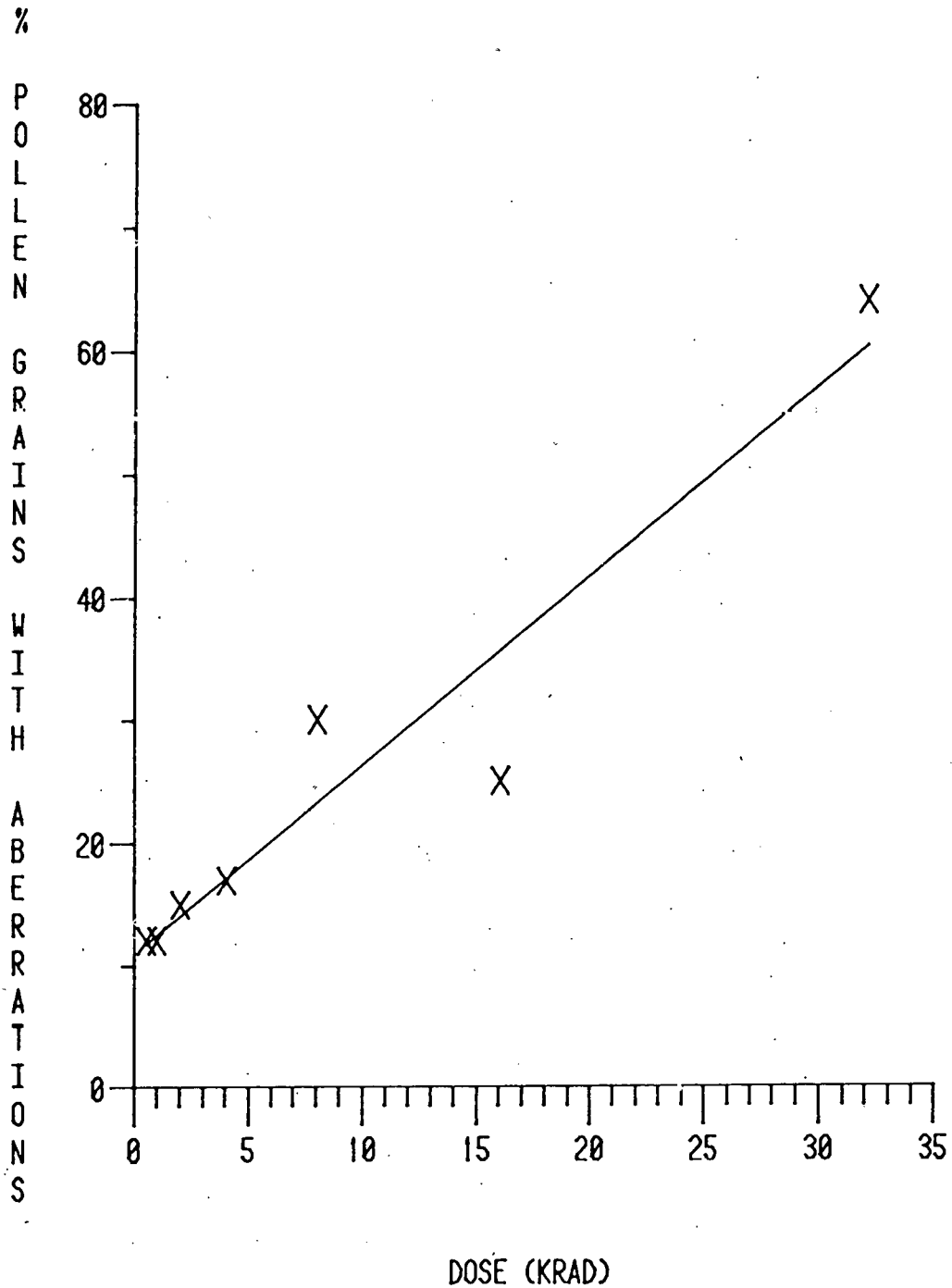
Figure 8. The linear relationship between the dose and the percent of pollen grains with any form of chromosome aberration from plants of the acute gamma radiation study (see Appendix G for data, controls subtracted from each dose level).

$$y = 10.9 \ (\pm \ 2.92) + 1.54 \ (\pm \ 0.21) \ X$$

where 10.9 was the intercept of the y axis (percent total aberrations) and 1.54 was the slope of the line. The plus and minus values are one standard deviation of the coefficients. This linear relationship was only determined for doses of 0.5 to 32 krad and had a coefficient of determination (r^2) of 0.916. Based on this linear relationship, chromosome aberrations in generative cells appear to be a useful measure of the effects of acute gamma radiation and constitued a more reliable indicator of radiation effects than growth observations.

Chronic Gamma Radiation Field Study

In this study, the pollen and root tip cells were scored for the same aberrations described earlier. All types of aberrations were seen in the pollen grains. However, the chromosomes of the root tips collected when the plants were brought in from the field and when the bulbs were dried showed only some clumped chromosomes. The lack of aberrations probably was due to the lower dose received by the roots as a result of shielding by the pots and soil.

In the pollen grains (generative cells), there was a trend for increasing chromosome aberrations with total accumulated dose. Figure 9 is a linear plot of the relationship between percent total aberrations and dose.

Figure 9. The linear relationship between the total accumulated dose and the percent of pollen grains with any form of chromosome aberration from plants of the chronic gamma radiation study (see Appendix G for data, controls subtracted from each dose level).

The coefficient of determination was 0.873 and the
relationship was

$$y = 6.77 \ (\pm 1.69) + 0.207 \ (\pm 0.046) \ X$$

where 6.77 was the intercept of the y axis (percent total
aberrations) and 0.207 was the slope of the line. This
linear relationship was only determined for doses of
4.2 to 70 krad. Again, in this study, chromosome
aberrations in the pollen were a more reliable indicator
of radiation damage than growth observations of the plants.

Internal Beta Radiation Study

Chromosome aberrations in both pollen and root tips
were the only effect of radiation investigated in this
study. Although the same types of aberrations were
observed here as in the two gamma studies, the doses
received by the plants of this study using internal beta
radiation were much lower than the doses received by the
plants in the gamma studies (see Appendix E for approxi-
mate doses).

While divisions were frequent in germinating pollen
grains, almost no cells in the root tips were in division
and, for this reason, almost no data were available for
analysis of aberrations in root tips. This apparent
inhibition of division may have been due to radiation
effects since it has been reported as an effect of

internal beta irradiation by incorporated tritrated
thymidine (Pollack et al., 1979).

In pollen grains, the number of total aberrations
increased as the dose accumulated over time. The linear
relationship between total aberrations and dose is shown
in Figure 10. The regression equation was

$$y = -3.60 \ (\pm 1.57) + 1.23 \ (\pm 0.179) \ X$$

where -3.60 was the intercept of the y axis (percent
total aberrations) and 1.23 was the slope of the line.
The coefficient of determination was 0.840 and the rela-
tionship was only determined for 3.7 to 13 rad. Since
here the y intercept was less than zero while in the
chronic gamma radiation study it was more than zero,
there may or may not be a threshold value necessary to
produce chromosome damage. Also in this study, there
appeared to be a disproportional increase in the percent
of pollen with aberrations at higher doses. This may
have indicated that the relationship was only approxi-
mately linear, or that the radiation increased the
length of mitotic cycles resulting in an increase in
dose to the chromosomes in any one pollen grain. However,
in spite of some deviation of the linear relationship at
the higher doses, chromosome aberrations appear to be a
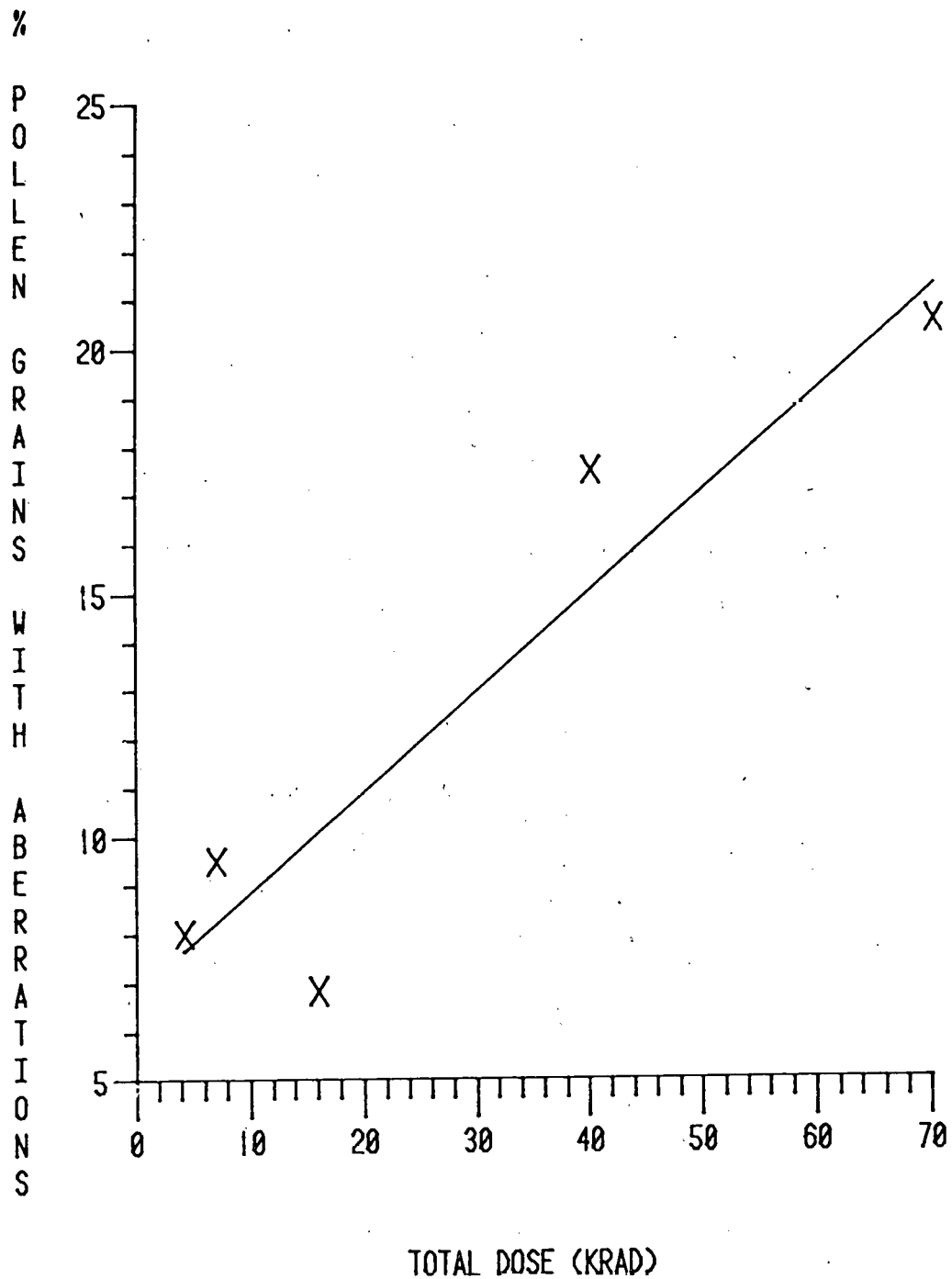useful measure of radiation damage at doses between 3.7
and 13 rad.
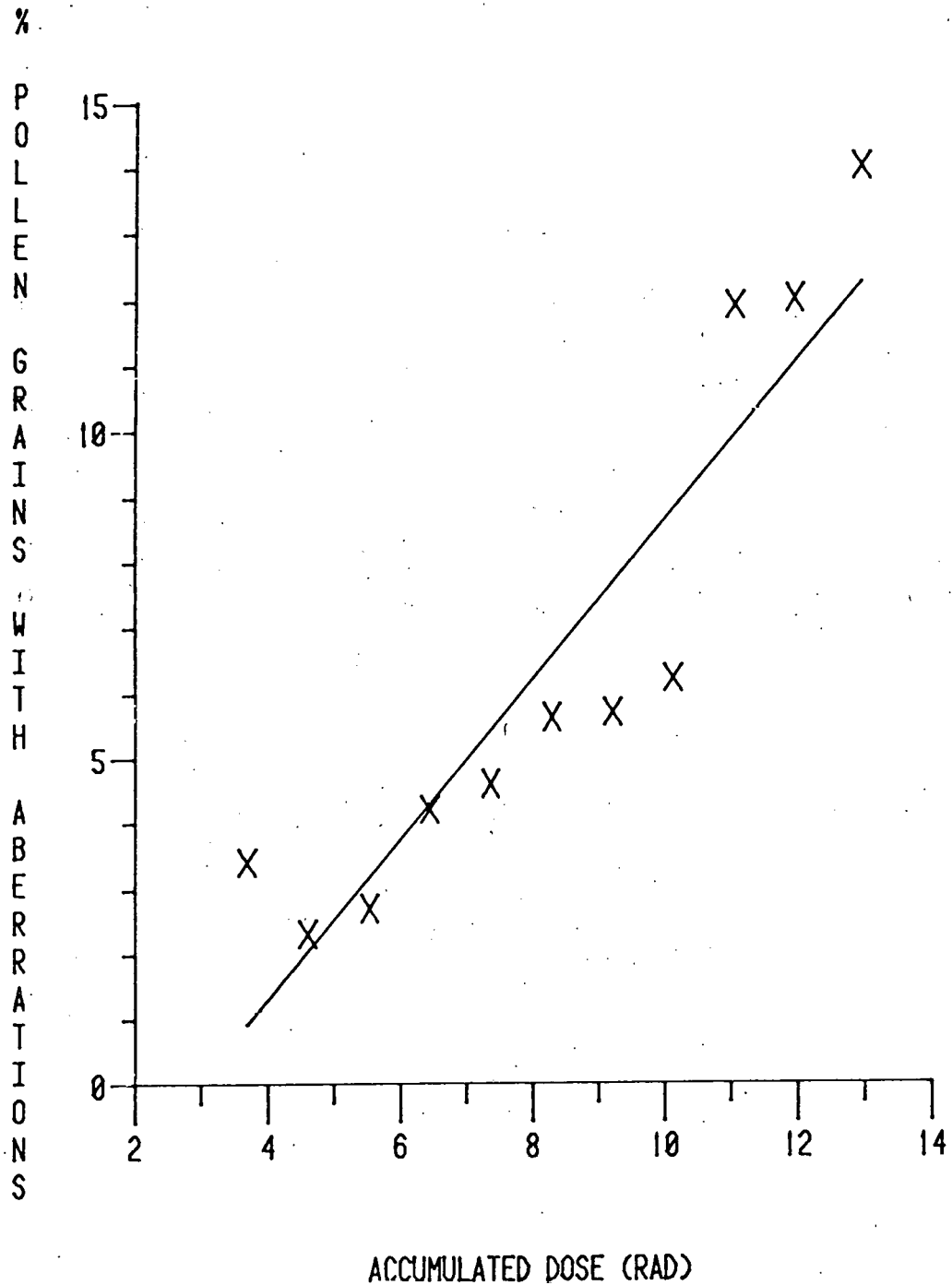
Figure 10. The linear relationship between the accumulated dose and the percent of pollen grains with any form of chromosome aberration from plants of the internal beta radiation study (see Appendix G for data, controls subtracted from each dose level).

Comparison to Past Studies

The linear relationships betwen total aberrations and dose found in the present study appear to be in conflict with the dose squared relationship reported by Sax (1940) and others (see Literature Review). Other researchers (Wolff and Luippold, 1957) have reported linear relationships for specific aberrations such as anaphase bridges, but no past studies have investigated all the types of aberrations examined here. There are at least two possible explanations for this conflict. One is that the linear relationships found here may have been due to a conversion at higher doses of some types of aberrations into other types (fragments into pulverized chromosomes, see Figure 11 and 12). The dose squared relationships for specific aberrations may fail at higher doses because of this conversion, and the result may be a linear relation for all observations. Another possible explanation is that methods of reporting aberrations are different and thereby result in changing the apparent relationships between aberrations and dose. For instance, aberrations have been reported as aberrations per chromosome (Sax, 1940) or aberrations per cell (Wolff and Luippold, 1957) or more recently, aberrations as a percent of total abnormal cells (Ojomo and Chheda, 1971) or percent of cells with aberrations (Lindgren et al., 1976). In agreement with the present study, Lindgren et al. (1976) found linear relationships between percent of cells with a
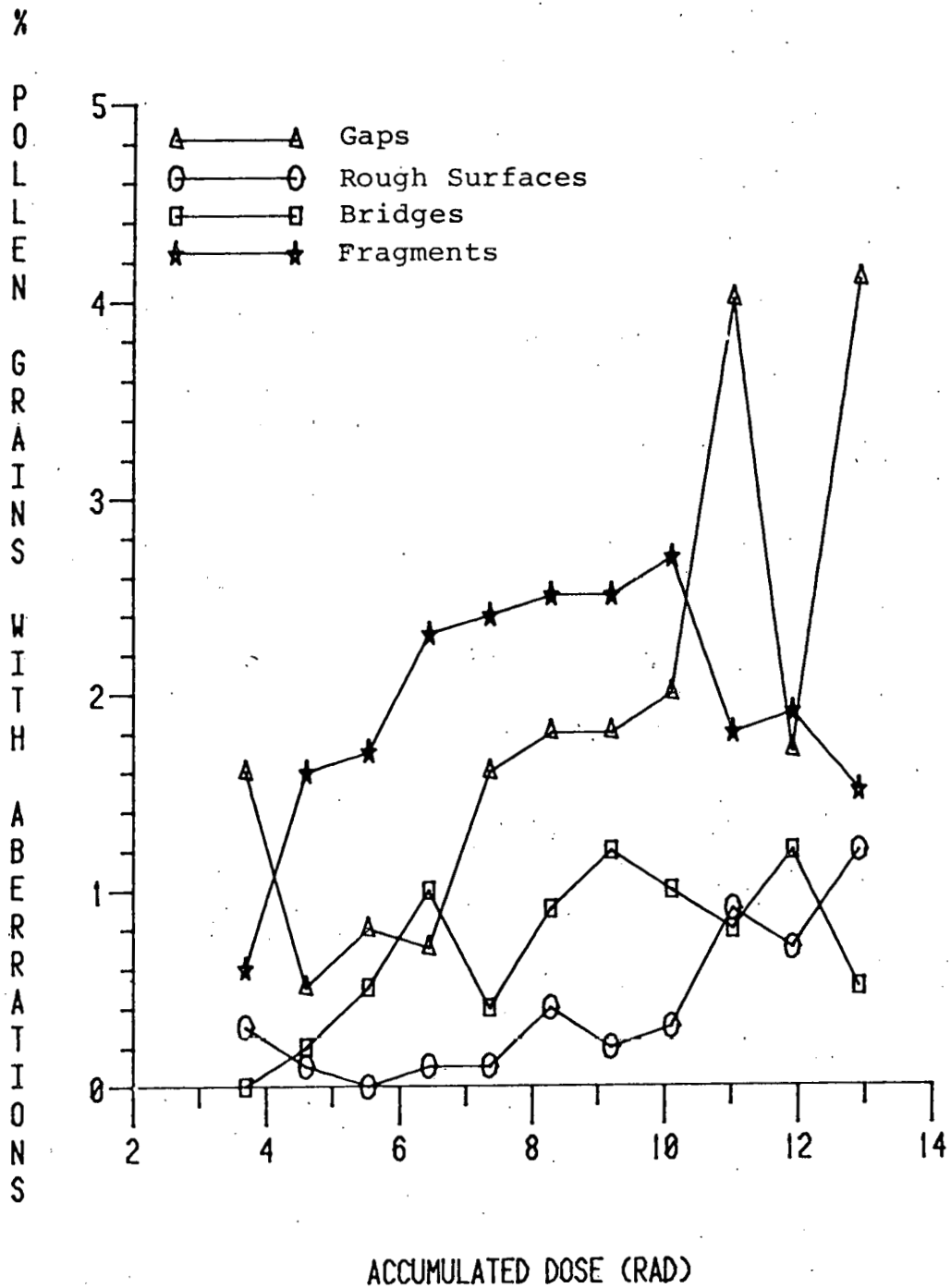
Figure 11. The percents of pollen grains with gaps, fragments, bridges, and rough surfaces from plants of the internal beta radiation study as a function of accumulated dose (see Appendix G for data).
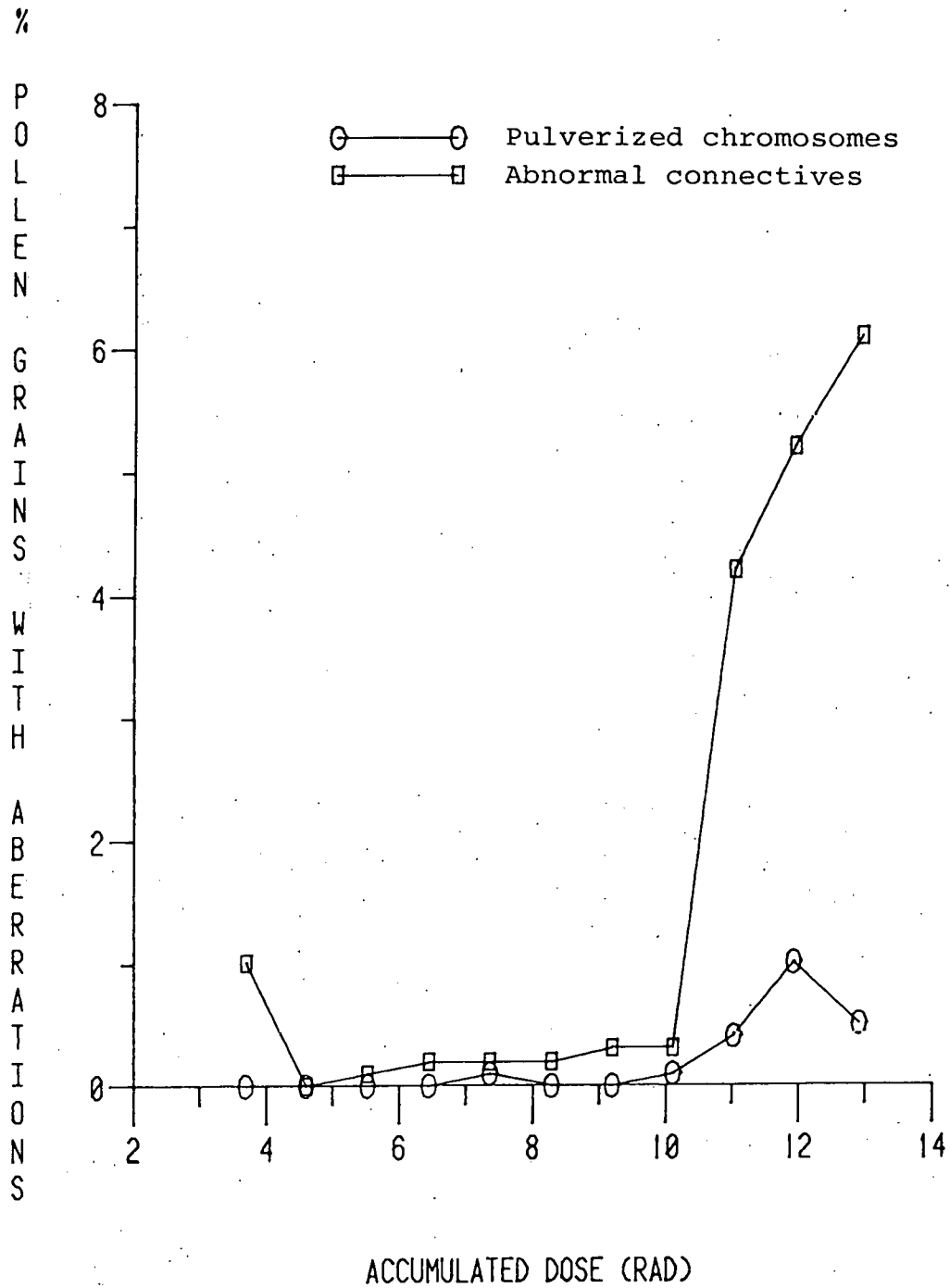
Figure 12. The percents of pollen grains with abnormal connectives and pulverized chromosomes from plants of the internal beta radiation study as a function of accumulated dose (see Appendix G for data).

variety cf different chromosome aberrations and dose of mutagenic chemicals.

Least and Most Sensitive Indicators

One of the objectives of these radiation studies was to determine the types of chromosome aberrations that are the least and most sensitive indicators of radiation damage. The least sensitive indicator is the type of aberration that occurs at the highest level of damage while the most sensitive indicator is the type that occurs at the lowest level of damage.

The doses received by the plants of the acute gamma radiation study were higher than those in the other two studies. At the highest dose (32 krad), most of the damage was expressed as pulverized chromosomes. Figure 13 shows the increase in pulverized chromosomes with increasing dose. It appeared that pulverized chromosomes were the least sensitive indicator of radiation damage.

The most sensitive aberration(s) should be the most frequently observed aberration(s) in cells minimally exposed to radiation. In this regard, since fragments, bridges, gaps, and rough surfaces were the only aberrations observed in the control plants of all three studies, it seems these aberrations can occur even at minimal levels of radiation. Of the three studies, the lowest doses of controlled radiation were received by the pollen in the internal beta radiation study (Appendix E). In
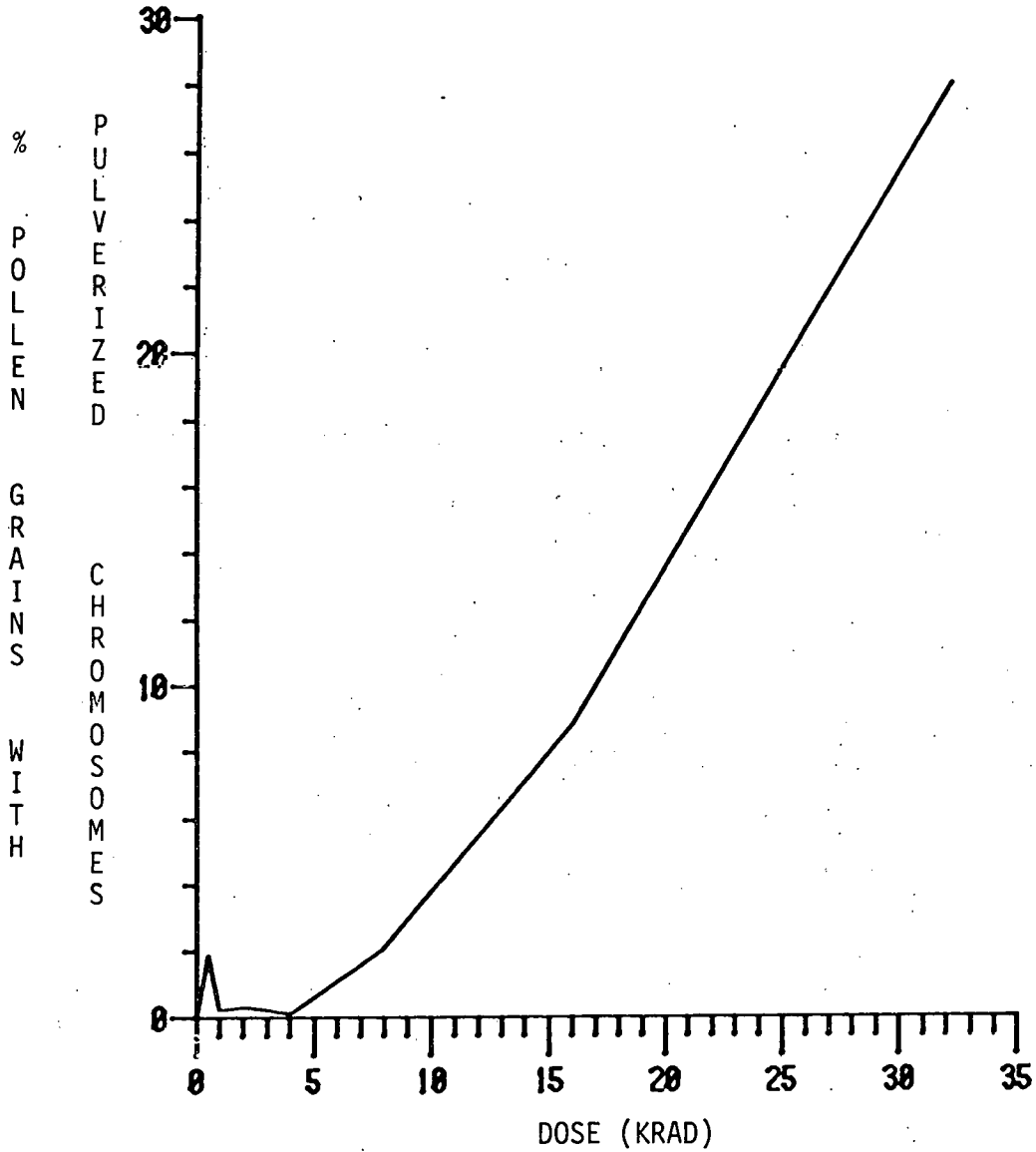
Figure 13. The percent of pollen grains with pulverized chromosomes from plants of the acute gamma radiation study eight days after irradiation (see Appendix G for data).

the first few days after removing the plants from the
^{32}P solution, i.e., after minimal cumulative radiation
dose, most of the damage was in the form of fragments,
bridges, gaps, and rough surfaces as might have been
predicted (Figure 11). Only a few cells with abnormal
connectives and pulverized chromosomes were observed
until the late days of the experiment (Figure 12). If
the frequencies of gaps, fragments, bridges, and rough
surfaces are compared to each other, it can be seen
that the majority of the damage at lower doses was
expressed as fragments (Figure 11). The same distribu-
tion of aberration frequencies based on dose was observed
to some extent in the acute and chronic gamma radiation
studies. From these observations, it seemed likely
that fragments were the most sensitive indicator of low
level radiation damage. In support of this conclusion,
other researchers have found that fragments were the
most frequent aberration (Riley, 1936) and that the
occurrence of fragments was frequent enough to study in
natural populations (Whitaker, 1936).

Biological Assay

The purpose of this study was to determine if
Ornithogalum virens can be used as a biological assay
for the effects of radiation. The linear relationships

between percent pollen grains with chromsome aberrations and dose indicated that *O. virens* is useful as a biological assay for beta and gamma radiation effects within the dose range of this study.

CHAPTER FIVE   SUMMARY AND CONCLUSIONS

Ornithogalum virens plants were exposed to two types

of radiation: acute (^{60}Co) and chronic (^{137}Cs) gamma

radiation and internal beta radiation (^{32}P). Lethality

(LD_{50}, LD_{100}), growth inhibition and chromosome aber-

rations were investigated after irradiation. To predict

the radiation sensitivity of *O. virens*, the nuclear and

interphase chromosome volumes were determined for pollen

and root tip cells.

In the two gamma radiation studies, lethality of the

plants was investigated. In the acute gamma radiation

study an estimated LD_{50} of 0.91 to 1.8 krad was found.

In determining the LD_{50}, a procedure in which the irra-

diated bulbs were first dried and then given the oppor-

tunity to sprout was found to be more practical than

the classical method of waiting for death of irradiated

plants. In connection with this procedure, since leaf

growth is variable even for nonirradiated *O. virens*

plants, root growth was found to be the better measure

of mortality. The LD_{100} was estimated to be less than

3.6 krad based on the drying and sprouting procedure.

There was general agreement between the predicted values

found by the use of the interphase chromosome volume in

relationships established by other researchers and the

results found in this study. Based on the study of
Sparrow and Schwemmer (1974) *O. virens* was found to
be more radiation sensitive than the majority of the
herbaceous plant species examined. In the study in
which plants were exposed to chronic gamma radiation
in the field for 30 days, the LD_{50} could not be deter-
mined because of the lack of mortality presumably due
to the low doses received by the roots and bulbs.

Though growth inhibition and abnormal growth were
observed in the two gamma radiation studies, the
changes in the growth of the plants were found to be
so variable that these parameters were considered
unreliable measures of radiation damage.

Chromosome aberrations were examined in all three
radiation studies. In the chronic gamma radiation study
chromosome aberrations were not observed in the root
tips again apparently because of the low doses received
by the bulbs and/or roots. Also, the aberrations in the
root tips of the plants of the internal beta radiation
study could not be analyzed because of few cells in
division. However, strong positive correlations between
the frequency of total aberrations in the pollen and the
dose were found in the gamma (acute and chronic) and
internal beta radiation studies. Since linear relation-
ships between the dose and total aberrations were found,
total aberrations can be used as a reliable indicator
of radiation damage. In an analysis of the types of

aberrations in the pollen, pulverized chromosomes (extreme fragmentation) and fragments seemed to be the least and most sensitive indicators of radiation damage, respectively.

In conclusion, *Ornithogalum virens* appears to be a useful biological assay system for the effects of beta and gamma radiation within the dose range used in this study.

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APPENDIX A

APPROXIMATE DOSES TO BULBS OF ACUTE GAMMA

RADIATION STUDY

In the acute gamma radiation study, the bulbs of the
plants were partially shielded by the vermiculite and
paper cups. To determine the approximate doses to the
bulbs, the following equation was used,

$$\frac{I}{I_o} = e^{-\mu_m \rho x}$$

(Radiological Health Handbook, 1970). In this equation,
I_o is the original radiation exposure rate, I is the
attenuated radiation exposure rate, e is the base of the
natural logarithm, μ_m is the mass absorption coefficient
(approximately 0.06 cm^2/g for wet vermiculite), ρ is the
absorber density (approximately 0.38 g/cm^3 for wet vermi-
culite) and x is the absorber thickness (4 cm from the
edge of the cup to the center of the bulb). The dose
to the bulbs is approximately 91% of the dose delivered
to the tops of the plants.

APPENDIX B

FORMULAS FOR NUTRIENT SOLUTION

The following two formulas were used alternately about every two months to water the plants grown in vermiculite in the greenhouse.

Formula One

The following were dissolved in 9.5 l water and diluted at the rate of 100:1 – 12.4 kg KNO_3, 12.4 kg $Ca(NO_3)_2$, 2.5 kg NH_4NO_3, 110 g $Na_2B_4O_7 \cdot 10\ H_2O$, 15 g $ZnSO_4$ and a trace of chelated iron. The $Na_2B_4O_7 \cdot 10H_2O$ and $ZnSO_4$ were dissolved in boiling water.

Formula Two

The following were dissolved in 9.5 l water and diluted at the rate of 100:1 – 12.4 kg KNO_3, 5.9 kg $MgSO_4$, 2.5 kg NH_4NO_3, 400 ml H_3PO_4, 110 g $Na_2B_4O_7 \cdot 10H_2O$ 15 g $ZnSO_4$ and a trace of chelated iron. The $Na_2B_4O_7 \cdot 10H_2O$ and $ZnSO_4$ were dissolved in boiling water.

APPENDIX C

DESCRIPTION OF GREENHOUSE SOIL

The following is a description of the greenhouse soil used in the chronic gamma radiation study.

Soil Mixture

4 parts top soil

2 parts sand

1 part perlite

1 part peat moss

Top Soil

70% sandy loam

25% organic matter

5% sewage sludge

APPENDIX D

APPROXIMATE DOSES TO BULBS OF CHRONIC GAMMA

RADIATION STUDY

In the chronic gamma radiation study, the bulbs of
the plants were placed approximately 8 cm below the sur-
face of the soil. Due to shielding by the soil, the
doses received by the bulbs were lower than the doses
received by the tops of the plants. The approximate
doses to the bulbs were calculated by using previously
determined dose rates for 8 cm below the surface of the
soil (Fraley, 1971) and 30 days of irradiation.

APPENDIX E

DOSE CALCULATIONS FOR INTERNAL BETA

RADIATION STUDY

In the interal beta radiation study, uptake of the ^{32}P by the plants was 71%. Plating was not a problem and 4% of the ^{32}P was rinsed from the roots when the plants were removed from the ^{32}P solution. Based on the Cerenkov counting of ^{32}P, the average activity concentrations (AC) of the root tips and buds were found to be 1.99 nCi/mg and 3.57×10^{-2} nCi/mg, respectively. The dose rates were calculated using the equation

$$\dot{D} = (AC) \, (\frac{37 \text{ s}^{-1}}{1 \text{ nCi}}) \, (\frac{3600 \text{ s}}{h}) \, (\frac{10^{6} \text{ mg}}{kg}) \, (0.7 \text{MeV})$$

$$(\frac{1.6 \times 10^{-13} \text{ J}}{\text{MeV}}) \, (f) \, (\frac{1 \text{ rad}}{0.01 \text{ J kg}^{-1}})$$

(personal communication with Leslie Fraley). In the equation, AC is the average activity concentration and f is the fraction of the beta particles absorbed by the root tips (0.2) and the buds (0.72) determined from Figure 1 of Parmley et al. (1962). The calculated dose rates for the root tips and buds were 0.59 rad/h and 3.8×10^{-2} rad/h, respectively. The accumulated doses were calculated by using the dose rates and the time after removal

of the plants from the ^{32}P solution. The actual doses

received by any one root tip cell or pollen grain could

not be determined because the beta particle from the

^{32}P can travel through several cells and because the

chromosomes of the cell are the product of several cell

cycles.

APPENDIX F

FORMULA FOR POLLEN GROWTH MEDIUM

The following were dissolved in 100 ml distilled water and used to germinate the pollen before squashes of the pollen were made.

> 10.00 g sucrose
>
> 0.01 g boric acid
>
> 0.03 g $Ca(NO_3)_2$
>
> 0.02 g $MgSO_4$
>
> 0.01 g KNO_3

APPENDIX G

RAW DATA TABLES OF CHROMOSOME ABERRATIONS

IN POLLEN GRAINS

The following tables contain the raw data on pollen grain chromosóme aberrations for the acute and chronic gamma and beta radiation studies. In all cases, pollen grains that contained more than one type of chromosome aberration were counted in the totals of each type of aberration.

Table 5a. The number of pollen grains with different types of chromosome aberrations from the plants of the acute gamma radiation study, six days after irradiation.

Dose (krad)	Clumped Chromosomes	Undamaged Chromosomes	Gaps	Rough Surface	Bridges	Fragments	Pulverized Chromosomes	Abnormal Connectives	Total Aberrations	Grand Total
					Number of Pollen Grains / Chromosome Aberration Type					
0	128	719	0	1	1	1	0	0	3	850
0.5	204	633	16	1	16	11	3	16	63	900
1	128	524	9	2	36	28	6	49	130	782
2	275	773	15	1	35	40	3	39	133	1181
4	101	294	9	1	56	58	1	22	147	542
8	231	241	9	0	12	40	1	31	93	560
16	293	108	3	0	15	37	47	60	162	537
32	383	38	24	0	2	13	21	64	124	525

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Table 5b. The number of pollen grains with different types of chromosome aberrations from the plants of the acute gamma radiation study, eight days after irradiation.

Dose (krad)	Clumped Chromosomes	Undamaged Chromosomes	Gaps	Rough Surfaces	Bridges	Fragments	Pulverized Chromosomes	Abnormal Connectives	Total Aberrations	Grand Total
			Number of Pollen Grains / Chromosome Aberration Type							

Dose (krad)	Clumped Chromosomes	Undamaged Chromosomes	Gaps	Rough Surfaces	Bridges	Fragments	Pulverized Chromosomes	Abnormal Connectives	Total Aberrations	Grand Total
0	120	548	0	0	0	1	0	0	1	669
0.5	177	658	15	18	6	18	18	39	114	945
1	231	802	25	3	39	24	3	53	147	1180
2	173	864	14	0	73	67	4	28	186	1223
4	187	999	26	3	46	57	1	108	241	1427
8	187	436	27	0	33	94	18	92	264	865
16	663	48	4	0	2	62	76	70	214	864
32	369	33	0	0	10	93	194	152	449	698

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Table 6. The number of pollen grains with different types of chromosome aberrations from the plants of the chronic gamma radiation study.

Total Dose (krad)	Clumped Chromosomes	Undamaged Chromosomes	Chromosome Aberration Type							Grand Total
			Gaps	Rough Surfaces	Bridges	Fragments	Pulverized Chromosome	Abnormal Connectives	Total Aberrations	
0.0072	66	1005	1	0	1	3	0	0	5	1076
4.2	163	1059	14	3	40	53	2	2	114	1336
7.0	180	968	19	3	60	46	0	5	133	1281
16	106	983	26	2	19	35	0	4	86	1175
40	122	775	45	32	5	30	9	70	191	1080
70	153	571	32	18	25	28	11	77	191	915

Number of Pollen Grains

Table 7. The number of pollen grains with different types of chromosome aberrations from the plants of the internal beta radiation study.

Accum. Dose (rad)	Clumped Chromosomes	Undamaged Chromosomes	Chromosome Aberration Type							Grand Total
			Gaps	Rough Surfaces	Bridges	Fragments	Pulverized Chromosomes	Abnormal Connectives	Total Aberrations	
0	382	9879	0	0	3	12	0	0	15	10276
3.7	182	1331	25	5	0	9	0	16	55	1567
4.6	61	1038	6	1	2	18	0	0	27	1126
5.5	61	1020	9	0	6	19	0	1	35	1116
6.4	54	1034	8	1	12	26	0	2	49	1137
7.4	49	990	18	1	5	26	1	2	53	1092
8.3	37	976	20	4	10	27	0	2	63	1076
9.2	33	964	19	2	13	27	0	3	64	1061
10	60	958	22	3	11	29	1	3	69	1087
11	144	1091	57	13	12	26	6	60	174	1409
12	207	1071	25	10	18	28	15	76	172	1450
13	97	1143	59	17	7	22	7	88	200	1437

Number of Pollen Grains