5-1977

Effects of Low-Level Chronic Radiation on Plant Nuclear Parameters as Related to Successional Patterns

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Recommended Citation

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EFFECTS OF LOW-LEVEL CHRONIC RADIATION ON PLANT NUCLEAR PARAMETERS AS RELATED TO SUCCESSIONAL PATTERNS

by

Eric A. Rechel

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

UTAH STATE UNIVERSITY
Logan, Utah

1977

ACKNOWLEDGMENTS

I would like to acknowledge with gratitude the assistance and guidance given me by Dr. William F. Campbell while serving as my major professor and advisor during the course of this study. His assistance in elucidating and explaining the methodology and rationale behind the scientific procedure is especially appreciated. Appreciation is also extended to Dr. I. Palmblad for his assistance and guidance throughout the writing of this thesis, and to Dr. W. Grenney for his suggestions and improvements.

I also extend thanks to A. Holmgren for his assistance in the taxonomic identification of the immature plants that were a major part of the study. Acknowledgment is also extended to the late Dr. A. H. Sparrow for his help and advice throughout the course of this study.

The financial support from the Utah State University Water Laboratory, contract WA-20, and from the Utah State Experimental Station, fund 051-0065-0113-76001, is greatly appreciated.

I am very indebted to my mother and father, Shirley and Paul, and my sisters, Holly, Jenny, and Kathy for their encouragement, patience, and who instilled in me the love of Biology.

Eric A. Rechel

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ABSTRACT

Effects of Low-Level Chronic Radiation on Plant Nuclear Parameters as Related to Successional Patterns

by

Eric A. Rechel, Master of Science
Utah State University, 1977

Major Professor: Dr. W. F. Campbell
Department: Plant Science

A major issue facing nuclear power stations is how to effectively deal with radioactive waste. This waste, as it comes from a reactor, is emitting large quantities of ionizing radiation which is usually confined. Another form of radioactive wastes is the mill tailings from uranium processing plants. These tailings are sites characterized by low-level chronic radiation.

The mill tailings of the Vitro Chemical Plant, in Salt Lake City, Utah, have been a point of radionuclide concentration and environmental contamination for 20 years. These tailings may adversely affect both surrounding ecosystems and any biological systems seeking to become established on the site. To test the potential hazard of this site to the succession of plant species I examined the interphase chromosome volume and relative amounts of DNA per chromosome from plants growing on this site and those on a control site. These nuclear parameters indicate the relative radiosensitivity of a species and would demonstrate the total effectiveness of the low-level chronic radiation in altering plant succession. The radiosensitive plant Tradescantia clone 02 was also grown in five soil samples from the mill tailings which represented a progressive increase in radioactivity. The purpose was to determine how effective these radiation levels are in altering reproductive integrity, fecundity, and somatic mutation rates in radiosensitive plant species.

There was a difference in species composition between plant communities growing on the mill tailings as compared to the controls as determined by coefficient of community. However, there was no difference in interphase chromosome volume or relative amounts of DNA per chromosome between plants growing on these two sites. The difference in species composition is attributed to the length of time each site has been undergoing succession, with the control site in a more advanced stage. Tradescantia grown in soil with a radiation dose greater than 0.10 mR/hr had significantly reduced reproductive integrity and fecundity, as measured by the number of stunted hairs on a stamen and pollen viability, and increased numbers of somatic mutations. Based on these data the radioactive mill tailings from the Vitro Chemical Plant have the potential to alter plant successional patterns due to their detrimental effect on any species that is relatively radiosensitive.

(71 pages)

INTRODUCTION

The use of nuclear power as an energy resource is being debated frequently on the national and international scene. In 1976, the outcome of the presidential election in Sweden was determined by the candidates' stand on nuclear power, major opposition to nuclear reactors as a source of energy surfaced in Great Britain, and the United States' role as a supplier of nuclear energy was critically questioned (Douglas, 1976). This controversy has led to the publication of papers assessing the quality and quantity of safety standards imposed on nuclear reactors (Cohen, 1976; Wald, 1976). Studies specifically involved with radioactive fallout, artificial radiation fields, and areas naturally high in ionizing radiation, and their effects on the environment and biological systems have also been conducted (Kaaz, Wallace and Romney, 1971; Mericle and Mericle, 1965a; Woodwell and Oosting, 1965). Moreover, the effects of uranium mill pollutants on aquatic communities have been examined by Anderson, Tsivoglow and Shearer (1963), Martin, Helm and Sigler (1969), Sigler et al. (1966), and more recently by the Federal Government (Sears et al., 1975). The work of Sparrow and colleagues (Sparrow, Rogers and Schwemmer, 1968; Sparrow and Schwemmer, 1974; Sparrow, 1966; Sparrow, Schwemmer and Bottino, 1971) has revealed several innate properties of communities and species that explain their radioresistance. These properties are related to the site where ionizing radiation has the most detrimental effect i.e., the nucleus, with interphase chromosome volume (ICV), amount of DNA, and degree of polyploidy being the predominant characteristics involved. There is, however, relatively little information on the successional pattern of higher plants as they colonize uranium mill tailings, which emit low-level chronic radiation. Therefore, the following hypotheses have been formulated: (1) Plant succession on a radioactive waste site will consist of polyploid species characterized by small ICV and comparatively small relative amounts of DNA per chromosome, as compared to species colonizing a waste site subjected to background levels of radiation. (2) A threshold dose at the waste site must exist before changes in these nuclear parameters can be detected. (3) The ionizing radiation that exists on the tailings has the potential to increase the number of somatic mutations and decrease both pollen viability and the overall reproductive integrity of selected plants that come in contact with the mill tailings.

REVIEW OF LITERATURE

Radioecology

Radioecology deals with many aspects of radionuclides and their role in biological systems, ranging from acute exposures by atomic bombs and fallout, to chronic exposures from nuclear waste and artificially designed radiation fields. Since the cessation of above-ground atomic explosions at the Nevada test sites in 1958, a number of authors have examined the plant communities inhabiting the area to determine the ecological ramifications of these detonations. These studies have been hampered by difficulties in distinguishing damage caused by the mechanical and thermal stress from those of radiation stress (Shields, 1959). However, several years of study on plant recovery have shown no marked effects of radiation damage due to the blasts (Shields and Wells, 1962; Shields, Wells and Rickard, 1963; Beatley, 1966).

It was not until detonations were conducted below ground, with the radioactive fallout escaping through a vent, that radiation damage to plant species and plant communities was detectable (Wallace and Romney, 1972). Damage to *Artemisia* included defoliation and a decrease in the number of inflorescences (Rhoads et al., 1971). Observations by Wallace and Romney (1972) revealed similar radiation damage to *Atriplex*, *Ephedra*, and *Juniperus* from the radiation blast that escaped through the vent and from the subsequent fallout containing beta particles.

Murphy and McCormick (1970) exposed granite outcrop communities to artificial fallout. Within communities treated with 700 rads terminal bud mortality, decreased leaf area index, and a reduction in total biomass were prominent consequences. The extent of damage suffered by an individual plant or species was related to physical position in the community, tolerance to radiation, and ability to adjust to naturally occurring environmental stresses.

The study of acute and chronic radiation and how it alters plant composition and structure has led to the development of several artificially irradiated plant communities. Gamma radiation fields in the oak-pine forest at Brookhaven National Laboratory (Sparrow, 1966), on granite outcrop communities (McCormick and Platt, 1962; McCormick, 1963), chaparral (Fabries, Grauby and Trochain, 1972), short grass prairie (Fraley and Whicker, 1973), and old fields (Woodwell and Oosting, 1965), are a few examples. Extensive reviews on the effects of irradiation on various communities may be found in the following: Platt, 1963, 1965; Woodwell, 1962, 1967; Woodwell and Whittaker, 1968; Woodwell and Sparrow, 1963; Woodwell and Holt, 1970; Martin, 1964; Sparrow and Woodwell, 1962; Dubinin, Shevchenko and Pomerantseva, 1973; Whicker and Fraley, 1974. This paper will contain a brief summary on the response of individuals and ecosystems to ionizing radiation.

When the irradiation is of an external nature, as it is in most experiments, the plants' ability to reproduce vegetatively from underground parts determines the rate of its recovery after the irradiation exposure ceases (Chappell, 1961). McCormick (1963) and McCormick and Platt (1962), working with annuals, observed morphological, physiological, and ecological differences in the parents and progeny of plants irradiated with 8,000 to 13,000 roentgens. These were exemplified by changes in the onset and length of anthesis of the species, changes in vigor, and a rearranging of dominance among community members.

In examining the difference between irradiated and nonirradiated plots, coefficient of community and percent similarity are the indices most often used (Fraley and Whicker, 1973; Whicker and Fraley, 1974). As the dosage increases, per day or cumulative, there is a change in both of the above indices that foretell the development of a simplified community i.e., a reversal of succession (Woodwell and Oosting, 1965; Woodwell and Rebuck, 1967; Fraley and Whicker, 1973; Holt and Bottino, 1972; Wagner, 1965; Garrett, 1969; Daniel, 1963). This change in species composition has revealed the relative radiosensitivity of various ecosystems and successional stages. In the continuum of plant succession the most radioresistant communities are those represented by colonizing populations e.g., lichens and weeds, with a climax forest of deciduous or evergreen trees being the most radiosensitive (Woodwell, 1970; Woodwell and Gannutz, 1967; Woodwell, 1963).

While the above may represent the generality, there are deviations. These may be caused by unseen environmental factors that either amplify the radiation effect or interact synergistically to bring about unforeseen responses (Woodwell and Oosting, 1965; Woodwell and Rebuck, 1969). Populations or ecosystems, undergoing stress from ionizing radiation react in a similar manner when subjected to other forms of stress that occur naturally (Woodwell, 1970; Garrett, 1969). Thus, when examining plant communities exposed to low-level chronic radiation, the possibility of concurrent environmental variation or any recovery and repair mechanism must be considered (Fabries, Grauby and Trochain, 1972; Woodwell, 1963; Gunkel and Sparrow, 1953; Woodwell and Marples, 1968).

Some of the first ecological work on areas naturally high in uranium and vanadium was conducted by Cannon (1952). This study dealt with the description and physiology of uranium uptake by various plants. Mericle and Mericle (1965a,b), assessing sites high in natural radioactivity, recorded a significant increase in somatic mutations in *Tradescantia* clone 02 when exposed to 0.25 and 0.10 mR/hr, thereby attesting to the potential hazard of low-level radiation. Examining plant adaptations to radiation, Mewissen, Damblon and Bacq (1959) discovered an ecotype of *Andropogon filifolius* inhabiting uraniferous soils characterized by low-level irradiation. When exposed to large doses of acute radiation, seeds from the plants grown on the uraniferous soil showed a higher percent germination than seeds of other ecotypes. The roots and stems of the developing seedlings were also longer for the uraniferous ecotype. Thus, plant populations exposed to low-level chronic radiation may evolve an array of adaptations allowing them to survive under this form of stress.

Nuclear parameters and radiosensitivity

The history of correlating radiosensitivity of higher plants with the various nuclear parameters has spanned more than a quarter of a century. Qualification, quantification, and application of these parameters have been resolved primarily by Sparrow and colleagues at

Brookhaven National Laboratory (Sparrow, 1966; Sparrow, Rogers and Schwemmer, 1968; Sparrow and Schwemmer, 1974). This information is now contributing to the understanding of DNA and chromosome structure and function, plus providing insight into individual and community radiosensitivity.

Establishing correlations between nuclear characteristics and radiation damage is founded on the principle that the actual target for biological injury due to ionizing radiation is the genetic material. Evidence comes from Read's review on the radiation biology of *Vicia faba* (1959), studies conducted by Sparrow and Evans (1961) correlating numerous nuclear characteristics of plant tissue to radiosensitivity, and Epstein's work with viruses (1953). Relating interphase chromosome volume (ICV), DNA per chromosome, and nuclear volume (NV) of plants subjected to irradiation and then assessing mutation rates and number of chromosome deletions, have given additional evidence for this basic principle (Baetcke, Sparrow and Nauman, 1968; Sparrow et al., 1968).

Sparrow, Moses and Dubow (1952), working with the meiotic cycle of *Trillium erectum*, observed large differences in the radiosensitivity of the various phases. Concurrent observations with *Tradescantia paludosa* showed germ cells, in general, were ten times as susceptible to irradiation as somatic cells. Later, chromosome size was suspected of being involved in plant radiosensitivity with large chromosomes highly sensitive and smaller chromosomes highly resistant (Sparrow, 1955).

Interphase NV was one of the first nuclear parameters found to affect plant radiosensitivity (Sparrow and Miksche, 1960). Their conclusions, based on cells located in the apical meristem, state that as NV increases radiosensitivity also increases i.e., a smaller dose was required to reach a specific growth or developmental end point for a species characterized by a large NV. The graph of these two variables (log dose X log NV) did not show a slope of -1. However, a slope of -1 was desired since it would mean that the product of the two would be a constant. Sparrow and Evans (1961) reiterated this concept, and presented other hypotheses relating a variety of nuclear characteristics to radiosensitivity.

It has since been shown that ICV varies inversely with plant radioresistance and demonstrates less scattering of points for the regression when compared to NV (Sparrow, 1964; Sparrow, Underbrink and Sparrow, 1967). This negative correlation persists using several biological end points of radiation damage under acute or chronic regimes. Lethal dose and ICV show a strong negative relationship among a number of plant species (Donini et al., 1967). The mutation rates of five plant species also revealed an inverse relationship to ICV (Sparrow et al., 1968). Yamakawa and Sparrow (1965, 1966) using chronic gamma irradiation, examined percent seed set and pollen viability in horticultural and agronomic species. Their results helped to substantiate the role of ICV in determining the dosage necessary to reach specified end points. This inverse relationship is not only applicable among species, but within individuals as well. Miller and Sparrow (1965, 1966), working with Marchantia thalli found that meristematic cells have larger ICV than non-apical cells. As expected the meristematic cells had a higher sensitivity to ionizing radiation.

The slope of the regression corresponded to previous work where correlations with different species were performed.

The effects of ionizing radiation on plant tissue is largely due to its interaction with DNA molecules and chromosomes as discussed previously. Parameters involving amounts of DNA per nucleus or per chromosome were then expected to coincide with the results from ICV and NV (Sparrow and Miksche, 1960). Baetcke et al. (1967) have shown an inverse correlation between DNA per chromosome and species radioresistance. However, when they compared the amount of DNA per nucleus to radioresistance a poor relationship was found. The interaction of these two variables has been applied to mutation rate and lethal dose with the results confirming the above inverse characteristic (Sparrow et al., 1968).

Polyploidy appears to be a chromosomal phenomenon that would enhance radioresistance. The genetic redundancy, attributed by the polyploid condition, is the prime factor behind this hypothesis. Sparrow and Schairer (1958), working with Chrysanthemum and Sedum, stated that in respect to chronic radiation polyploidy increased the tolerance for individuals and species. During the early 1960s this concept gained acceptance and seemed to be well established (Sparrow and Evans, 1961). It was not until a profuse number of experiments relating ICV, DNA per chromosome, and polyploidy to radioresistance that the above theory was dispelled (Ichikawa and Sparrow, 1966). Polyploidy by itself has no effect on a species tolerance to ionizing radiation as demonstrated in woody plants (Sparrow et al., 1970a), viruses, bacteria, and herbaceous species (Sparrow, Underbrink and Sparrow, 1967). The protective effect, if any, afforded a polyploid species is due to reduced ICV and not to genetic redundancy (Sparrow et al., 1965). Studies with the genus Rumex have shown that polyploid level and ICV can sometimes act as independent variables (Ichikawa et al., 1971).

There exist many nuclear, cellular, and environmental factors affecting the tolerance of plants to irradiation concurrent with those mentioned above (Sparrow, 1955; Gunckel and Sparrow, 1961; Sparrow, Miksche and Evans, 1961). Different phases of cell division have distinct tolerance levels to irradiation with interphase one being the most resistant (Sax and Swanson, 1941; Sparrow, 1948). Upon examining the G_1, S, and G_2 stages in interphase of Allium fistulosum, there occurred a two fold increase in NV, thus demonstrating that the time of sampling has an effect (Sparrow, 1965). Changes in NV and ICV have been observed between dormant and nondormant tissue (Taylor, 1965; Sparrow et al., 1963). The position of the centromeres (Evans and Sparrow, 1961), the time required to complete mitosis (Van't Hof and Sparrow, 1963), and meiosis (Sparrow and Evans, 1961) are other nuclear characteristics affecting radioresistance. Thus, numerous physiological and environmental parameters can account for the differential radiosensitivity within a species or individual.

The determination of the relationship between the above nuclear parameters and radiosensitivity has allowed prediction of the extent of radiation damage to an individual or population. The first applications of these relationships used NV and polyploidy as the basis for the comparisons (Sparrow, Miksche and Evans, 1961; Sparrow, Schairer and Sparrow, 1963). Through continued research it became apparent that ICV and amount of DNA per chromosome were more suitable in predicting the extent of radiation damage. Interphase chromosome volume has now become the major index in predicting radiosensitivity (Sparrow et al., 1965). The radiosensitivity for a variety of end points can now be found for woody plants (Sparrow et al., 1970a,b; Sparrow, Rogers and Schwemmer, 1968), herbaceous plants (Sparrow and Sparrow, 1965; Baetcke, Sparrow and Nauman, 1968), bacteria and viruses (Underbrink, Sparrow and Pond, 1968; Sparrow and Schwemmer, 1974), with these values being used on the community and ecosystem level (Woodwell and Whittaker, 1968). A number of reviews have been published on this subject and may be useful for those wishing more information (Sparrow and Woodwell, 1962; Underbrink, Sparrow and Pond, 1968; Sparrow, Rogers and Schwemmer, 1968; Gunckel and Sparrow, 1961; Sparrow, 1961, 1965).

METHODS AND MATERIALS

Location

The Vitro Chemical Plant, located at 33rd South and 9th West in Salt Lake City, Utah, refined uranium ore from 1953 to 1967. During the time of operation the tailings were deposited on 45 hectares of adjoining land in large mounds and ridges (Figure 1). In June 1975, a radiological survey was made with a Victoreen Thyac III survey meter. The purpose was two fold. First, establish two vegetative plots to test hypothesis One i.e., that plant succession on a radioactive waste site will differ as to species composition when compared to plant succession on a site with no increase in radioactivity. Hypothesis Two, a threshold dosage must exist before any changes in plant succession can be detected, was also tested by using these same two plots. Second, to choose an additional five sites where the soil would represent a progressive increase in radioactivity. These soil samples were used to test hypothesis Three i.e., the ionizing radiation emitted from the tailings could affect the mutation frequency, reproductive integrity, and pollen viability of selected plants that come in contact with the mill tailings.

The vegetative plot with the greatest radioactivity was designated hot and the other, half way between this and background, was designated medium. Two control plots were chosen one kilometer to the east of the two treatments. Each plot was nine square meters in area. The activity of the hot site was 2.20 mR/hr, the medium 1.00 mR/hr, and the controls 0.01 mR/hr.

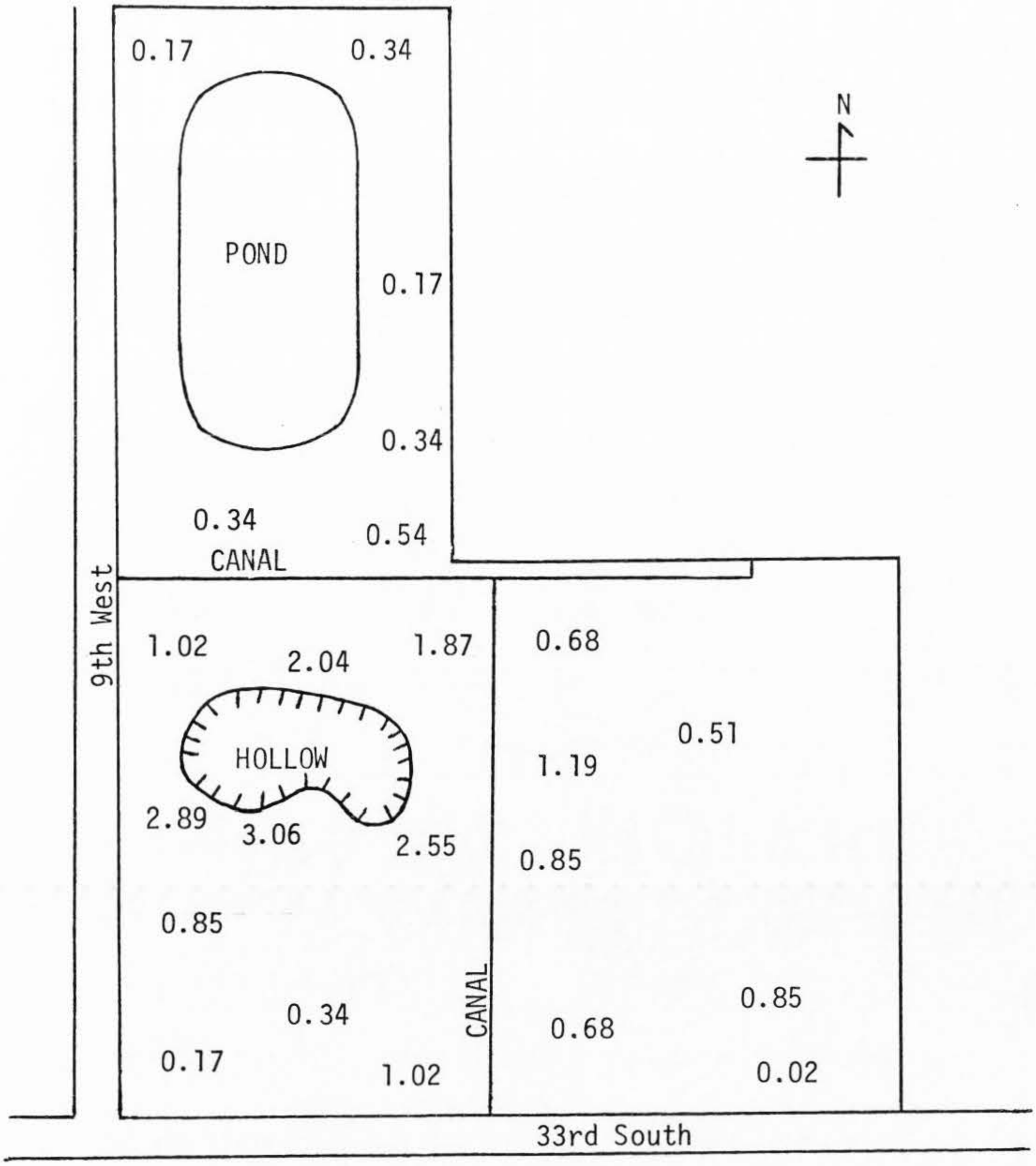

Figure 1. Schematic diagram of mill tailings from the Vitro Chemical Plant showing the levels of radioactivity in mR/hr as of June 1975.

The control site was a relatively large area and probably contained numerous microhabitats. To compensate for this variation two plots were selected for the control and designated One and Two. These plots also represented a more extensive array of species to compare with the radioactive communities. Coefficient of community (Whittaker, 1975) was used to detect plant community differences between these two controls, along with detecting differences between the two treatments, and between the controls and treatments.

It is assumed that there will be no atmospheric variations, such as amount of precipitation and solar radiation, between the two sites since they are located in the open and the distance separating them was only one kilometer. However, there could have been large differences in the soil due to their dissimilarity in morphogenisis. Specifically, the treatment plots were composed of mill effluents, whereas the controls resulted from natural geological processes. The pH, EC_e, and soil texture were used to quantify any variation in soil composition between the plots. The pH and EC_e were determined by the Soil, Plant, and Water Analysis Laboratory at Utah State University under the direction of Dr. Lamborn. Soil texture was ascertained by the method outlined by Bouyoucos (1962) with the exclusion of subjecting the soil to a high speed mixer. Instead a buret containing the sample was inverted by hand six times to suspend the particles.

ICV, relative DNA amounts, and polyploidy

Interphase chromosome volume, relative amount of DNA per chromosome, and polyploidy were used in determining the validity of hypothesis One i.e., the increased stress from ionizing radiation will alter the successional pattern on radioactive waste sites. In addition, the validity of hypothesis Two i.e., a threshold dosage must exist before ionizing radiation will affect plant succession, was also tested. Below is the procedure for the quantification of these nuclear parameters.

In June 1975, the apical meristems of seven individuals from every plant species present at each plot were collected. Only individuals in the active stages of growth were sampled. Meristems were collected over a two day period between 0900 and 1300. Five of the meristems were fixed in FAA for ICV determination, and the remaining two in Carnoy fixative for DNA determination.

Meristematic tissue used in calculating ICV were prepared according to Galigher and Kozloff (1971) using Safranin O and Fast Green for staining, and ethanol-tertiary butylalcohol in dehydration. Median longitudinal sections of shoot apices, ten microns thick, were then mounted in Canada Balsam and examined under a Zeiss Universal Light Microscope at 1000 X. The damage caused by ionizing radiation has its most crucial effect in active meristematic tissue (Sparrow and Miksche, 1961; Sparrow et al., 1961). Thus all nuclei used in determining ICV were selected from the actively dividing region of the shoot apex (Figure 2). When the nuclei were not spherical, as in most cases, both major and minor axes were measured and averaged to give a mean diameter. The volume of each nucleus, in cubic micrometers (μ^3), was determined using the formula for a sphere, $V = D^3/6$, where D is the mean diameter. Ten nuclei from each individual, with a total of 50, were tallied to give the mean volume for each species. The NV for plants that were no longer actively growing were obtained from

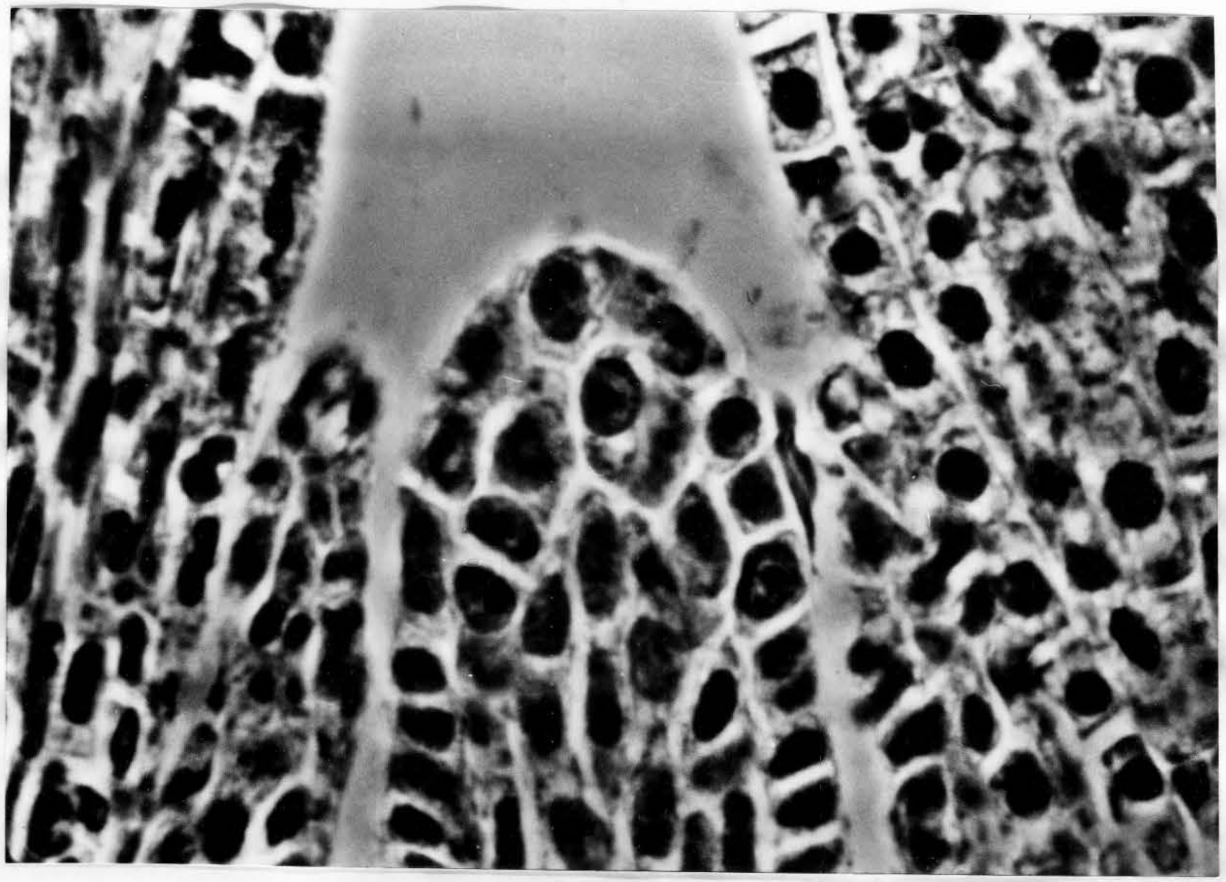

Figure 2. Apical meristem of Poa pratensis showing the nuclei used in determining nuclear volume (1216 X).

Sparrow (personal communication). Nuclear volume divided by the species chromosome number gave the ICV (Sparrow et al., 1968).

The histological technique outlined above is known to dehydrate and shrink the cell and nucleus. It is assumed that the amount of shrinkage is constant (Sparrow et al., 1965; Sparrow, 1965). Thus, when comparing different species this procedure is reasonably valid.

Chromosome numbers came from Love (1976, 1975, 1974, 1973, 1972, 1971a,b, 1970a,b, 1967, 1965), Darlington and Wylie (1956), Ornduff (1967), Cave (1957, 1956), and Cave and Hommersand (1962). Student's t test was used to detect any difference in the number of polyploid species inhabiting the treatment sites as compared to the control sites.

There is a well defined inverse relationship between the quantitative amount of DNA per chromosome and radioresistance (Baetcke et al., 1967). In the present study it was hypothesized that the relative amount of DNA per chromosome would demonstrate the same relationship to radioresistance as the quantitative amount of DNA per chromosome.

The histological procedure followed in staining and mounting apical meristems to determine relative amounts of DNA was taken from Campbell (1968). Measurements on percent transmission of interphase nuclei, using a Zeiss Microspectrophotometer, were taken at 555nm and 488nm according to the procedure described by Patau (1952). The final DNA content, which is dependent on the intensity of the stain (Patau, 1952), was divided by the chromosome number for that species to give relative amounts of DNA per chromosome. An analysis of variance was performed on the NV, ICV, and relative DNA quantities to detect any significant differences between the plant communities growing on the four plots.

Mutational analysis

The third hypothesis is a statement relating the low-level ionizing radiation from the radioactive mill tailings with changes in the viability and growth of somatic and germinal tissue. More specifically, somatic mutation frequency, reproductive integrity, and pollen viability of plants inhabiting these tailings are assumed to be affected adversely. Below is the procedure used in the verification of hypothesis Three.

Soil activity. There were five additional sites chosen on the uranium mill tailings for analysis of mutation potential. The levels of activity were 1.11, 1.62, 2.04, 2.72, and 3.32 mR/hr with the latter representing the highest radiation level detected on the tailings. The control had an activity of 0.01 mR/hr. Upon transporting the soil from the Vitro Mill site to the greenhouse there was a decrease in the amount of ionizing radiation as measured by the survey meter. The activity dropped to 0.07, 0.10, 0.14, 0.20, and 0.19 mR/hr respectively, with the control remaining at 0.01 mR/hr. This decrease may be caused by a decline in the amount of radioactive radon gas, a member of the uranium decay series. Radon gas is trapped in the soil layers by any mechanism that tends to seal the surface (Osburn, 1965) e.g., a lack of vegetation or very little precipitation. Upon transferring the soil samples to the greenhouse the soil layers were disturbed, thus releasing the radioactive radon gas which may have caused the decrease in activity. A second probable cause may be the result of fewer ionizing particles coming from the smaller soil sample as compared to the much larger Vitro Mill site.

Plant material. Plants of Tradescantia, clone 02 (obtained from A. H. Sparrow, Brookhaven National Laboratory) were used. Clone 02 is a perennial, herbaceous diploid plant (2n=12) with narrow tapering leaves not unlike some of the medium size grasses and reaches a height of about 60 centimeters. It is heterozygous for blue and pink flower color and is thought to be a putative hybrid between T. occidentalis X T. ohiensis (Sparrow et al., 1961).

Tradescantia, clone 02, has a number of characteristics that contribute to its versatility as a monitor of environmental mutagens: (1) Clone 02 is extremely sensitive to chemical mutagens (Rushton, 1969; Smith and Lofty, 1954), ultaviolet radiation (Chang and Campbell, 1976), and ionizing radiation (Sparrow et al., 1961; Ichikawa, 1971, 1972); (2) Plants of this variety can be grown in test environments for long periods of time and thus act as mutagen monitors (Underbrink, Schairer and Sparrow, 1973); (3) There are a number of morphological features produced by ionizing radiation which can be easily studied: (a) chromosome aberrations in microspores, root tips, ovaries, and stamen hairs; (b) somatic mutations in petals and stamen hairs in Tradescantia clones heterozygous for flower color; (c) pollen abortion; (d) loss of reproductive integrity in stamen hairs; and (e) whole plant or seedling death (Underbrink, Schairer and Sparrow, 1973); (4) The Tradescantia clone 02 test system can be used in experiments requiring a short time period and still produce adequate cell populations for statistical analysis (Underbrink, Schairer and Sparrow, 1973); (5) The radiobiological data from the stamen hairs e.g., mutation rate and reproductive integrity, can be compared with similar data from microorganisms and mammalian cell cultures (Davies, 1963; Ichikawa, Sparrow and Thompson, 1969; Sparrow, Underbrink and Sparrow, 1967; Underbrink, Sparrow and Pond, 1968); and (6) The handling of the plants and the scoring of the different radiation induced changes requires little time and is fairly easy.

The stamen hairs of Tradescantia clone 02 were used in analyzing for somatic mutations and reproductive integrity. Advantages of the stamen hair system for monitering a particular environment such as radioactive waste sites include: (1) Stamen hairs are produced mainly by division of the terminal cell with only a few subterminal or interstitial divisions. Thus, at certain developmental stages they appear to have a single- or two-celled meristem and are analogous to a small single-celled colony (Davies, 1963; Ichikawa and Sparrow, 1967b, 1968b; Ichikawa, Sparrow and Thompson, 1969); (2) Individual inflorescences contain a compact cluster of small flower buds which permit uniform exposure to treatments; and (3) Bud development in the inflorescence proceeds in an orderly and continuous sequence from bud initiation to anthesis with the youngest buds at the base of the inflorescence (Figure 3) (Underbrink et al., 1970).

Experiment and analysis. Thirty-six plants of equal size and containing young inflorescences were selected from a stock population and their root systems washed in water to remove the existing soil. Each treatment consisted of six plants repotted in either radioactive soil from one of the five sites or the control soil and grown under greenhouse conditions with a 16 hr light cycle and a day temperature of 35C and a night temperature of 20C. The pH of the radioactive soil

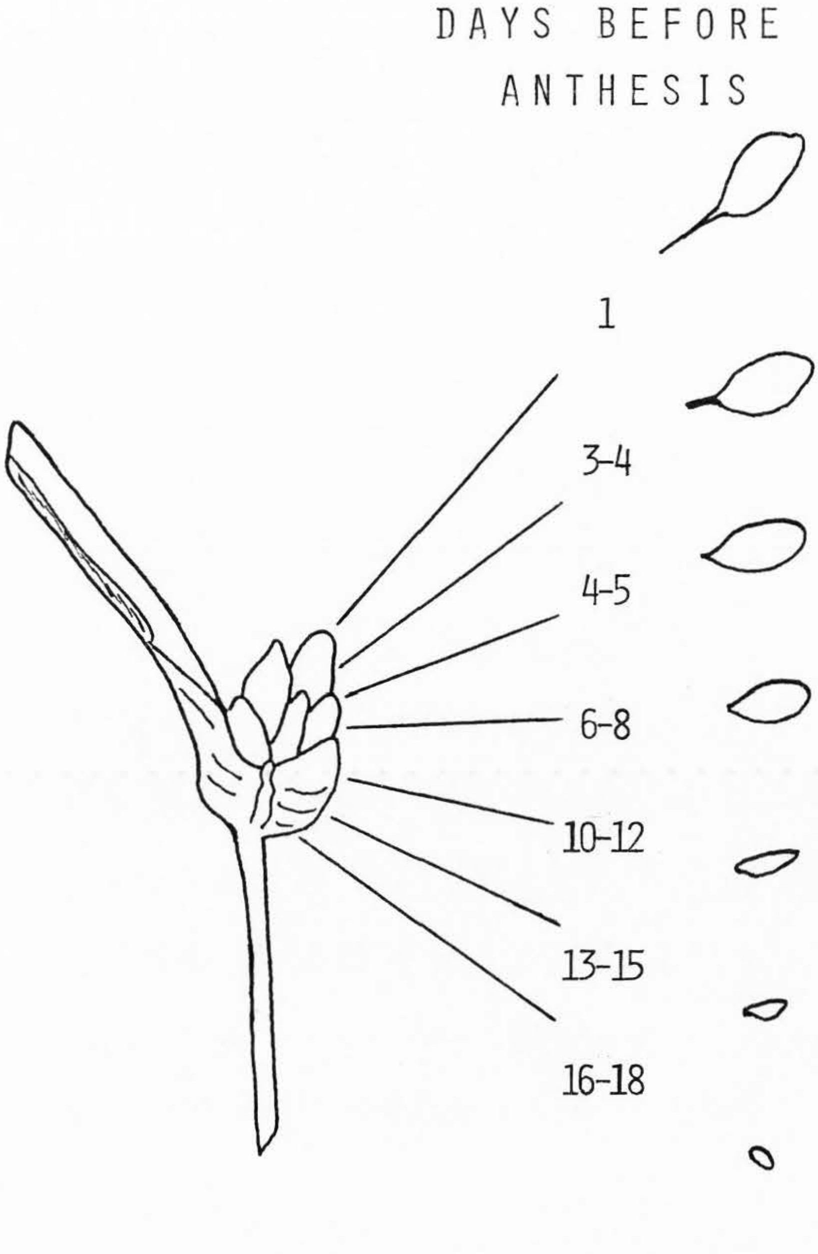

Figure 3. Approximate blooming order for individual flowers from an inflorescence of _Tradescantia_ clone 02.

was adjusted to that of the control (pH 8.0) with $CaCO_3$. A nutrient solution was added to each pot once every five days for the first two weeks (Blankedaal et al., 1972). Plants at each radiation level were separated by 30 cm on the same greenhouse bench, while those in the control soil were located in an adjacent room.

Ten days after the plants were potted, data were collected for 20 continuous days on the number of stunted hairs, somatic mutations per stamen and percent pollen viability. This ten day lag period served two purposes. First, it allowed the plants to recover from the shock of transplanting. Second, effects from the radiation treatment are not seen until a period of one week to ten days has elapsed (Ichikawa and Sparrow, 1967a; Underbrink et al., 1970). A maximum of four flowers, from each treatment, were chosen randomly each day, and from these flowers one stamen was chosen at random for scoring. The stamens are numbered with an arbitrary antipetalous stamen being number one, and then in a clockwise fashion the next antipetalous stamen is two, the next three. The antisepalous stamen located between stamen one and two is designated four, with five and six located in a similar clockwise manner (Figure 4). The selected stamen was then divided into three arbitrary regions for ease of scoring (Figure 5). The types of somatic aberrations scored were blue to pink, blue to colorless, pink to colorless, colorless to pink, normal size cell to giant or dwarf, and branched hairs (Figure 6). Loss of reproductive integrity in stamen hairs was measured by the number of stunted hairs (Ichikawa and Sparrow, 1967a, 1968a). An average hair consisted of 20.5 cells and a stunted hair was defined as one with 12 cells or less (Ichikawa and Sparrow, 1967a; Underbrink, Schairer and Sparrow, 1973).

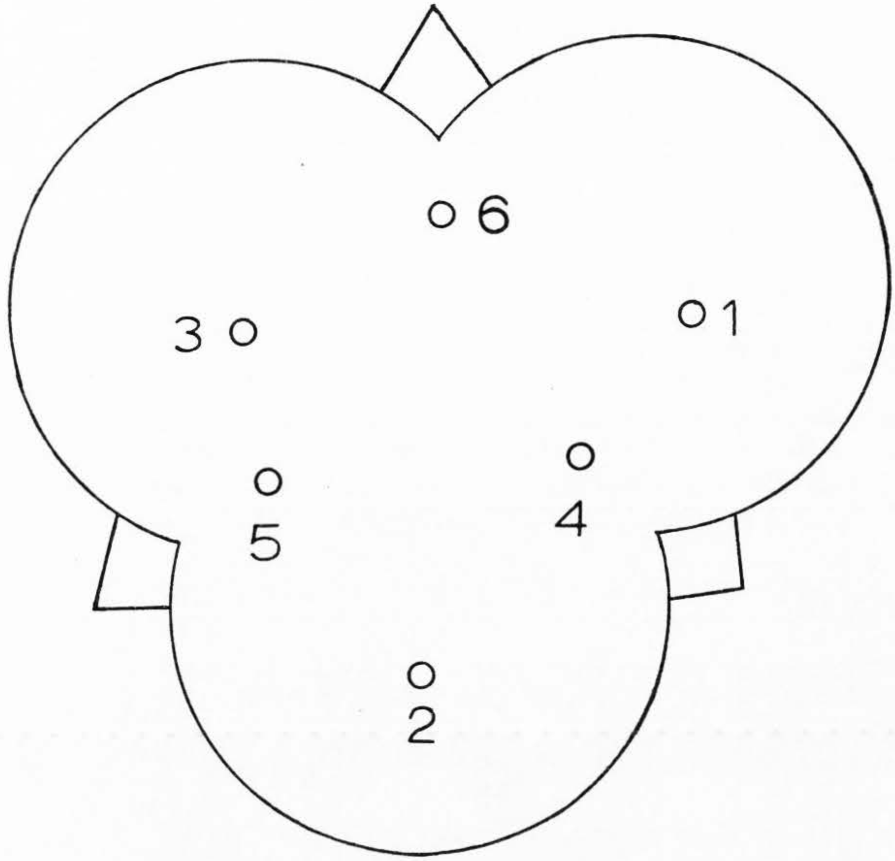

Figure 4. Schematic representation of a Tradescantia clone 02 flower demonstrating the procedure for numbering stamens.

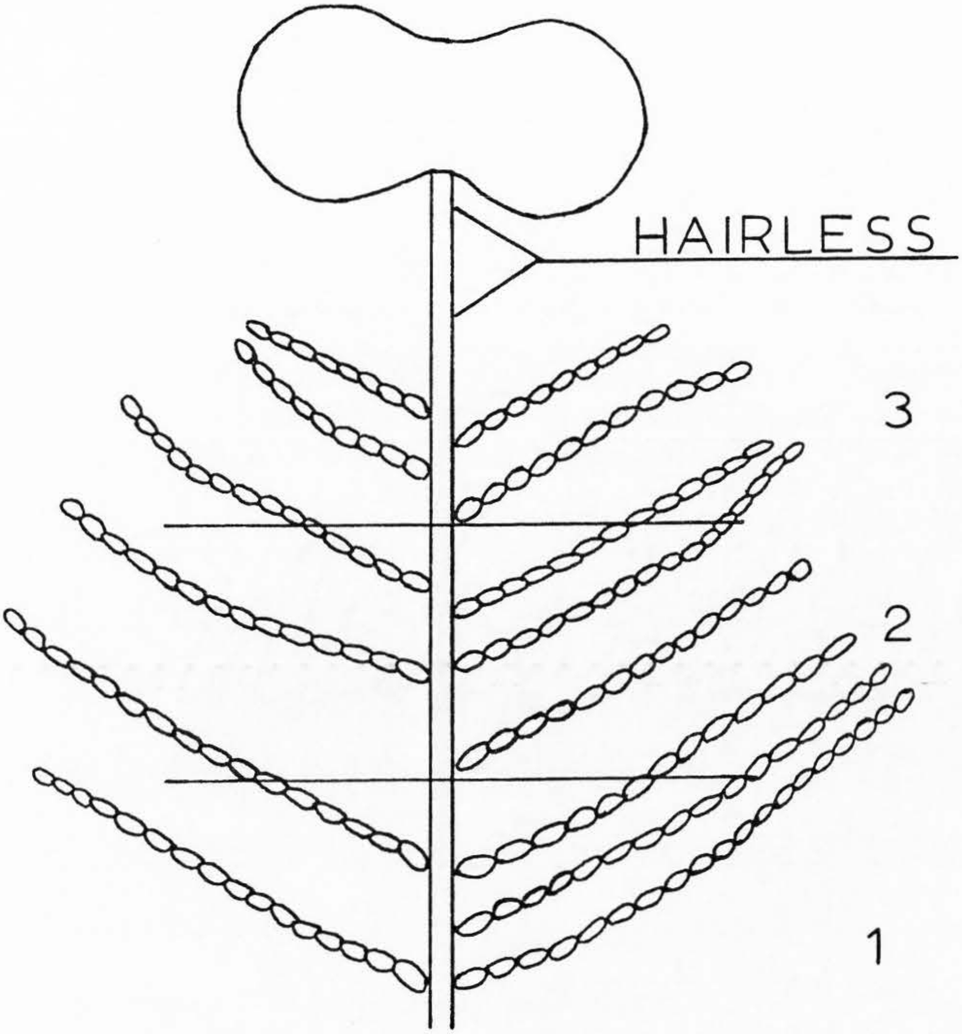

Figure 5. Schematic stamen with hairs, showing the three arbitrary divisions of the filament.

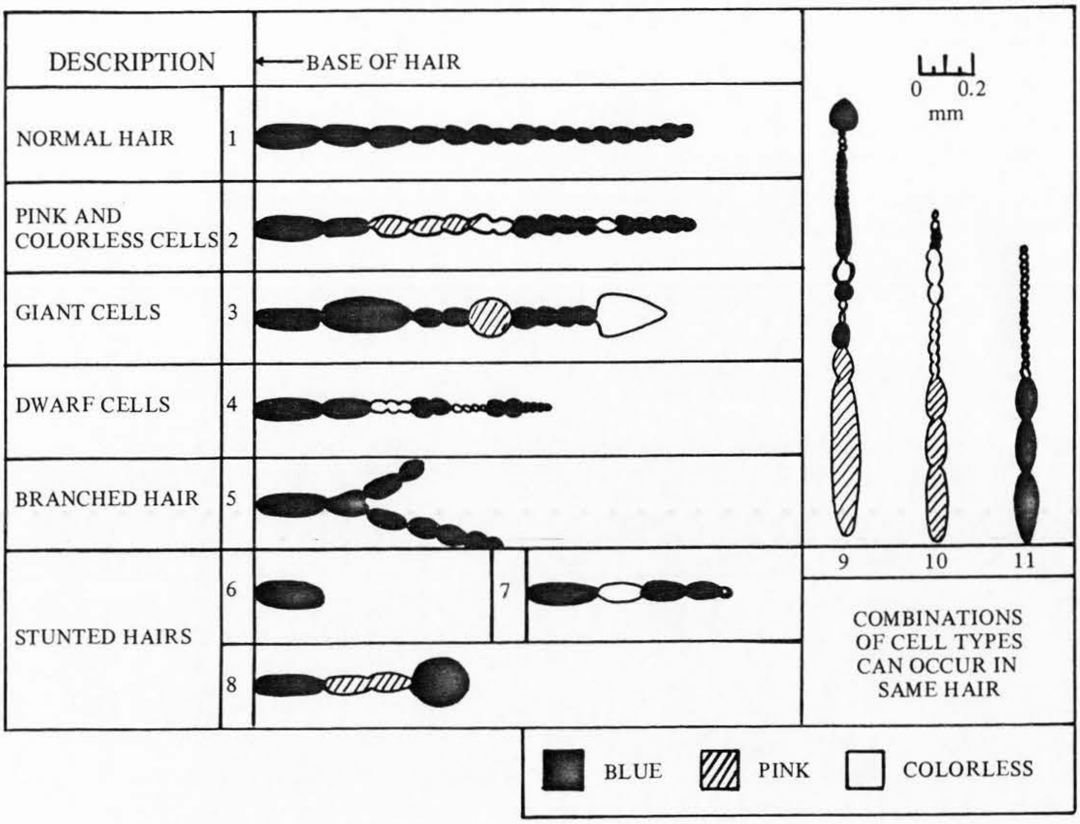

Figure 6. Diagram representing the different types of cell mutations scored and stunted hairs from the stamen of *Tradescantia* clone 02.

Reproductive integrity is the ability of somatic tissue to achieve the normal number of mitotic cycles characteristic for the species which will lead to its natural size and shape. The number of stunted hairs is correlated with reproductive integrity by demonstrating that these hairs have not obtained their normal length by a slow down or complete cessation of the mitotic cycle. Pollen viability was determined by randomly selecting two of the four flowers previously picked and smearing the remaining five anthers on a slide to release the pollen grains. The pollen was then stained with acetocarmine. The viable pollen absorbed the stain and the nucleus appeared red, whereas the aborted pollen remained yellow (Figure 7). Data were recorded in percent viable and nonviable pollen. Each parameter was subjected to an analysis of variance using a completely randomized design followed by statistical comparisons for locating significant differences among the treatments.

Radiation source analysis

Nayar, George, and Gopal-Ayengar (1970) have shown that the ionizations from radionuclides absorbed by roots of Tradescantia clone 02, from radioactive monazite sand, were more important than external radiation in increasing the frequency of somatic mutations. A similar experiment was conducted with a soil sample from the Vitro Mill tailings. The purpose was to determine whether the differences in the number of somatic mutations per stamen, stunted hairs per stamen, and pollen viability were due to internally absorbed radionuclides or to external ionizations.

Six plants of equal size and containing young inflorescences were potted in soil with a background radiation level of 0.01 mR/hr.

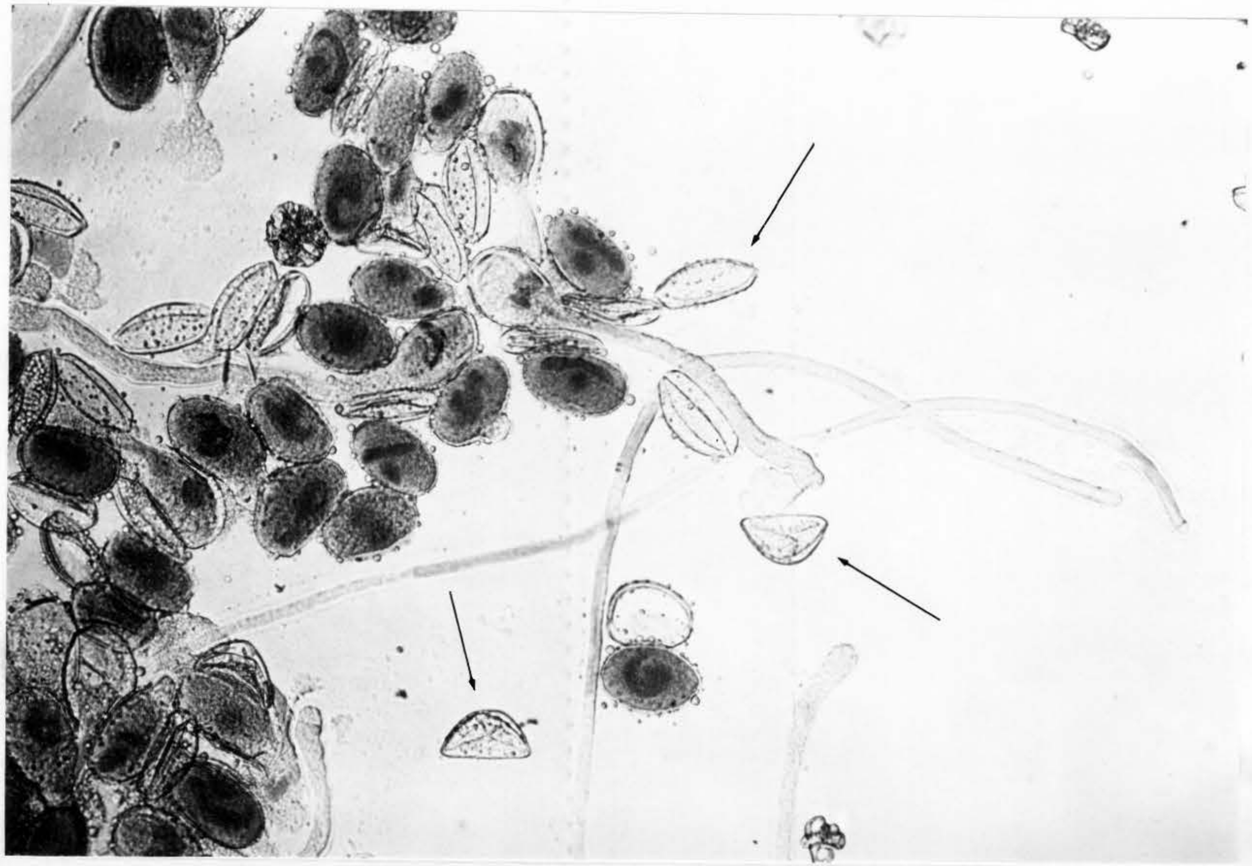

Figure 7. Pollen from Tradescantia clone 02 stained with acetocarmine to distinguish viable and non-viable grains. Note the non-viable pollen grains are lighter (arrows)(300 X).

A plastic bag was placed around each pot and all six arranged in a small wooden enclosure. The wooden enclosure contained a sample of soil that was analogous to the soil in the above 0.19 mR/hr treatment. In both experiments the treated plants were subjected to equal amounts of radioactive soil. Six different plants, representing the control, were potted in soil with a background radiation level of 0.01 mR/hr and located in an adjacent room. All plants were grown in the greenhouse. Ten days after potting, data were collected for 15 consecutive days on mutations per stamen, stunted hairs per stamen, and percent pollen viability in a manner similar to the above procedure. The data were subjected to Student's t test.

RESULTS

Analysis of the plant community and soil variables

A list of the species sampled, their corresponding chromosome number, nuclear volume (NV), interphase chromosome volume (ICV), and relative amount of DNA per chromosome, from each plot is presented in Table 1. The coefficient of community (Whittaker, 1975) was used in determining species similarity between the two controls, the two treatments, and between the controls and treatments. The results were .53, .76, and .09 respectively. The values obtained for the treatment plots and for the control plots are relatively high. The smallest coefficient of community was observed between the controls and treatments with Atriplex patula the only species occurring in both. These values, however, only reflected the similarity of the four plots at that specific time of year.

The pH, EC_e, (mmhos/cm), and soil texture for each plot is presented in Table 2. There was a slight decrease in pH from the control to the hot site, though neither of these values were extreme enough to affect normal plant growth. Electrical conductivity showed an increase from the controls to the hot site. Even with this increase these values demonstrate that the soil salt concentration will have no effect on plant growth. The soil texture analysis showed all four plots to be similar i.e., they were either a sandy or silt loam.

Table 1. Plant species collected from the four radiation levels and their corresponding chromosome number, nuclear volume, interphase chromosome volume, and relative amount of DNA per chromosome.

Radiation Level	Plant Species	Chromosome Number	NV μm^3	ICV μm^3	DNA (relative amount per chromosome)
Control 1 (0.01 mR/hr)	*Cardaria draba* L.	64 (8n)	67	1.0	---
	Ambrosia artemisiaefolia L.	36 (6n)	106	2.9	---
	Lactuca serriola L.	18 (2n)	141	7.8	---
	Carex nebraskensis Dewey	--	145	---	---
	Poa pratensis L.	77 (10n)	169	2.2	0.7
	Nepeta cataria L.	34 (2n)	81	2.4	---
	Asclepias speciosa Torr.	22 (2n)	61	2.7	---
	Juncus balticus Willd.	40 (4n)	65	1.6	---
	Rumex crispus L.	60 (6n)	204	3.4	---
	Asteraceae (spp.)	--	99	---	---
Control 2 (0.01 mR/hr)	*Cardaria draba* L.	64 (8n)	103	1.6	0.2

Table 1. Continued

Radiation Level	Plant Species	Chromosome Number	NV μm^3	ICV μm^3	DNA (relative amount per chromosome)
	Ambrosia artemisiaefolia L.	36 (6n)	229	6.4	0.3
	Lactuca serriola L.	18 (2n)	152	8.4	1.6
	Carex nebraskensis Dewey	--	144	---	---
	Poa pratensis L.	77 (10n)	328	4.3	0.2
	Cirsium sp.	--	348	---	---
	Atriplex patula L.	36 (4n)	303	8.4	0.5
	Agropyron repens L.	42 (6n)	425	10.1	0.7
	Unknown	--	105	---	---
Medium (1.00 mR/hr)	*Medicago lupulina* L.	16 (2n)	44	2.8	0.8
	Atriplex patula L.	36 (4n)	56	1.6	0.2
	Bassia hyssopifolia Pall.	18 (2n)	68	3.8	0.6
	Salsola kali L.	36 (4n)	116	3.2	0.3

Table 1. Continued

Radiation Level	Plant Species	Chromosome Number	NV μm^3	ICV μm^3	DNA (relative amount per chromosome)
	Hordeum jubatum L.	52 (4n)	254	4.8	0.6
	Helianthus annuus L.	34 (2n)	153	4.5	1.0
	Grindelia squarrosa Pursh	--	89	---	---
	Descurainia sophia L.	28 (4n)	--	---	---
	Lepidium perfoliatum L.	--	--	---	---
	Bromus tectorum L.	14 (2n)	--	---	---
Hot (2.20 mR/hr)	Medicago lupulina L.	16 (2n)	65	4.1	0.5
	Atriplex patula L.	36 (4n)	69	1.9	0.4
	Bassia hyssopifolia Pall.	18 (2n)	81	4.5	0.7
	Salsola kali L.	36 (4n)	123	3.4	0.3
	Hordeum jubatum L.	52 (4n)	271	5.2	0.7
	Sisymbrium altissimum L.	14 (2n)	34	2.4	---

Table 2. The pH, EC_e, and soil texture for the control and treatment plots.

Plot designation	pH	EC_e	Soil texture
Control 1	8.0	0.1	Silt loam
Control 2	8.0	0.1	Silt loam
Medium	7.6	0.9	Sandy loam
Hot	7.3	1.4	Silt loam

Comparisons of nuclear parameters between the four plots

Comparisons made on NV, ICV, polyploidy, and relative amount of DNA per chromosome between the treatments and controls were performed to establish the validity of hypotheses One and Two. There are, however, a number of species lacking all the assessed nuclear parameters. *Descurainia sophia*, *Lepidium perfoliatum*, and *Bromus tectorum*, located on the medium site had already set seed at the time of sampling. Thus no apical meristems were sampled and only the chromosome numbers of *D. sophia* and *B. tectorum* are shown. The chromosome numbers for *L. perfoliatum*, *Carex nebraskensis*, and *Grindelia squarrosa* were at this time, as far as the author was aware, unpublished. It was not possible to identify three of the samples to the species level e.g., *Cirsium*, a total unknown, and an Asteraceae. An attempt was made, by returning to the sites later in the summer, to acquire the adult forms for identification. This was met with little success owing to: (1) Possible differences between the juvenile and adult forms; and (2) The plant species may have matured and senesced in the intervening time. However, Grindelia squarrosa was identified in this manner.

Levels of ploidy ranged from eight diploids, five tetraploids, four hexaploids, one octaploid, to one pentaploid which appeared to be an aneuploid. There was a significant difference, using Student's t test at the .95 level, in the ploidy number between the control and treatment plots. Higher ploidy levels occurred more frequently in the control plots than in the treatment plots with the averages 5.0 and 3.1 respectively.

Nuclear volumes ranged from 34 μm^3 for Sisymbrium altissium to 425 μm^3 for Agropyron repens. There was good correlation between the NV for the same species as they occurred in the medium and hot sites. When comparing NV for similar species in the two control plots some major deviations were apparent. Cardaria draba, Ambrosia artemisiaefolia, and Poa pratensis all had considerably larger NV in control Two as compared to control One. However, Lactuca serriola and Carex nebraskensis had similar NV values from each of the two controls. Atriplex patula was the only species which occurred in the two treatment plots and control Two, as previously mentioned. There were similar NV values for this species in the treatments, 56 μm^3 and 69 μm^3. However, the NV observed in control Two was fairly large, being 303 μm^3.

Statistical analysis, adjusted for unequal sample size, gave adjusted means of 113 μm^3 for control One, 262 μm^3 for control Two, 119 μm^3 for the medium, and 121 μm^3 for the hot sites. The analysis of variance (ANOV) showed control Two to be significantly larger than the others. There was no significant difference between the other three.

Interphase chromosome volume showed relatively little difference between similar species as they occurred in the medium and hot sites. The one exception was Medicago lupulina with a larger ICV in the hot treatment. Species comparisons among the controls showed that only Ambrosia artemisiaefolia and Poa pratensis had relatively larger ICV in control Two. Atriplex patula showed the same trend with ICV as it did with NV.

Statistical analysis, again adjusted for unequal sample size, gave averages of 3.3 μm^3 for control One, 6.6 μm^3 for control Two, 4.3 μm^3 for the medium, and 3.7 μm^3 for the hot site. The ANOV showed no significant difference between any of the plots. Thus, the differences observed in polyploidy level and NV among the different plots may have been depressed when these two parameters were combined in calculating ICV.

Relative amounts of DNA per chromosome are only shown for control Two and both medium and hot treatments. Poa pratensis is the only species that appears in control One, the other species were not analyzed due to technical accidents and a lack of material. All DNA values were very similar for species occurring in both medium and hot sites. Medicago lupulina possessed the largest difference in relative DNA amounts per chromosome. Poa pratensis had a much larger DNA value in control One than in control Two. The means for the relative amounts of DNA per chromosome were 0.58 for control Two, 0.61 for the medium, and 0.51 for the hot site. The ANOV showed no significant difference between these plots.

Effects of ionizing radiation on stamen hairs and pollen of *Tradescantia*

The procedure for testing hypothesis Three uses the stamen hairs and pollen from *Tradescantia* clone 02. It involved determining if the radiation levels on the mill tailings were sufficient to change the normal growth and reproductive patterns of radiosensitive plant species.

Average number of mutations per stamen for each level of radiation is presented in Figure 8. The ANOV revealed a significant difference between the treatments and the statistical comparisons revealed exactly where the differences occurred. Three subsets appeared within the six radiation treatments (Figure 8). A linear regression analysis with adjusted treatment means demonstrated a significant correlation, $R = .78$ and $r^2 = .62$. The comparisons, regression ANOV, and model are presented in Table 3.

Figure 9 shows the average number of stunted hairs per stamen for each treatment. Again a significant F value for treatments was obtained through the ANOV, with the statistical comparisons determining the exact locations of significance. Three major subsets were apparent with different treatments appearing in more than one subset. A linear regression, using adjusted means, gave a significant $R = .88$ and $r^2 = .77$. The comparisons, regression ANOV, and model are presented in Table 4.

Pollen viability, averaged for each treatment, is shown in Figure 10. Analysis of variance showed a significant difference between the different radiation levels. The statistical comparisons grouped the six treatments again into three subsets. There was some overlapping of subsets among the treatments. The linear regression, on the adjusted

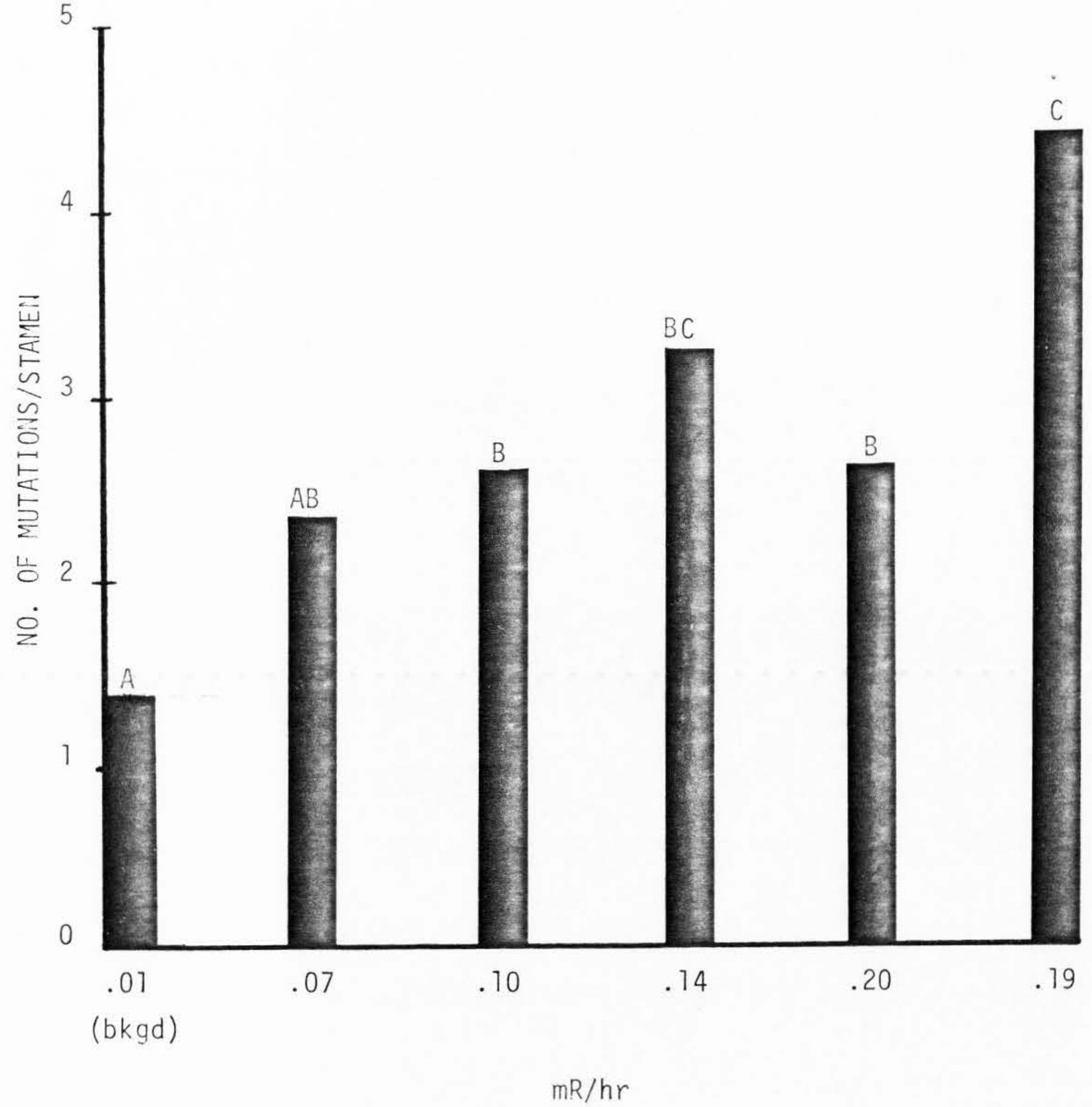

Figure 8. Mutations per stamen in *Tradescantia* clone 02, as affected by ionizing radiation from radioactive mill tailings. Columns with the same letter above are not significantly different.

Table 3. Analysis of variance for statistical comparisons between the six treatment levels for the average number of mutations per stamen, and ANOV regression.

ANOV	d.f.	S.S.	M.S.	$F_{(cal)}$
Treatments	5	444.7	88.9	4.6**
0.01 vs 0.07 (mR/hr)	1	18.6	18.6	0.9
0.07 vs 0.10 (mR/hr)	1	1.9	1.9	0.1
0.01 vs 0.10 (mR/hr)	1	59.5	59.5	3.1*
0.14 vs 0.19 (mR/hr)	1	35.9	35.9	1.8
0.01-0.07 vs 0.10-0.14-0.20 (mR/hr)	1	93.6	93.6	4.8**
0.01-0.07-0.10-0.14-0.20 vs 0.19 (mR/hr)	1	195.0	195.0	10.0**
Residual	338	6557.4	19.4	
Corrected Total	343	7001.9		

ANOV (regression)	d.f.	S.S.	M.S.	$F_{(cal)}$
Model	1	3.2	3.2	6.5*
Residual	4	1.9	0.5	
Corrected Total	5	5.1		

MODEL $Y = (10.86)(X) + 1.50$

* Significant at the 90 per cent confidence level
** Significant at the 95 per cent confidence level

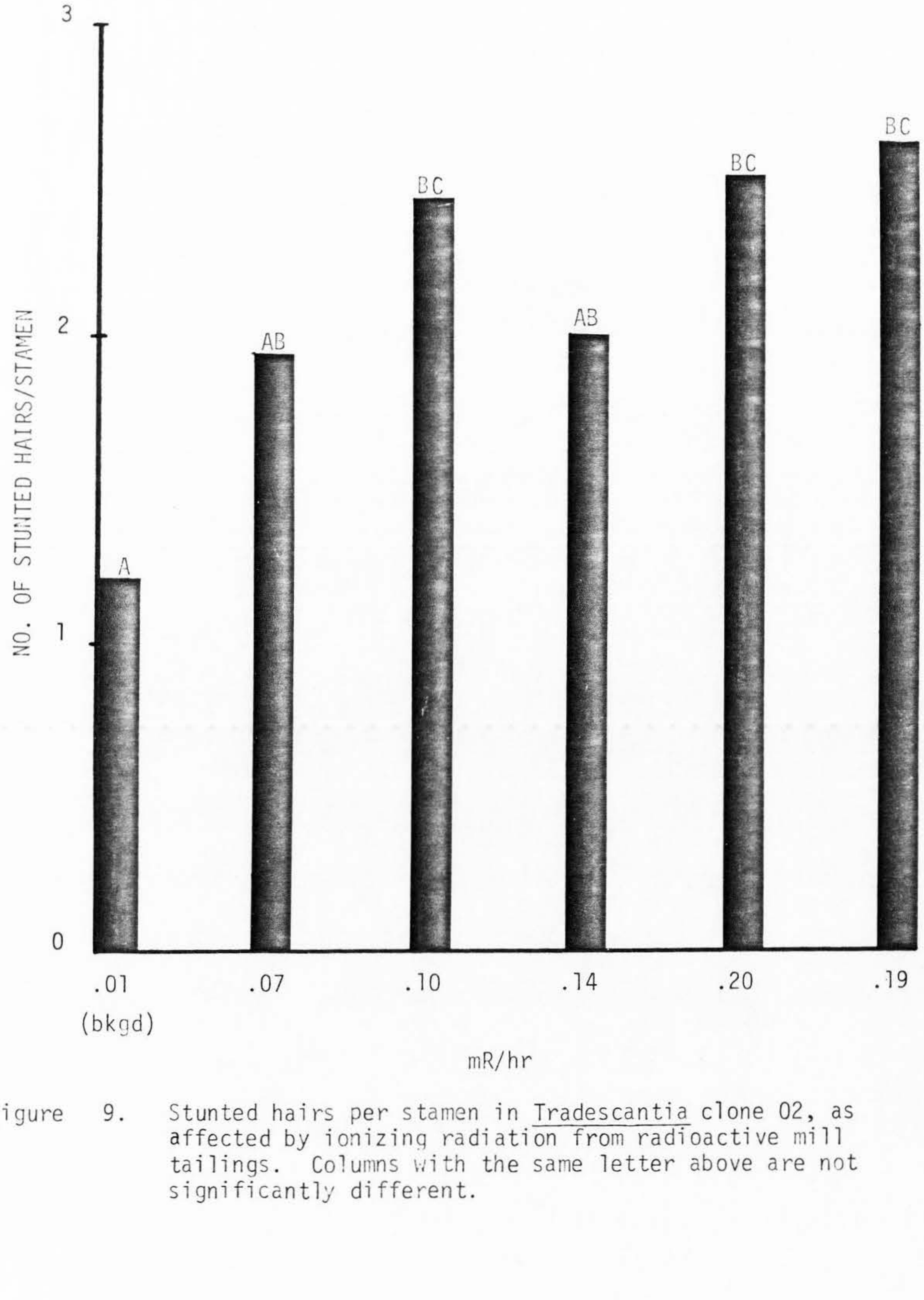

Figure 9. Stunted hairs per stamen in Tradescantia clone 02, as affected by ionizing radiation from radioactive mill tailings. Columns with the same letter above are not significantly different.

Table 4. Analysis of variance for statistical comparisons between the six treatment levels for the average number of stunted hairs per stamen, and ANOV regression.

ANOV	d.f.	S.S.	M.S.	$F_{(cal)}$
Treatments	5	101.6	20.3	2.8*
0.01 vs 0.07 (mR/hr)	1	9.5	9.5	1.3
0.01 vs 0.10 (mR/hr)	1	50.2	50.2	6.8*
0.01 vs 0.14 (mR/hr)	1	18.6	18.6	2.5
0.01-0.07-0.14 vs 0.10-0.20 (mR/hr)	1	53.7	53.7	7.4*
0.10-0.20 vs 0.19 (mR/hr)	1	1.7	1.7	0.2
Residual	338	2467.4	7.3	
Corrected Total	343	2569.0		

ANOV (regression)	d.f.	S.S.	M.S.	$F_{(cal)}$
Model	1	1.08	1.08	13.7*
Residual	4	0.32	0.08	
Corrected Total	5	1.39		

MODEL $Y = (6.36)(X) + 1.36$

* Significant at the 95 per cent confidence level

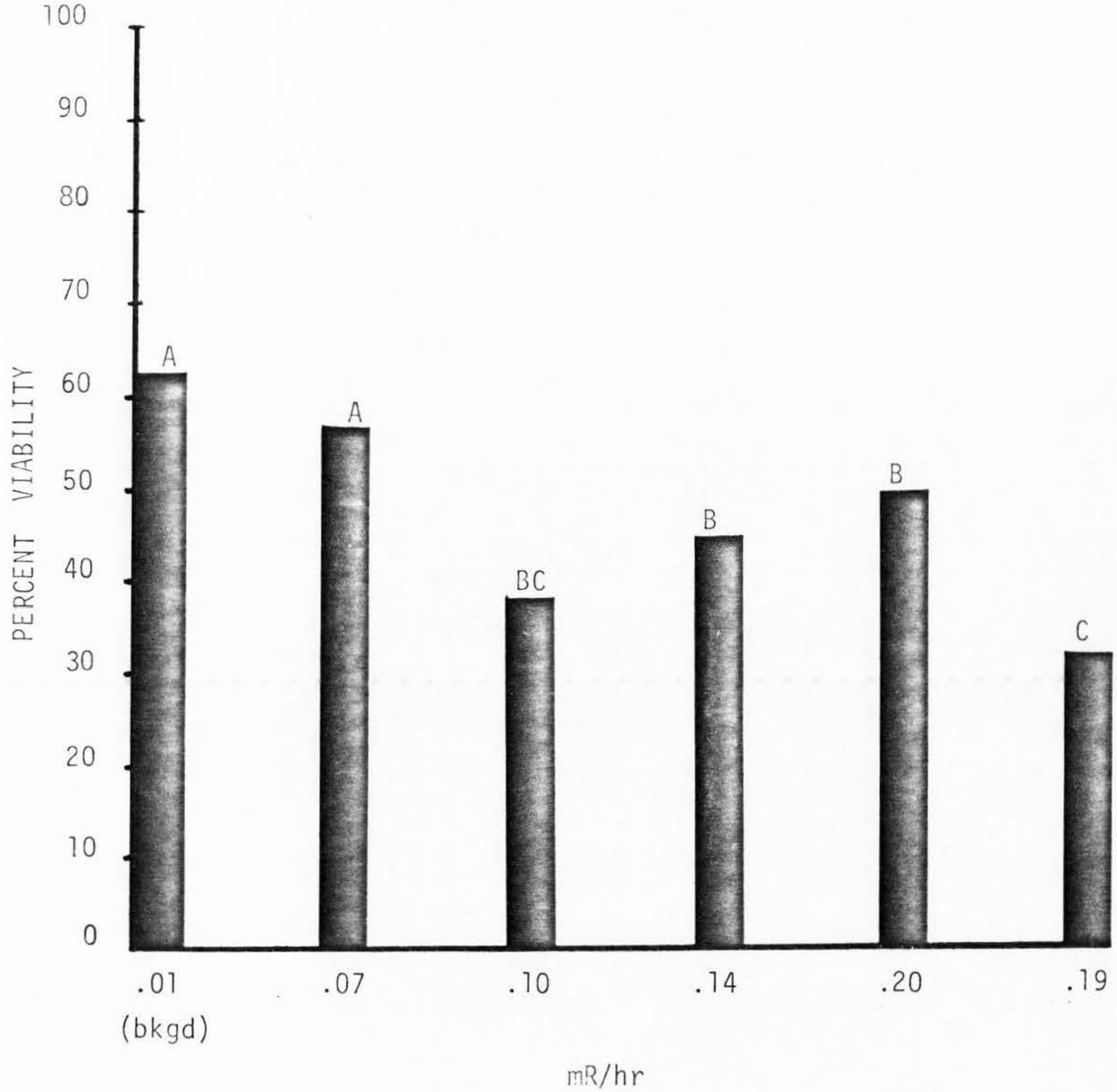

Figure 10. Pollen viability in Tradescantia clone 02, as affected by ionizing radiation from radioactive mill tailings. Columns with the same letter above are not significantly different.

Table 5. Analysis of variance for statistical comparisons between the six treatment levels for the average percent pollen viability, and ANOV regression.

ANOV	d.f.	S.S.	M.S.	$F_{(cal)}$
Treatments	5	27828	5565	15.0**
0.01 vs 0.07 (mR/hr)	1	293	293	0.8
0.07 vs 0.20 (mR/hr)	1	1030	1030	2.9*
0.07 vs 0.14 (mR/hr)	1	2076	2076	5.8**
0.10-0.19 vs 0.14-0.20 (mR/hr)	1	5520	5520	15.4**
0.14-0.20 vs 0.19 (mR/hr)	1	5560	5560	15.5**
Residual	200	71600	358	
Corrected Total	205	99428		

ANOV (regression)	d.f.	S.S.	M.S.	$F_{(cal)}$
Model	1	314	314	3.7
Residual	4	336	84	
Corrected Total	5	650		

* Significant at the 90 per cent confidence level
** Significant at the 95 per cent confidence level

means, revealed R = .69 and r^2 = .48, which were not significant (Table 5).

The results from the radiation source analysis experiment are presented in Table 6. There was no significant difference between the control or treated plants in the number of mutations per stamen, stunted hairs per stamen, or pollen viability.

Table 6. Student's t test for comparing stamen hairs and pollen from *Tradescantia* which were subjected to an external radiation dose of 0.18 mR/hr or 0.01 mR/hr.

Parameter	d.f.	$t_{(cal)}$
Somatic mutations per stamen	68	0.19
Stunted hairs per stamen	68	0.24
Pollen viability	22	0.88

DISCUSSION

The relationship between the nuclear parameters and succession

Statistical analysis of NV demonstrated a significant disparity between the plots e.g., the mean NV for plants in the treatments and control One were approximately equal, whereas the mean NV for plants in control Two was decidedly larger. Reasons for this dicotomy are associated with the plant species which occurred in both control plots. Half of these had an average NV 35 to 54 percent smaller in control One as compared to control Two. In addition, the NV of Atriplex patula in control Two was over three times as large as the corresponding NV from A. patula growing on the medium and hot sites. Sparrow, Rogers and Schwemmer (1968) stated that different sampling times, for actively growing meristems, can be the cause for a 30 to 50 percent variation in NV between individuals of the same species. In this experiment species from each control plot were fixed at different times over a two day period. This variation in sampling can affect NV in many ways, with the resulting changes occurring in G_1, S, and G_2 stages of interphase. Thus, the difference in NV between the four plots may best be explained as a result of sampling and fixing meristems from the same species at different times and at different mitotic stages.

The control plots contained more polyploid species than did the treatment plots. This is exactly opposite of what was hypothesized and, in retrospect, the ploidy level should have no effect on radiosensitivity. The difference in numbers of polyploids between the plots can be accounted for by the fact that polyploids are more often perennials than annuals (Stebbins, 1950). Moreover, there were more perennials sampled from the controls than from the treatments.

When ICV comparisons were made between the four plots no significant differences were found. The variation in NV and polyploidy has thus been negated by their incorporation into the ICV. Since radiosensitivity is more closely correlated with ICV than NV or polyploidy, the significance noted in the latter two variables would not reveal any notable relationships among the plots as to which one was more adapted to radiation.

Relative amounts of DNA per chromosome showed no distinction between control Two and the hot and medium sites. This does not verify, nor does it disprove the hypothesis that relative amounts of DNA per chromosome can demonstrate a similar relationship to radiosensitivity as the actual amount of DNA per chromosome has shown (Baetcke et al., 1967). With the plant species and radiation levels represented here there was no difference in the relative amount of DNA per chromosome.

By calculating ICV it is possible to predict the morphological and developmental changes a species may undergo when irradiated with a specified dosage. It is also possible to determine the amount of radiation applied by examining the resulting changes in individual or community development (Woodwell and Whittaker, 1968; Baetcke, Sparrow and Nauman, 1968). When there is a difference between one individual or community and another but they possess a similar ICV, the disparity cannot be explained in terms of radiation. In the current situation there was a difference in species composition between treatments and controls, thus possibly verifying hypothesis One. However, this difference was not reflected in the statistical comparisons involving ICV and relative amounts of DNA per chromosome. The disparity, between plots, was not due to radiation stress for they all possessed similar ICV, but instead it may be due to some other factor e.g., the differential length of time each site has been undergoing succession. There are two reasons for proposing the latter statement. First, the coefficient of community showed a greater diversity in control One and Two than in the hot and medium sites. This is a major premise in ecological thought; there is an overall increase in species diversity from early successional stages to later stages with varying degrees of diversity occurring at different times and under different environments (Whittaker, 1975; Odum, 1971). Second, a closer examination of the species listed in each plot disclosed more perennials in the controls than in the treatments, and more annual "weedy" species in the treatments than in the controls. This also lends support to the control and treatment sites being distinct with respect to the length of time succession has been proceeding (Whittaker, 1975). With respect to the present study, the results do not support hypothesis One which was a stated assumption relating low-level chronic radiation and its effects on specific nuclear parameters to species composition in plant communities inhabiting radioactive mill tailings. These results also nullify hypothesis Two i.e., a threshold dosage exists before effects in these nuclear parameters can be detected for the plant communities and the radiation levels under examination.

Although radioresistance and ICV show an inverse relationship (Baetcke, Sparrow and Nauman, 1968), this quality was not expressed in the present study, even though the treatment plots were definitely exposed to more radiation than either of the two controls. The actual radiation levels detected on the treatment sites were in milliroentgens whereas the dosages used to determine the correlation between ICV and radiosensitivity were in either roentgens or killiroentgens (Sparrow, Schairer and Sparrow, 1963; Sparrow and Sparrow, 1965; Sparrow et al., 1967). The dosage observed on the Vitro Mill tailings may not be large enough to initiate a response which can be correlated to ICV, as seen in the latter studies. In addition, there was no large distinction between the treatments and controls in terms of radiation dosage when compared to studies by Sparrow (1964; 1965), where differences of 100 to 1,000 roentgens were common.

Sensitivity of Tradescantia clone 02 to low-level ionizing radiation

Early studies conducted by Sparrow and Pond (1956), using two Antirrhinum clones, demonstrated that exposure levels of 16 mR/hr significantly increased mutation rates. They also suggested that an increase in mutations could be detected at much lower doses.

A more comprehensive analysis, correlating low-level radiation and its effect on the stamen hairs of Tradescantia clone 02, has been conducted by Mericle and Mericle (1963, 1965a,b). Their procedure involved exposing plants to geological dikes where the radioactivity was two to five times higher than normal. They detected significant changes in the number of somatic mutations and stunted hairs per stamen at 0.10 and 0.25 mR/hr. Since the plants were potted in normal soil this increase is attributed primarily to external radiation.

Results of this study closely paralleled those obtained by Mericle and Mericle (1963). All plants subjected to 0.10 mR/hr and above showed a significant difference from the control plants. The only exception was in the number of stunted hairs per stamen. Here, plants subjected to 0.10 mR/hr did show statistical distinction from the controls, but plants grown in soil at 0.14 mR/hr showed no significant difference from the controls. This anomaly may be due to biological variation, and could perhaps be substantiated if more data were collected (Underbrink, Schairer and Sparrow, 1973). Although there appeared to be variation throughout the six treatments in the number of mutations per stamen and stunted hairs per stamen, the trend was for an increase as illustrated by the regression ANOV (Table 3 and 4). Pollen viability, however, did not show a significant trend with increasing radiation, but there was a definite decrease in viability when comparisons were made against the two extremes (Table 5 and Figure 10). In contrast to the studies by Mericle and Mericle (1965a,b), there was no disparity in the qualitative analysis of the stamen hairs or pollen from 0.10 mR/hr to the hottest treatment levels 0.20 and 0.19 mR/hr. The present study also revealed that plants grown in the 0.07 mR/hr soil demonstrated similar results as the control plants according to the ANOV (Table 3, 4, 5). Thus, the threshold dosage in the present study seems to lie in the vicinity of 0.07 mR/hr. Again if it had been possible to collect more data this too might have shown significance over the control (Underbrink, Schairer and Sparrow, 1973).

The data analyzed from the Tradescantia stamen hairs and pollen lend support to hypothesis Three, but only to selected plants i.e., those with a high radiosensitivity. Stated in another way, species characterized by low radioresistance e.g., Tradescantia clone 02, will have to adjust to an increase in somatic mutations, and a decrease in reproductive integrity and pollen viability when growing on the radioactive mill tailings from the Vitro Chemical Plant.

The radiation source experiment was an attempt to verify the origin of the ionizations that contributed to the change in mutation rate, reproductive integrity, and pollen viability in Tradescantia clone 02. Student's t test confirmed evidence that the source was internal. This contradicts the results of Mericle and Mericle (1965a), though absorption of radioactive radon gas through the leaves could not be eliminated. In the present study, all internal radiation must be a product of root absorption. Work by Mistry et al. (1965) and D'Souza and Mistry (1970) examined the uptake of various radionuclides by plants inhabiting radioactive monazite sand and those growing in hydrosolutions. Radium-226 was transported more readily and accumulated in higher concentrations in stems and leaves of treated plants than any other radionuclide. This does not eliminate the fact plants can absorb other radionuclides such as uranium-238, thorium-230, lead-210, and polonium-210 (Dinse and LaFrance, 1953; D'Souza and Mistry, 1970). The ionizing radiation, from the Vitro Chemical Plant mill tailings, is due, in part, to these same elements, which represent the uranium decay series (Sears et al., 1975). Thus, Tradescantia probably absorbed radionuclides through its root system, and these nuclides may have caused the internal ionizations, with radium-226 most probably the predominant element involved.

Also the levels of radiation measured in the greenhouse were considerably lower than that recorded on the mill tailings. If it had been possible to grow these plants on the site the differences between the radiation levels and the controls might have been more pronounced due to the greater divergence of treatment dosages (Nayar and Sparrow, 1967; Ichikawa, 1971).

Radiological surveys taken on the five radioactive soils as they existed in the greenhouse revealed the two hottest samples to be approximately equal in activity, whereas in the field there was a much larger difference. Reasons for the initial gross decrease have already been discussed. Subtle similarities and variations are related to the Vitro Chemical Plant and its milling operations. First, ore for the mill was appropriated from numerous mines and is thus composed of several different mineral types. Second, the procedure for extracting the uranium was altered in 1956. Thus the soil texture and its chemical makeup varied from one site to another. This affects the presence and availability of numerous radionuclides, brought about by differential leaching due to changes in soil types. Consequently the radioactivity in the soil samples will vary, and the degree to which these radionuclides are available to the plants will now differ (Osburn, 1965; Prokhorov, 1973). These factors help explain the similarity between the two hottest samples. Furthermore, in presenting Figures 8, 9, and 10 the six radiation treatments were arranged as to their activity observed on the mill tailings and not as they occurred in the greenhouse.

Although the radiation levels from the tailings of the Vitro Chemical Plant were enough to cause detrimental effects in Tradescantia stamen hairs, one must realize that not all plant species exhibit this degree of radiosensitivity, especially when the stamen hairs themselves are more sensitive to radiation than the rest of the plant (Sparrow and Schwemmer, 1974). There are many physiological and morphological attributes a species may possess which will allow it to exist in an environment subjected to above normal radiation levels e.g., nutritional state, growth hormone concentrations, plant size and depth of roots, time requirement for mitosis and meiosis, percentage of cells dividing in the meristem, and most importantly ICV (Gunkel and Sparrow, 1961). However, radioactive uranium mill tailings, which represent a source of low-level chronic radiation, are a potential hazard to any species trying to establish on these soils. If the species has any mechanism that conveys a relatively high radioresistance it will not be affected by the radiation. The other possibilities are a lower fecundity, a higher somatic mutation rate, and a decrease in reproductive integrity. These three aspects, working together, will incur a disadvantage onto a radiosensitive population by decreasing its competitive ability, and thus altering the chances of becoming successfully established. The radiosensitive species will not contribute as it normally would in the successional sequence, but will be selected against, allowing only the more radioresistant species to colonize such sites. Due to this fact, succession on radioactive uranium mill tailings, over a long period, may not proceed in the same direction or at the same rate as would a similar area lacking radiation stress. And in a situation where there is low-level radiation the change will not be apparent until succession has proceeded to the point where the natural turnover time in species composition is greater than the time required for radioselection to be effective.

SUMMARY AND CONCLUSIONS

Even though the treatments and controls represent two different successional stages subjected to three different levels of radiation, there was no disparity between the species in ICV and relative amounts of DNA per chromosome. The ionizing radiation emitted from the mill tailings of the Vitro Chemical Plant does not represent sufficient dosage to alter species composition in the plant communities studied. Thus, there was no supporting evidence for hypotheses One and Two in relationship to the existing environmental conditions. Hypothesis One is: plant succession on a radioactive waste site will consist of polyploid species characterized by small ICV and comparatively small relative amounts of DNA per chromosome, as compared to species colonizing a waste site subjected to background levels of radiation. Hypothesis Two is: a threshold dose at the waste site must exist before changes in these nuclear parameters can be detected. However, the analysis from the stamens and pollen of Tradescantia revealed that the radiation emitted from the tailings has the potential to select against species with high radiosensitivity as determined by ICV. This affirms hypothesis Three: the ionizing radiation that exists on the tailings has the potential to increase the number of somatic mutations and decrease both pollen viability and the overall reproductive integrity of selected plants that come in contact with the mill tailings.

Although there was no difference between plant community composition from the radioactive site to the control site, the radiation emitted from the tailings has the potential to alter normal plant succession. Increased radiation stress will decrease the ability of radiosensitive species to compete against other species and decrease their ability to survive in these types of environments. The effects from the increased radiation stress would also be long range in nature with the extinction of plants due to radiation not noticeable until local extinction by succession is proceeding at a slower rate.

Revegetating programs, aimed specifically at uranium mill tailings, must consider the stress from low-level chronic radiation. The species listed in this study as growing on the hot and medium sites will grow on similar sites given the same environmental conditions. The problem may come later when these sites are approaching a climax situation because these latter successional stages are more sensitive to radiation (Woodwell and Whittaker, 1968; Sparrow and Woodwell, 1962). The program must consist of species with relatively small ICV to insure their radioresistance.

Uranium mill tailings are not only a source of increased radiation stress to the flora and fauna inhabiting the site, but also to surrounding biological systems. Examples from aquatic communities are the Jordan River as it passes near the Vitro Chemical Plant (Pendleton et al., 1964), the Animas River as it flows past an old uranium mill in Durango, Colorado, and the San Miguel in Western Colorado (Martin, Helm and Sigler, 1969). An area needing investigation is the effect this low-level chronic radiation has on adjacent terrestrial communities. These communities may be exposed to: (1) radioactive dust (Sears et al., 1975); (2) water that has drained through uranium mill tailings and may be contaminated with radionuclides; and (3) increases to radioactive radon gas escaping from the mill tailings. Crops, irrigated with contaminated water, may undergo abnormal growth and development. Finally, it is important to consider the following ramifications on human populations: (1) How are they affected by culinary and irrigation water contaminated with radionuclides; (2) Does the radioactive dust and radon gas in the surrounding air occur in quantities large enough to pose a threat to health; and (3) Are the radionuclides, present in the crops and animals they consume, a health hazard?

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